The Biomedical Basis of Elite Performance
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Contribution of respiratory and locomotor muscle afferents to the cardio-ventilatory responses to rhythmic exercise in humans - The Physiological Society's 2012 Bayliss-Starling Prize Lecture

J.A. Dempsey

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When studied in isolation, stimulating muscle afferents clearly increases ventilation and sympathetic vasoconstriction – but their contribution remains uncertain when activated in a physiologic setting, i.e. in the presence of other powerful influences from central command and other reflex receptors. We and others have studied exercising canines and humans to determine that metaboreflexes from the diaphragm and expiratory muscles are activated in heavy exercise in health and during moderate exercise in disease states, thereby enhancing sympathetic vasoconstrictor outflow and affecting blood flow distribution between limb and respiratory muscles. These influences on oxygen transport exacerbate the rate of exercise-induced locomotor muscle fatigue and reduce exercise performance. A unique vasoreactivity of the diaphragm vasculature contributes importantly to these selective responses. Blockade of opiate-sensitive spinal afferents from the legs at all cycling intensities caused hypoventilation and reduced MAP and heart rate. Endurance exercise performance was also impaired primarily because of reduced O₂ transport. On the other hand, loss of feedback via afferents from fatiguing limbs also meant less inhibition of central locomotor command, resulting in a “choice” to continue exercise (or augment power output), thereby precipitating excessive peripheral fatigue. The relative importance of muscle afferents is likely altered in the presence of CHF and COPD, with aging, in hypoxic environments and with training. Our understanding of exactly which stimuli / conditions during rhythmic exercise activates these metaboreceptors remains incomplete, as does our knowledge of additional influences arising from interactive effects between feedback and feedforward mechanisms.

Supported by NHLBI.

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The biological basis for exercise and health

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Epidemiology has associated physical inactivity with 35 detrimental conditions/diseases; they are: accelerated secondary aging, premature death, low cardiorespiratory fitness, sarcopenia, metabolic syndrome, obesity, insulin resistance, prediabetes, type 2 diabetes, nonalcoholic fatty liver disease, coronary heart disease, peripheral artery disease, hypertension, stroke, congestive heart failure, endothelial dysfunction, arterial dyslipidemia, hemostasis, deep vein thrombosis, cognitive dysfunction, depression and anxiety, osteoporosis, osteoarthritis, balance, bone fracture/falls, rheumatoid arthritis, colon cancer, breast cancer, endometrial cancer, gestational diabetes, preeclampsia, polycystic ovary syndrome, erectile dysfunction, pain, diverticulitis, constipation, and gallbladder diseases.

Deteriorating function with physical inactivity underlies some of the above conditions. After 20 days, healthy young men in the classical Dallas bed rest study had 11% lower heart volumes, 26% lower maximal cardiac outputs, 29% lower maximal stroke volumes, and 28% lower maximal oxygen consumptions. Microgravity of 1-yr space flight produces hipbone loss that would take 10 years to occur on Earth, illustrating gravity’s role with physical inactivity. A 3rd example is reduced daily steps. Healthy young men who reduced their daily steps from 10,501 to 1,344 for a 2-wk period displayed a clustering of metabolic alterations that included an increased insulin response to an oral glucose tolerance test, increased plasma triglyceride response to an oral fat tolerance test, a 7% increase in visceral fat, and a 0.5-kg loss of lean leg mass. A cellular basis was decreased insulin-stimulated ratio of pAkt(thr308)/total Akt in vastus lateralis muscle after step reduction.

Two animal models utilized by us to delineate the molecular basis of inactivity will be described. In the first model, rats have been selectively bred now for 7 generations to separate and contrast the phenotypes of motivation to voluntarily run long distances (HVR) vs. the motivation to voluntarily run low distances (LVR). An average 6-fold distance in voluntary running now separates HVR and LVR selected lines. HVR had reduced voluntary running after injection of D1 dopamine receptor agonists and antagonists into the nucleus accumbens of the rat brain, while LVR had no responses (Physiol Behav 105:661, 2012). The second model locks wheels for voluntary running (WL) resulting in elimination of daily running. The mRNAs and proteins for the mechanosensor genes Ankrd2 and Csrp3 fell in young, growing rats. Relative to WL5h controls, plantaris muscle Ankrd2 and Csrp3 mRNAs were lower at WL53h, WL173h, and SED; Ankrd2 protein tended to decrease at WL53h (p = 0.054) and Csrp3 protein was less in WL173h and SED rats (J Appl Physiol Epub Jan 26, 2012).

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How science, medicine and engineering has changed how athletes train and perform

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Arguably sport science is in its infancy, with the first robust publications truly directed at the subject appearing many years after physiological and medical related treatises relevant to similar subject areas. While understanding in biomedical sciences has progressed almost exponentially since the advent of modern biology of the Watson and Crick era, the same can’t really be said for sport science at its application edge. Often sport science appears undertaken with little reference to more progressive and better work in fundamental fields of biomedical endeavor.

This does not necessarily need to be the case. Much work of high caliber in biomedicine and engineering can be piggy-backed on to do innovative work at the applied end of sport science. While innovation is not always viewed as novel or pure in the academic sport science perspective it can both escalate and accelerate applied adoption of good fundamental concepts and indeed correct poorly used and extrapolated ones. A good example of the latter is the understanding (and application) of the hormone testosterone. The clear results in muscle hypertrophy from supraphysiological abuse led to the sport science concept that small changes in natural levels would be equally important, probably an erroneous notion of what testosterone does. Excellent work at the biomedical level has challenged this and as a consequence in turn driven elite athlete applied work demonstrating other important sporting applicable roles of natural testosterone more in keeping also with a biological evolutionary perspective. Similarly an examination of a wealth of biomedical data compared to new descriptive data collected in elite athletes suggest that elites function quite differently to the often used student population sport science study.

Rapid progress in engineering and technology has also driven sporting applications that have undoubtedly assisted in the color of medals obtained – while it has become a sporting cliché –centimeters, hundreds of seconds and minimal percentages that appear marginal do increment to a measurable gain. Knowledge on athletic performance is rapidly evolving through the miniaturization of electronics, growth in processing power and automation methods which are now common place in the field. Such technological advances permit the rapid testing and application of fundamental biomedical ideas in an ecologically valid environment in which the athlete and coach practice and thrive. These advances provide a platform for greater adherence and commitment from athlete and coach to a systematic process of investigation in pursuit of performance development. Although much work still remains, technology is beginning to provide the capability that allows an integrated systems approach to understanding how individuals respond to the stressors of training and competition in a continuous and longitudinal manner.

Our talk will discuss some of these examples in detail and argue that harvesting fundamental work in bio-medicine and engineering can promote high caliber applied sport science research that is both more scientifically robust and more quickly adoptable to sporting gains. These two make extremely good partners and have, and can further, push elite athlete practice forward.

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A life-time contribution to our understanding of the elite athlete

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Sports today are closely linked with sport science; a research field with roots in many early Nobel Prize winners' work in physiology or biochemistry with Hill being a key pioneer. The primary goal at the time was to understand basic functions of the human body during exercise, and not the least providing answers to which organs or functions of the body that set a limit for human performance. Three examples will be presented, hopefully demonstrating that it is worthwhile and challenging to study the basic mechanisms for human performance and that the inclusion of elite athletes in this research adds crucial insights.

Human skeletal muscle plasticity. The potential for a marked elevation in mitochondrial capacity is similar in type 1 and 2 fibres of human skeletal muscle. Usage of the two major motor units during the training is the critical factor. The triceps brachii muscle having the highest relative percentage of type 2 fibres may reach as high a mitochondrial capacity as a muscle with a dominance of type 1 fibres, as demonstrated in world class cc skiers. Thus, there is a clear dissociation between phenotype expressions of the contractile proteins as compared with the proteins regulating energy metabolism. Humans may be quite different from other species where there is a closer link between contractile and metabolic characteristics of the specific fibre types.

Muscle glycogen and fatigue. The link between the need for a large intake of carbohydrates to maintain large stores of glycogen in the human body for good endurance performance has a long history. Only recently have these studies reached the subcellular level. Glycogen granula are stored at different locations in a muscle fibre with two main sites around the mitochondria and close to the transverse tubuli (TT) and the sarcoplasmic reticulum (SR). Both stores are crucial, but the one close to the TT and SR systems appears to be essential not only to secure proper propagation of the action potential but also for the maintenance of the SR kinetics. With too low amounts of glycogen, the reuptake of Ca2+ from the cytosol by the SR system is retarded and peak tension development reduced; a mechanistic link, which is the same in both the main fibre types. It is worth highlighting that experimental evidence for the intake of ample amounts of CHO playing a role for endurance has been available since the late 1800. However, it has taken more than a century to explain why glycogen plays this crucial role.

Was Hill right or wrong? In 1923 Hill and Lupton wrote: “The volume of oxygen actually used by the heart is almost equal to that required (but not obtained) by voluntary muscle during very violent exercise. The muscle has to stop, owing to oxygen want”.

A view heavily debated through the years up to our time without reaching a consensus. Our data demonstrate that Hill was right. When elite cc skiers work either with their legs (roller skies on a treadmill), with their upper bodies (double pooling), or by using the ordinary diagonal style (whole body exercise) it was demonstrated that in the latter exercise muscle blood flow and O2-delivery were markedly reduced from peak levels, giving support to the notion once proposed by Hill that the heart was unable to deliver the blood flow that the muscles were asking for.

What is the value of the above given findings? Primarily they serve as tools in the understanding of regulations and limitations of basic human functions. They may also be of some interest to the “curious” athlete or coach, but they are hardly of any “help” in the athlete’s preparations to reach Olympic level performance.

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Vascular adaptation in athletes: Is there an “Athlete’s Artery”?

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Whilst the existence of a specific phenotype characterised as “Athlete’s Heart” is generally acknowledged, the question of whether athletes exhibit characteristic vascular adaptations has not been specifically addressed. To do so in this symposium, studies which have assessed the size, wall thickness and function of elastic, large muscular and smaller resistance arteries in athletes have been reviewed. Notwithstanding the caveats pertaining to cross-sectional comparisons between athletes and “matched” controls, these studies reveal increased conduit artery size, including enlargement of epicardial arteries and those supplying skeletal muscle. Evidence that peak limb blood flow responses are enhanced in athletes further suggests that resistance arteries undergo increases in total cross-sectional size. Such increases can be localised to those arteries supplying active muscle leading to speculation, supported by exercise training studies in humans and animal and cellular data, that arterial enlargement is associated with repetitive episodic increases in arterial shear stress which elicit endothelium-mediated remodelling. Such structural remodelling at conduit and resistance artery level may play a role in accommodating the substantial increase in cardiac output apparent in endurance athletes; arterial pressure is not increased at rest or during exercise in athletes (vs controls). Arterial wall remodelling also occurs in athletes but, in contrast to the impact of shear stress on remodelling of arterial lumenal dimensions, the impact of endurance athletic status on wall thickness may be a systemic, rather than localised, phenomenon. Finally, the question of whether the arteries of athletes exhibit enhanced function is moot. Somewhat paradoxically, measures of conduit and resistance artery endothelial function may not be enhanced, compared to healthy controls. This may relate to the inherent difficult of improving artery function which is already normal, or the time-course and transient nature of functional change. It may also relate to the impact of compensatory structural remodelling, as artery lumen size and wall thickness both affect functional responsiveness. In summary, there is clear evidence for an impact of athletic status on arterial structure and function, at least with respect of the impact of endurance training. Arterial adaptation may, to some extent, emulate that evident in the hearts of endurance athletes and it is tempting to speculate that similar mechanisms may be at play.

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The athlete's heart

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Endurance athletes have hearts that are large, compliant, relax fast, and pump a lot of blood. Indeed, a large maximal stroke volume is the key defining feature of the athlete’s heart. Much of the phenotypic elements of the athlete’s heart can be induced by a year of training in previously sedentary young individuals, though changing compliance may require much longer periods of training or starting at a young age during growth and development. Interestingly, the elaboration of the athlete’s heart begins with right ventricular eccentric hypertrophy, and left ventricular concentric hypertrophy; only with very prolonged and intense training does truly eccentric LV hypertrophy appear to develop in a longitudinal fashion. Females also develop physiologic cardiac hypertrophy, but despite virtually identical training, their hypertrophic response is blunted, presumably due to a reduced amount of physiologic anabolic steroids. A life-long pattern of competitive exercise seems to be highly protective against the cardiac atrophy of age, with preservation of youthful cardiac compliance. However even life-long training is not sufficient to prevent the slowing of relaxation which seems to be an inevitable consequence of senescence. Finally, some reports have suggested that extraordinary training can be injurious to the heart, and chronic left atrial dilation may increase the risk of atrial fibrillation. It is also possible that training in the presence of coronary artery disease, especially for older, ultra-endurance athletes may be deleterious; in fact, the term “athletes heart” was originally coined to reflect pathology. Nevertheless, long term follow of Olympic athletes suggest that this level of training does not have adverse consequences on the heart, and it is likely that adverse cardiac effects of training are the exception rather than the rule.

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Pulmonary system limitations to endurance exercise performance in humans

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Accumulating evidence over the past 25 years depicts the healthy pulmonary system as a limiting factor of whole body endurance exercise performance. This brief overview emphasizes three respiratory system-related mechanisms which impair O\textsubscript{2} transport to the locomotor musculature \([\text{arterial O}_2 \text{ content (CaO}_2) \times \text{leg blood flow (QL)}]\), i.e. the key determinant of an individual’s aerobic capacity and ability to resist fatigue. First, the respiratory system often fails to prevent arterial desaturation substantially below resting values and thus compromises CaO\textsubscript{2}. Especially susceptible to this threat to convective O\textsubscript{2} transport are well-trained endurance athletes characterized by high metabolic and ventilatory demands and, likely due to anatomical and morphologic gender differences, active females. Second, fatiguing respiratory muscle work (W\textsubscript{resp}) associated with strenuous exercise elicits sympathetically-mediated vasoconstriction in limb-muscle vasculature which compromises QL. This impact on limb O\textsubscript{2} transport is independent of fitness level and affects all individuals, however, only during sustained, high-intensity endurance exercise performed above \(~85\%\) VO\textsubscript{2max}. And third, excessive fluctuations in intrathoracic pressures accompanying W\textsubscript{resp} can limit cardiac output and therefore QL. Exposure to altitude exacerbates the respiratory system limitations observed at sea level and further reduces CaO\textsubscript{2} and substantially increases exercise-induced W\textsubscript{resp}. Taken together, the intact pulmonary system of healthy endurance athletes impairs locomotor muscle O\textsubscript{2} transport during strenuous exercise by failing to ensure optimal arterial oxygenation and compromising QL. This respiratory system-related impact exacerbates the exercise-induced development of fatigue and compromises endurance performance.

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The cardiovascular system and the ageing athlete

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The demographics of ageing are changing dramatically such that there will be many more older adults in the near future. This setting likely will produce a new “boomer-driven” epidemic of physiological dysfunction, disability and risk of chronic degenerative disorders, including cardiovascular diseases (CVD). Standing out against this dreary biomedical forecast are Masters athletes, a group of middle-aged and older adults who engage in regular vigorous physical training and competitive sport. Compared with their sedentary/less active (untrained) peers, Masters athletes who perform endurance training-based activities demonstrate a more favorable arterial function-structure phenotype, including lower large elastic artery stiffness, enhanced vascular endothelial function and less arterial wall hypertrophy. As such, they may represent an exemplary model of healthy or “successful” vascular ageing. In contrast, Masters athletes engaged primarily/exclusively in intensive resistance training exhibit less favorable arterial function-structure than their endurance-trained peers and, in some instances, untrained adults. These different arterial properties likely are explained in large part by the different intravascular mechanical forces generated during endurance vs. resistance exercise-related training activities. The more favorable arterial function-structure profile of Masters endurance athletes may contribute to their low risk of clinical CVD.

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Cardiovascular limitations in the Paralympic athlete with a spinal cord injury

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This report briefly summarises the cardiovascular factors that influence exercise physiology, and eventually sports performance, of athletes with a spinal cord injury (SCI). The consequences of a SCI are numerous and concern voluntary muscle function, deep and superficial sensitivity, and autonomic function to a degree determined by the level and completeness of the spinal lesion. Athletes with SCI perform with their upper body, which limits their maximal exercise capacity and puts them at a disadvantage compared to leg exercise in terms of mechanical efficiency and physiological adaptations to exercise. Studies generally find that maximal oxygen consumption and mechanical power output are inversely related to spinal lesion level. Athletes with cervical or dorsal lesions down to Th6 have limited maximal heart rates due to a lack of sympathetic drive to the heart. Blood redistribution from body areas lacking autonomic control will be impaired, thus reducing venous return and limiting cardiac stroke volume during exercise. Thermoregulatory function is affected through a lack of afferent neural feedback and limited efferent vasomotor and sudomotor control below the lesion. Strategies to support venous return and to promote body cooling potentially improve physiological responses and athletic performance, especially in individuals with high lesion levels. The latter are subject to autonomic dysreflexia, a generalised sympathetic vasoconstriction below the lesion resulting from nociceptive stimulations in insensate body regions. Acute episodes induce high blood pressure, may enhance exercise performance and must be treated as a clinical emergency. Deliberate triggering of this reflex is prohibited by the International Paralympic Committee.

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The evolving science of detection of ‘blood doping’

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Blood doping practices in sport have been around for at least half a century and will likely remain for several years to come. The main reason for the various forms of blood doping to be common is that they are easy to perform, and the effects on exercise performance are gigantic. Yet another reason for blood doping to be a popular illicit practice is that detection is difficult. For autologous blood transfusions for example, no direct test exists, and the direct testing of misuse with recombinant human erythropoietin has proven very difficult despite a test exists. Future blood doping practice will likely include the stabilization of the transcription factor Hypoxia Inducible Factor which leads to an increased endogenous erythropoietin synthesis. It seems unrealistic to develop specific test against such drugs (and the copies hereof originating from illegal laboratories). In an attempt to detect and limit blood doping the World Anti Doping Agency (WADA) has launched the Athlete Biological Passport (ABP) where indirect markers for all types of blood doping are evaluated on an individual level. The approach seemed promising but a recent publication demonstrates the system to be incapable of detecting even a single subject as “suspicious” while treated with rhEpo for 10-12 weeks. Sad to say, the hope that the 2012 London Olympics should be cleaner in regards to blood doping seems faint. We propose that WADA strengthens the quality and capacities of the National Anti Doping Agencies (NADA) and that they work more efficiently with the international sports federations in an attempt to limit blood doping.

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Drugs that are abused in sport

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Misuse refers to wrong or improper use whereas its synonym abuse has the connotation of a corrupt practice or custom especially one that has become chronic [Oxford English Dictionary online]. Indeed, intent is also usually implied with abuse. In the context of drugs in sport the term doping defines misuse or abuse. Evidence obtained from anti-doping analysis can clearly only identify misuse by an individual. Recently, surveillance programmes in the anti-doping context and interviewing individuals thought to be misusing drugs have provided clear evidence of abuse but it is still very rare for an athlete to admit the use of a drug.

The World Anti-Doping Agency (WADA) is the international body that produces a Prohibited List each year[1] clarifying what would constitute misuse in sport thereby providing the detail to be able to control misuse. The WADA list uses a variety of standard pharmacological categories for prohibited substances plus some less widely used terms to classify the types of substance that are prohibited. The prohibition of use may be at any time or only during competition (in-competition), which is defined as being within 12 hours of a competition until the end of doping control sample collection.

The main category of substances prohibited at all times is the so-called anabolic agents. This category is subdivided into anabolic androgenic steroids and other anabolic agents, that is substances with anabolic activity that do not have a steroidal structure. Other prohibited categories comprise peptide hormones, growth factors and related substances, beta-2 agonists, hormone and metabolic modulators, diuretics and other masking agents A new category called non-approved substances was added last year to the Prohibited List in order to be able to control designer drugs or drugs under development or that have been discontinued. WADA also prohibit a variety of methods some of which may involve drug administration. These methods are categorised as enhancement of oxygen transfer, chemical and physical manipulation and gene doping.

The categories of substances prohibited in-competition only comprise stimulants, narcotics, cannabinoids and so-called glucocorticosteroids. Some sports also prohibit alcohol and some beta-blockers.

The findings from WADA accredited laboratories are summarised each year. These figures obviously only document what has been found and not what is being misused and there is very limited reliable data relating to prevalence of misuse. Furthermore the reported findings require careful interpretation since they may be heavily influenced by apparently minor changes in the rules and/or by the sports that have been tested and by the type of competition.

Over the years since figures were first compiled (initially by the International Olympic Committee), nandrolone, testosterone and salbutamol appeared to be the most widely misused of all of the substances representing about 0.3 % of samples each against an average of between 1-2 % of all samples containing a Prohibited Substance. Indeed the reporting of testosterone rocketed in 2005 probably because WADA reduced the reporting threshold, based on the ratio of testosterone (T) to its inactive epimer epitestosterone (E) in urine, from 6 to 4. Presumably this was to make the test of administration more sensitive. Although the modal T/E is about 1, the ratio of 4 is exceeded in about 3 % of the normal population (see e.g. [2]). On the other hand, Asians in particular have a lower modal value of about 0.2 and some individuals can administer testosterone without exceeding a ratio even of 4. Internationally, nandrolone findings have remained fairly consistent over the years after a relatively large number of findings in the late 1980s thought to be caused by the use of nandrolone esters especially nandrolone decanoate that has a particularly long biological half-life. In the UK, an increase to 0.29 % in 1999 from the previous twelve-year average of 0.09 % was thought to have arisen from the use by athletes of contaminated supplements[3] coupled with the fact that the International Olympic Committee reduced the reporting threshold for the nandrolone metabolite 19-norandrostenedione after the Atlanta Olympic Games in 1996; thus even misuse in its generally understood sense cannot necessarily be proved by a doping control result. On the other hand, reported findings for salbutamol have dropped significantly in the last few years thought to be due simply to the fact that the reporting threshold has now been raised.

The pharmacology of most of the drug classes prohibited by WADA has been covered in a special edition of the British Journal of Pharmacology[4] and diuretics in a later issue.[5] This presentation will focus on how the abuse of drugs in sport may be reduced before the forthcoming Olympic Games.


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Testosterone in sport

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Anabolic androgenic steroids have powerful effects on lean body mass, which can be associated with an enhancement of performance. Intramuscular injection of testosterone induces an increase in net protein synthesis. At the cellular level, testosterone is associated with the hypertrophy of muscle fibres. However, there is evidence suggesting a more pronounced effect on slow type I muscle fibres. Testosterone is able to stimulate the mitotic activity of muscle satellite cells. By promoting the entry of satellite cells in the cell cycle, testosterone induces the generation of new myonuclei, which supports the hypertrophy of muscle fibres. The myotrophic effects of testosterone are mediated by androgen receptors expressed both by myonuclei and satellite cells. New studies suggest the existence of an androgen receptor-independent mode of action for testosterone. A signalling cascade dependent upon Erk and mTOR is activated in response to treatment with testosterone. Currently, the question related to the durability of the myotrophic effects after stopping testosterone usage has not been settled.

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The Athlete Biological Passport

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The Athlete biological passport (ABP) is an individual and longitudinal follow up in the fight against doping which came naturally with the implementation of tests out of the competition period of the athletes. For an individual, it has been observed that the homeostasis of biosynthesis and metabolism of endogenous hormones was not disturbed by sports activity, but of course will be influenced by the intake of similar substances. Actually, the passport has been defined as an individual and longitudinal observation of biomarkers. These markers need to belong to the biological cascade influenced by the application of forbidden hormones or more generally, affected by biological manipulations which can improve the performance of the athlete.

Nowadays, only the hematologic passport of an athlete has been officially set up. This is a statistical representation of the longitudinal follow up of some blood biomarkers. This individual and longitudinal follow up of blood parameters is of interest, because the intra-individual variability is lower than the corresponding inter-individual variability. Among the key points for the implementation of the athlete biological passport is its possibility to resist to the legal and scientific challenges. The ABP should be implemented in the most possible transparent way in the process and with the necessary independence between planning, interpretation and result management of the passport.

To reach this transparency and efficiency, a new major actor has been introduced in the system to create a framework of independence: The athlete Passport Management Unit (APMU). The World Antidoping Agency (WADA) did implement new dedicated technical documents associated to the passport (hematological module). This was done in order to allow a correct implementation of a profile which can resist to any scientific or legal critics in following strictly some steps in the chain of production of the results and in the management of the interpretation of the passport. Four technical documents have been then associated to the guidelines which correspond to the requirements for passport operation.

The ABP will be completed very soon by the steroid profile module. The same philosophy of individual and longitudinal follow up will be applied and the interpretation steps will also be managed by a specific APMU in a similar way as applied in the hematologic module. Thus, after exclusion of any possible pathology, specific deviations from the individual norms would be then considered as a potential manipulation of performance with hormones or other modulators.

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Central fatigue

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The term ‘central’ fatigue recognises that some important processes and limitations accompanying different types of human physical activity reside in the central nervous system (CNS). Furthermore, some of these processes lead to suboptimal force output from muscles in maximal voluntary tasks and hence it is important to determine the underlying mechanisms. The definition of central fatigue is a progressive exercise-induced failure of voluntary activation of the muscle. A subset of this type of fatigue is ‘supraspinal’ fatigue which refers to failure to generate output from the motor cortex. For reviews of the development of these concepts see Gandevia (2001), Taylor & Gandevia (2008) and Enoka & Duchateau (2008). The latter type of fatigue can be quantified with transcranial magnetic stimulation of the motor cortex. In terms of impaired force capacity, the development of supraspinal fatigue has been documented for both upper and lower limb muscles. It arises not only with isometric contractions but also with rhythmic exercise such as cycling. It can represent a large fraction of force loss in a maximal effort. Many factors are involved in the development of central fatigue. Muscle contraction alters the sensory input reaching the spinal cord and supraspinal sites. There are resultant changes in the drive to the motor cortex in sustained efforts as revealed by studies using electromagnetic stimulation and neuroimaging. Changes also occur in motor cortical circuits. In addition to changes in spinal reflexes, recent work using corticospinal stimulation (at a subcortical location) shows that exercise can profoundly depress motoneuronal responses to descending excitatory inputs (e.g. Butler et al. (2003); McNeil et al. (2009)). Ultimately, with exercise, the CNS must overcome many fatigue and activity-dependent processes occurring at different levels of the neuraxis and it must adapt its output to cope with changes in the size and dynamics of muscle force production.


Supported by the National Health and Medical Research Council

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Satellite cells in skeletal muscle growth, homeostasis and repair

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Skeletal muscle has primarily evolved to facilitate coordinated voluntary movement in animals. The functional cells of skeletal muscle are the long, cylindrical shaped, syncytial myofibres. Each is packed with myofibrils composed of thousands of sarcomeres, containing the actin and myosin filaments that interact to generate force. These multinucleated myofibres often contain hundreds of post-mitotic myonuclei, and are formed by the fusion of many myoblasts during developmental myogenesis.

Postnatal muscle growth in mouse is initially achieved by both further increasing the number of myonuclei, combined with myofibre hypertrophy. The myoblasts that supply these new myonuclei are generated by muscle satellite cells. After approximately 3 weeks of age though, further muscle growth occurs solely by myofibre hypertrophy. Satellite cells become mitotically quiescent as muscle matures, residing in a niche on the surface of the myofibre. However, these stem cells can be readily activated to enter the cell cycle to provide further myoblasts for the routine needs of myofibre homeostasis and hypertrophy, or the more sporadic demands for muscle fibre repair and regeneration.

There are distinct parallels between developmental and regenerative myogenesis, both in the mechanism, myoblasts fusing to damaged myofibres to replace lost myonuclei, or fusing together for de novo formation of new myofibres, and the regulatory networks that control satellite cell function.

Here, I will give an overview of the role of satellite cells in skeletal muscle biology and discuss the progress that we have made in understanding how satellite cell function is regulated.

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Fibre type transition and training

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The diversity of skeletal muscle fibers has been recognized since long time and it is now traditional to distinguish four major fiber types in mammalian muscles, type 1 or slow and fast 2A, 2X and 2B fibers, based on myosin heavy chain (MyHC) isoform composition. The difference between muscle fibers is not restricted to myosin and myofibrillar proteins, but involves metabolic enzymes (predominance of glycolytic or mitochondrial activities), and any subcellular system, including transmembrane ionic fluxes and intracellular calcium signaling (Schiaffino & Reggiani, 2011). Fiber type profiles vary according to species, most human muscles containing only type 1, 2A and 2X fibers, which differ from the corresponding mouse fibers, thus making dangerous any extrapolation of results from transgenic or knockout studies (Schiaffino, 2010). Muscle fibers differ in speed of shortening and maximal power output, which is ten times greater in human type 2X compared to type 1 fibers, and in the resistance to fatigue, which is much greater in type 1/slow fibers. Although the presence of intermediate fiber types may suggest that muscles consist of a continuous spectrum of fibers rather than distinct fiber types, the existence of clusters of molecular, structural and functional parameters indicate that fiber types do exist. Preferential combinations of specific molecular and functional properties presumably reflect the need to obtain consistent values of specific functions, for example, matching energy production with energy consumption. Gene expression profiles must therefore be compatible with the constraints imposed by electrical, mechanical and metabolic influences.

The muscle fiber type composition can change in response to variations in activity levels, however the degree of transformation is limited by intrinsic constraints within specific “adaptive ranges” of possible transitions. This may reflect a relative inflexibility of motor unit properties, which control the muscle phenotype. On the other hand, the finding that regenerating fast and slow muscles respond differently to the same electrical stimulation pattern suggests that intrinsic differences between muscle cell precursors in fast and slow muscles may contribute to restrict muscle plasticity (Khalovde et al, 2005). Human skeletal muscle can also undergo significant fiber type changes in response to training, for example MyHC-2X isoform is downregulated with increased activity with corresponding up-regulation of MyHC-2A, however fast-to-slow fiber type transformations have not been unambiguously demonstrated in athletes (Harridge, 2007). The signaling pathways which control the muscle fiber phenotype are presently the object of intensive research that will benefit not only sport science but also clinical medicine (Schiaffino et al, 2007; Gundersen, 2011).


Supported by grants from the European Commission (FP7 MYOAGE Integrated Project) and the Italian Space Agency (ASI, Project OSMA)

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Neural and muscular limitations in the Paralympic athlete

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Sports for people with disabilities and the Paralympic Movement is an evolving area. Since the start some 60 years ago, the Paralympic Movement and its governing body the International Paralympic Committee (IPC) has experienced exponential growth in the number of sports as well as the number of athletes competing in the Paralympics, the second largest sporting event in the world. Research involving the Paralympic athlete is also an evolving area encompassing basic science, applied science, social science, nutrition, and performance enhancement in both hot and cold environments. At the same time, training of aerobic, anaerobic, strength and endurance can be done similarly to other athletes. However, for certain groups of athletes there are neural and muscular limitations, which have to be considered when developing a training program. Examples are those athletes with a spinal cord injury, cerebral palsy or amputations. As with sports for able bodied individuals, knowledge from the field of athletics can be transferred to the area of rehabilitation. It is now known that sport specific training can enhance neural and muscular performance in people with stroke and other neurological disabilities. Thus, research into the area of the Paralympic athlete can enhance the design of rehabilitation program for many other people.


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Ageing human muscles and tendons: The master athlete

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Ageing of the musculoskeletal system is characterised by a loss of muscle mass and strength (sarcopenia). This process has a multiple aetiology, involving neuropathic changes, inflammation and oxidative stress, hormonal, nutritional, metabolic and lifestyle factors (Narici and Maffulli 2010). Amongst the lifestyle factors, inactivity is a main cause of sarcopenia.

Tendons are also affected by ageing and inactivity, as both conditions lead to a decline in stiffness, mostly due to a deterioration in material properties rather than to tendon atrophy (De Boer et al. 2007, Onanbele et al 2006). On the other hand, regular training, and in particular resistive exercise, has been found to be highly effective for recovering muscle mass, strength and tendon stiffness in older people (Reeves et al 2003). Probably, the best example of the efficacy of regular exercise in combating musculoskeletal frailty in old age, is the case of master athletes. These athletes, normally ranging from the age of 40 to 100+ years, not only achieve remarkable sporting records for their age but also display a smaller reduction in many physiological parameters linked to performance than their sedentary age-peers.

In terms of musculoskeletal characteristics, from 20 to 55 years of age, muscle volume of master sprinters (MS) is greater than those of master endurance (ME) runners and of untrained controls (UC) but beyond 55 years of age the difference tapers off progressively, becoming non significant at the age of 75 years (Grassi et al. 1999). In line with these observations, Type I and II fibre cross-sectional area (CSA) of ME runners is not different from that of UC, whereas type II fibre CSA of MS is consistently larger than that of ME and, particularly for the Type IIX fibres, larger than of UC (Aagaard et al. 2007). Fibre type proportion, shows a prevalence if type I fibres in the ME runners and a lower proportion of type IIX fibres in ME than in MS, while UC show the highest proportion of the fastest fibres. This high proportion of type IIX fibres in UC is consistent with the suggestion that physical activity level modulates myosin heavy chain composition in old age (D'Antona et al. 2003). Instead, type IIA fibre proportion is similar in all groups. Single fibre contractile properties of master runners are also different from those of controls. Widrick et al. (1996) found that gastrocnemius muscle type I and IIA fibres of the runners produced 15% and 22% less force than of controls while maximal shortening velocity (Vmax) was not different between the two groups. Muscle power of type I and type IIA fibres was 13% and 27% lower in the master runners than in the sedentary controls. Comparing single fibres of young and old sprint runners, Korhonen et al. (2006) reported no difference in specific tension between the two age groups.

However, maximum unloaded shortening velocity (Vo) of fibres expressing type I MHC was lower in the older runners while no difference in Vo of type IIA MHC fibres was found. Hence these data suggest that the decrease in Vo of slow fibres with old age is not due to a reduction in physical activity, as master athletes are highly active, but are mostly the result of ageing per se.

Whole muscle strength and power of power-trained athletes has also been found to be higher than those of endurance athletes and of untrained controls, with some studies reporting greater values also for endurance athletes. Also, the rate of force development of MS and ME is faster than that of UC. This preservation of muscle strength and power in master athletes seems also related to a protection from the age-related loss of motor units, probably due to a decrease in oxidative stress afforded by regular physical activity.

Scanty data exist on tendon mechanical properties of master athletes but it has been shown that resistive training in older men reverses the age-related decline in tendon stiffness. Reeves et al (2003) have indeed shown a 73% increase in tendon stiffness after 14 week resistive training in septuagenarian men. While no data exists on tendon stiffness in master athletes, recent measurements performed by our group show tendon strain values of master runners similar to those of young untrained controls; these seem due to a greater tendon CSA in the master runners. However, tendon hypertrophy in master runners seems a pathophysiological compensation to tendon injury and is correlated to the hours of training.

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Muscle Protein Synthesis (MPS) in response to nutrition & exercise

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MPS is the driving-force behind adaptive responses to exercise and represents a widely adopted proxy for gauging chronic efficacy of acute interventions i.e. exercise/nutrition. Recent findings in this arena have been progressive. Nutrient-driven increases in MPS are of finite duration (~1.5 h); switching-off thereafter despite sustained amino acid availability and intramuscular anabolic signaling. Intriguingly, this “muscle-full set-point” is delayed by resistance exercise (RE) (i.e. the feeding×exercise combination is ‘more anabolic’ than nutrition alone) even ≥24 h beyond a single exercise-bout; casting doubt on the importance of nutrient timing vs. sufficiency per se. Studies manipulating exercise intensity/workload have shown that increases in MPS are negligible with RE at 20-40% but maximal at 70-90% 1-RM when workload is matched (according to load×repetition number). However, low-intensity exercise performed to failure equalises this response. Analysing distinct sub-cellular fractions (e.g. myofibrillar, sarcoplasmic, mitochondrial) can provide a readout of chronic intervention efficacy in addition to effect size in MPS per se i.e. while ‘mixed’ MPS increases similarly with endurance and RE, increases in myofibrillar MPS are specific to RE; prophetic of adaptation (i.e. hypertrophy). Finally, the molecular regulation of MPS by exercise and its regulation via ‘anabolic’ hormones (e.g. IGF-1) has been questioned leading to discovery of alternate mechanosensing-signalling to MPS.

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Regulation and limitations to fatty acid oxidation during exercise

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Fatty acids (FA) as fuel for energy utilization during exercise originate from different sources: FA transported in the circulation either bound to albumin or as triacylglycerol (TG) carried by very low density lipoproteins (VLDL) and FA from lipolysis of muscle TG stores (IMTG). Despite a high rate of energy expenditure during high intensity exercise the total FA oxidation is suppressed to below that observed during moderate intensity exercise. Although this has been known for many years, the mechanisms behind this phenomenon are still not fully elucidated. A failure of adipose tissue to deliver sufficient FA to exercising muscle has been proposed, but evidence is emerging that factors within the muscle might be of more importance. The high rate of glycolysis during high intensity exercise might be the "driving force" via the increased production of acetyl CoA which in turn is trapped by carnitine. This will lead to decreased availability of free carnitine for long chain FA transport into mitochondria. This review summarizes our present view on how FA metabolism is regulated during exercise with a special focus on the limitations in FA oxidation in the transition from moderate to high intensity exercise in humans.

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Carbohydrate metabolism during exercise and training

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Utilization of carbohydrate in the form of intramuscular glycogen stores and glucose delivered from plasma becomes an increasingly important energy substrate to the working muscle with increasing exercise intensity. The talk gives an update on the molecular signals by which glucose transport is increased in the contracting muscle and how glucose uptake is affected by training. The talk also deals with the signalling relaying the well-described increased sensitivity of glucose transport to insulin in the post-exercise period which can result in an overshoot of intramuscular glycogen resynthesis post exercise (glycogen supercompensation).

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High intensity intermittent training

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High-intensity interval training (HIT) induces numerous physiological adaptations that resemble traditional endurance training despite a low total exercise volume (2). As little as six sessions of HIT over two weeks, totaling ~15 min of “all out” cycle exercise (~600 kJ total work), increases the maximal activity of mitochondrial enzymes and improves performance during tasks that rely heavily on aerobic energy provision (3). Low-volume HIT also promotes improvements in markers of metabolic control during matched-work exercise that are comparable to endurance training despite marked differences in total exercise volume and training time commitment (1). These data suggest that HIT may be a potent and time-efficient strategy to induce skeletal muscle metabolic adaptations and improve functional exercise capacity. Little is known regarding the molecular processes that regulate mitochondrial biogenesis in response to HIT but evidence is accumulating to suggest that peroxisome proliferator-activated receptor γ coactivator (PGC)-1α is involved. An acute bout of HIT (4 x 30 sec “all out” cycling interspersed with 4 min of recovery) increased the activation of 5’AMP-activated protein kinase and p38 mitogen-activated protein kinase, two kinases which can directly activate PGC-1α, and led to a robust increase in PGC-1α mRNA measured 3 hours into recovery (4). The majority of PGC-1α was detected in cytosolic fractions at rest but acute HIT increased nuclear PGC-1α protein 3 h into recovery, a time point that coincided with increased mRNA expression of mitochondrial genes, and this was followed by an increased mitochondrial protein content and enzyme activity at 24 h recovery (5). Many low-volume HIT studies have employed extreme variable-load exercise interventions (e.g., repeated Wingate Tests) that may not be safe or well tolerated by certain individuals. Little et al. (6) recently showed that 2 wk of a more “practical” model of HIT (6 sessions x ~10 x 1 min repeats at ~90% maximal heart rate, separated by ~1 min of recovery) increased muscle oxidative capacity and improved endurance performance. Low-volume HIT studies in persons who might be at risk for cardiometabolic disorders or patients with chronic disease are very limited. However, a recent study showed that six sessions of the practical HIT model over 2 wk improved estimated insulin sensitivity in previously sedentary, overweight individuals (7). Insulin sensitivity was calculated based on single fasting glucose and insulin measurements and therefore primarily reflects hepatic as opposed to peripheral insulin sensitivity. It was also recently shown that low-volume HIT was effective and well tolerated in people with type 2 diabetes (8). Two weeks of HIT reduced average 24-h blood glucose concentration and postprandial glucose excursions, measured via continuous glucose monitoring under standardized diet but otherwise free-living conditions. Given that “lack of time” is the most commonly cited barrier to regular exercise participation, it is tempting to speculate that low-volume HIT may represent a time-efficient alternative to traditional endurance training. While the preliminary evidence from small, short-term studies are intriguing, large-scale studies are clearly needed to resolve whether low-volume HIT is a realistic, time-efficient exercise alternative to improve health and reduce the risk of cardiometabolic disease.


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A key, but less understood, function of the cardiovascular system is to exchange heat between the internal body organs and the skin to maintain internal temperature within a narrow range in a variety of conditions that produce vast changes in external (exogenous) and/or internal (endogenous) thermal loads. Heat transfer via the flowing blood (i.e., vascular convective heat transfer) is the most important heat exchange pathway inside the body. This pathway is particularly important when metabolic heat production increases many fold during exercise. During exercise typical of many recreational and Olympic events, heat is transferred from the heat producing contracting muscles to the skin surrounding the exercising limbs and to the normally less mobile body trunk and head via the circulating blood. Strikingly, a significant amount of heat produced by the contracting muscles is liberated from the skin of the exercising limbs (González-Alonso et al. 1999, 2000). The local and central mechanisms regulating tissue temperature in the exercising limbs, body trunk and head are essential to avoid the deleterious consequences on human performance of either hyperthermia or hypothermia (González-Alonso et al. 2008; Taylor et al. 2008). This presentation focuses on recent evidence addressing: (i) the dynamics of heat production in contracting skeletal muscle, (ii) the influence of exercise and environmental heat and cold stress on limb and systemic haemodynamics, and (iii) the impact of changes in muscle blood flow on heat exchange in human limbs. The presentation highlights the need to investigate the responses and mechanisms of vascular convective heat exchange in exercising limbs to advance our understanding of local tissue temperature regulation during exercise and environmental stress and how temperature might limit human performance in extreme environments.


Recent author's work has been supported by the Gatorade Sports Science Institute and PepsiCo.

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High skin temperature and hypohydration impairs aerobic performance

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Heat stress and body water deficits (hypohydration) can impair both submaximal and maximal intensity aerobic exercise performance (Sawka et al., 2011). This paper examines the roles of hot skin (>35°C) alone and hypohydration (>2% body mass) on impairing submaximal aerobic performance. For this paper, we define hot skin as 35°C and above, warm skin as 30 to 34.9°C and cool / cold skin as <30°C. We recognize that skin temperature effects are a continuum and the Tsk to Tc gradient alters these relationships. Warm-hot skin is associated with high skin blood flow requirements and hypohydration is associated with reduced cardiac filling, both of which act to reduce aerobic reserve. In euhydrated subjects, hot skin alone (with a modest core temperature elevation) impairs submaximal aerobic performance (Ely et.al. 2009). Conversely, aerobic performance is sustained with core temperatures >40°C if skin temperatures are cool-warm when euhydrated (Ely et.al. 2010). No study has demonstrated that high core temperature (~40°C) alone, without coexisting hot skin, will impair aerobic performance (Cheuvront et.al. 2010). In hypohydrated subjects, aerobic performance begins to be impaired when skin temperatures exceed 27°C, and even warmer skin exacerbates the aerobic performance impairment (~1.5% for each 1°C Tsk) (Kenefick et.al. 2010). Hot skin and hypohydration may act through a variety of mechanisms including increased thermal discomfort, osmoreceptor / baroreceptor stimulation, reduced aerobic reserve, elevated perception of effort and altered afferent signal processing. We conclude that: 1) hot skin (high skin blood flow requirements from narrow Tsk to Tc gradients), is the “primary” factor impairing submaximal aerobic performance when euhydrated; 2) hypohydration impairs submaximal aerobic performance when skin temperatures ~27°C, and even warmer skin exacerbates (~1.5% for each 1°C Tsk) these decrements; and 3) high core temperature (~40°C) alone does not impair aerobic performance.


The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Army or the Department of Defense.

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Brain temperature and exercise performance

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Events arising within the central nervous system seem to play a major factor in the aetiology of hyperthermia-induced fatigue. Thus, various studies with superimposed electrical nerve stimulation or transcranial magnetic stimulation have shown that both passive and exercise-induced hyperthermia will impair voluntary motor activation during sustained maximal contractions. In humans the brain temperature increases in parallel with that of the body core making it very difficult to evaluate the independent effect of the cerebral temperature. Experiments with separate manipulation of the brain temperature in exercising goats indicate that excessive brain hyperthermia will directly affect motor performance. However, several homeostatic changes arise in parallel with hyperthermia including factors that may influence both peripheral and central fatigue and it is likely that these changes interact with the inhibitory effect of an elevated brain temperature.

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Genomic predictors of trainability

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The concept of individual differences in the response to exercise training or trainability was defined three decades ago. In a series of experimental studies with pairs of monozygotic twins, evidence was found in support of a strong genotype dependency of the ability to respond to regular exercise. In the HERITAGE Family Study, it was observed that the heritability of the VO2max response to 20 weeks of standardized exercise training reached 47% after adjustment for age, sex, baseline VO2max, and baseline body mass and composition. Candidate gene studies have not yielded as many validated gene targets and variants as originally anticipated. Genome-wide explorations have generated more convincing predictors of VO2max trainability. A genomic predictor score based on the number of favorable alleles carried at 21 single nucleotide polymorphisms appears to be able to identify low and high training response classes that differ by at least threefold. Combining transcriptomic and genomic technologies has also yielded highly promising results concerning the ability to predict trainability among sedentary people.

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Exercise therapy - the public health message

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Much of the evidence linking a sedentary way of life to morbidity and mortality has been derived from relatively healthy populations, but there are data on the effects of physical activity in individuals with health problems or with risk factors for chronic disease. Clinicians, and often the general public, frequently consider the primary value of physical activity as the contribution it makes to control of these other risk factors. This approach underestimates the value of physical activity in the prevention and treatment of numerous chronic health conditions. There is a steep inverse gradient of morbidity and mortality across categories of cardiorespiratory fitness and physical activity in all subgroups—women and men who are middle-aged or older, obese or normal weight, or healthy or unhealthy. The overall death rates vary by these subgroups, older individuals obviously have higher death rates than younger persons, but the pattern of association of fitness or activity to mortality is comparable for the various population subgroups. In fact, fit individuals with another risk factor often have lower death rates than unfit individuals without the risk factor.

Regular physical activity and moderate to high levels of cardiorespiratory fitness provide protection against numerous health problems and inactivity should be given increased attention by physicians and other health care professionals. Recent research on how to use cognitive and behavioral strategies to help sedentary individuals become more physically active has shown promising results. Exercise Is Medicine is a global initiative to address how to implement physical activity into clinical practice, and thereby help more patients improve their health.


Dunn AL, Marcus BH, Kampert JB, Garcia ME, Kohl HW III, Blair SN. Comparison of lifestyle and structured interventions to increase physical activity and cardiorespiratory fitness: A randomized trial. JAMA, 1999; 281:327-334.


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Training and bone – from health to injury

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Mechanical load through physical activity has been shown to be one of the best stimuli to increase the accrual of bone mass. But, also the structural skeletal adaptations associated with exercise contributes to bone strength. Exercise prescription also includes a “window of opportunity” in the late pre- and early peri-pubertal period, where exercise is supposed to insert the most obvious beneficial effects, even if physical activity provides recordable skeletal benefits during all growth. Adverse skeletal effects by exercise include exercise induced amenorrhea with low estrogen levels and low bone mass and mechanically induced stress fractures. These adverse effects could usually be avoided by adapting the level training to sufficient intensities. The reported exercise induced benefits in young years seems also to be at least partly maintained at advanced age. The notion that former male athletes have lower fracture risk than expected by age, support this view. Physical activity could therefore to be recommended at growth and adolescence as one possible strategy to reduce the future burden of fragility fractures.

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Tendon overuse and development of injury

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Tendons transmit skeletal muscle forces to bone, are essential for all movement, and can withstand considerable loads. Mechanical loading of tendon tissue results in an upregulation of collagen expression and an increased synthesis of collagen protein. The degradation of collagen proteins also rises after exercise, but seems to peak earlier than the synthesis. There are indications that this collagen-induction relates to the auto-paracrine action of collagen-stimulating growth factors, such as TGFβ-1 and IGF-I, which are expressed in response to mechanical stimuli. Further, months and years of mechanical loading can influence the gross morphology of tendon, seen as an increase tendon cross sectional area (CSA). Similarly, tendon stiffness appears to be affected by weeks to months of loading. The possible mechanisms behind alterations in tendon material properties include changes in collagen fibril morphology and levels of cross-linking between collagen molecules. Despite the ability of tendons to adapt to loading, repetitive use often results in injuries, such as tendinopathy, which is characterized by pain during activity, localized tenderness upon palpation, swelling and impaired performance. Tendon histological changes include reduced numbers and rounding of fibroblasts, increased content of proteoglycans, glycosaminoglycans and water, hypervascularization and disorganized collagen fibrils. At the molecular level, the levels of messenger RNA for type I and III collagens, proteoglycans, angiogenic factors, stress and regenerative proteins and proteolytic enzymes are increased. Tendon microrupture and material fatigue as well as apoptosis have been suggested as possible injury mechanisms, thus implying that one or more 'weak links' are present in the structure. Understanding how tendon tissue adapts to mechanical loading will help to unravel the pathogenesis of tendinopathy.

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Rehabilitation of muscle after injury - role of anti-inflammatory drugs

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Nonsteroidal anti-inflammatory drugs (NSAIDs) are widely consumed among athletes worldwide in relation to muscle injury and soreness. This presentation aims to provide an overview of studies investigating their effect on skeletal muscle adaptation, including the repair processes in injured muscle, adaptation to unaccustomed and/or resistance exercise and training. Muscle injury occurs in diverse situations and the nature of muscle injuries varies significantly, from classical muscle strain injuries to contusion and overload injuries. Strain injuries occur at the interphase between the muscle fibres and connective tissue most often in the myotendinous junction, whereas contusion or overload injury can damage both myofibres and intramuscular connective tissue. It is important to distinguish between types of muscle injury –and to keep in mind that unaccustomed (voluntary) exercise does not necessarily lead to muscle damage or injury, at least not to the muscle fibres per se. Likewise, delayed onset muscle soreness (DOMS) following unaccustomed exercise can occur in the presence or absence of actual damage to the muscle fibres. Investigations into the potential of NSAIDs to alleviate DOMS in exercised muscles are numerous, and the outcomes are inconsistent, with roughly equal numbers finding no effect of NSAIDs on DOMS or a significant attenuation of DOMS.

-NSAID effects on cells responsible for muscle repair –satellite cells

A population of muscle stem cells, known as satellite cells, is well recognised as being indispensable for the repair of skeletal muscle. The evidence, at least from animal studies, for an inhibiting effect of NSAID action on satellite cells is convincing (Bondesen 2004, 2006) and in addition, human studies have shown a reduction of satellite cell numbers 8 days after exercise by NSAIDs (Mackey 2007, Mikkelsen 2009).

-NSAIDs and muscle adaptation without injury –protein synthesis & hypertrophy

A blunting of the muscle hypertrophy response to overloading has been reported in animals treated with NSAIDs. In humans, NSAID ingestion has been reported to suppress the protein synthesis response to a single bout of exercise in young individuals (Trappe 2002), whereas NSAIDs appear to have positive effects on training induced hypertrophy and strength gains in elderly (Trappe 2011). Accordingly, it is possible that the influence of NSAIDs on muscle adaptation is different between young and old individuals, which may relate to the chronic level of systemic inflammatory markers.

In summary, evidence exists for a negative influence by NSAIDs on cellular activity during muscle repair, mainly comprising the muscle stem cell population (satellite cells), and as regards muscle protein balance and hypertrophy discrepant results may relate to differences in chronic inflammatory state.


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Ablation of p38γ-MAPK reduces cardiac remodelling following pressure overload

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Cardiac hypertrophy establishes itself when the heart has to pump against an increased afterload. Unlike exercise-induced hypertrophy, pathological hypertrophy is characterized by an increased amount of interstitial collagen, induction of foetal-type genes and an increased lumen size (dilatation). There is an increasing body of evidence to suggest a causative role of the p38-mitogen activated protein kinases (p38MAPKs, of which there are 4 isoforms; α, β, γ & δ) in cardiac dysfunction and progression to remodelling post-infarction or under increased afterload. The two most abundant isoforms of p38 in the heart are α and γ (1). Following aortic constriction, the γ isoform translocates into the nucleus, suggesting that the isoform may be involved in the de novo synthesis of proteins (1). It was hypothesised that inhibition/obliteration of p38γMAPK would result in an altered response to pressure overload. 11 WT and 10 p38γKO age and weight-matched mice underwent abdominal aortic banding surgery. Animals were anaesthetised using isoflurane (1.5% in 100% O2) and received post-operative analgesia (0.05mg/kg buprenorphine). Each animal had a constriction on the descending aorta using 8/0 equivalent to the diameter of a 28G needle. Cardiac function and morphometry was assessed every 14 days over a 10 week period using high resolution cardiac ultrasound (Veo770, Visualsonics, CA). Animals were anaesthetised using isoflurane (1.5% in 100% O2). Data were analysed, using a two-way ANOVA with repeated measures with Tukey’s pairwise post-hoc test. Over the course of the experiment both cohorts showed decreased ventricle performance in response to surgery. However, at 10 weeks, left ventricle (LV) ejection fraction (EF) and fractional shortening (FS) had decreased significantly more in WT mice compared to the KO cohort (35±3 vs. 55±3% & 18±2 vs.28±2%, respectively), suggesting better preservation of cardiac function in the absence of p38γ. Similarly, ventricular mass:body mass (LVM:BM) ratio (8.3±0.8 vs. 5.8±0.2) and LV volume (77±13 vs. 41±4μl) had increased significantly in the WT mice compared to those lacking p38γ. All hearts were subjected to antibody staining, using antibodies for β-myosin heavy chain (β-MHC) at the end of the experiment. β-MHC expression had increased more in WT mice by the end of the study. This strongly suggests the involvement of p38γ (or δ) MAPK in the development of cardiac remodelling. However, little is known about the downstream pathways involved of whether inhibition of this pathway may have other, more systemic effects. In the future, we aim to determine the effects of this pathway on exercise-induced cardiac adaptation.


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Former male elite athletes sustain fewer fragility fractures than expected


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Background: Physical activity during growth and adolescence is associated with high peak bone mass, and may as a result prevent osteoporosis later in life. It is therefore possible that physical activity during youth could serve as a strategy to reduce the risk of fragility fractures at advanced ages. However, it is yet currently unclear whether former athletes actually have fewer fractures than expected with age.

Purpose: This study aimed to evaluate fracture incidence in former male athletes.

Methods: In a retrospective matched controlled design lifetime incidence of fractures was registered through a mailed questionnaire sent to 709 former male athletes now with a median age of 70 years (range 50–93), who had given up regular sports activity a median 35 years (range 1–63) ago, and to 1368 male controls aged a median 70 years (range 51–93). Fragility fractures were defined as fractures due to a light trauma and after age 50 in proximal humerus, distal radius, vertebra, pelvis, hip, and tibial condyles. Both cohorts were normally distributed (Shapiro Wilk’s test). Data are presented as means with 95% confidence intervals (95% CI). Group differences were evaluated by Student’s t-test between means and chi-square test. Differences between athletes and controls in time to first fracture were evaluated by Kaplan-Meier survival curves, and rate ratios (RR) were estimated by Poison distribution.

Results: The anthropometrics and lifestyle factors were most similar between the groups. There was no group difference in current Body Mass Index and level of leisure-time physical activity. After retirement from sports (age 35 years), the former athletes had a lower risk of sustaining any fractures with a RR of 0.70 (0.52, 0.93), a lower risk of sustaining any fragility fractures (RR 0.50 (0.27, 0.89)), and a lower risk of sustaining a distal radius fracture (RR 0.29 (0.09, 0.74)), but did not reach statistical significance as regard to hip fracture after age 50 (RR 0.79 (0.28, 2.00)) (Figure 1).

Conclusions: Intense physical activity during growth and young adulthood is in retired sportsmen associated with a lower risk of sustaining a fracture than expected by age. In a wider perspective, this indicates that physical activity in younger years could be recommended as a feasible strategy to reduce the incidence of fragility fractures in older ages.

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Tyrosine supplementation does not influence the capacity to perform prolonged exercise in a warm environment

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Since dopamine and noradrenaline are intrinsically involved in motivation, arousal, reward and motor control, it might be expected that strategies to increase catecholaminergic neurotransmission would enhance exercise performance. With local ethics committee approval, eight trained males (Mean ± SD age 23 ± 3 y; height 1.82 ± 0.06 m; body mass 80.0 ± 9.6 kg; VO2max: 5.2 ± 0.3 L/min) were recruited to examine the effect of acute supplementation with tyrosine (TYR), the amino acid precursor of the catecholamines, on the capacity to perform prolonged exercise in a warm environment. Subjects entered the laboratory in the morning and remained seated for 1 h, before cycling to volitional exhaustion at 70% VO2max in a warm environment (30.2 ± 0.2°C, 50 ± 1% rh). Two 250 mL aliquots of a placebo or a TYR solution were ingested at 30 min intervals prior to exercise, with an additional 150 mL consumed every 15 min throughout exercise (total TYR dose: 150 mg/kg body mass). A series of computer-based tests was completed prior to drink ingestion, at the end of the rest period and at exhaustion. TYR ingestion had no effect on exercise capacity (placebo 61.4 ± 13.7 min; TYR 60.2 ± 15.4 min; P = 0.505). No differences in heart rate (P = 0.380), core temperature (P = 0.554), weighted mean skin temperature (P = 0.167) or perceived exertion (P = 0.790) were apparent between trials. Ingestion of TYR caused an elevation in plasma TYR concentration immediately prior to exercise (+236 ± 46 μmol/L; P < 0.001), resulting in a 5 ± 1-fold increase in the plasma concentration ratio of TYR to the remaining large neutral amino acids (P < 0.001). No change was apparent during the placebo trial (P = 0.924). Exercise caused an increase in error rate during the complex component of the Stroop test (P = 0.034), but there was no difference in the number of errors between trials at exhaustion (placebo 4 ± 1; TYR 4 ± 1; P = 0.106). No other aspect of cognitive function was altered by the protocol (all P > 0.05). Acute oral supplementation of 150 mg/kg BM TYR did not influence exercise capacity in the heat, measures of cognitive function, or the physiological response to exercise when compared to a placebo condition.

Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.
Repeated-sprint ability is further enhanced by intensive training in hypoxia than in normoxia

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Introduction

Specific muscular adaptations (1,2) potentially beneficial for endurance performance (3) were reported after high-intensity training (HIT) in hypoxia. Whether and how HIT in hypoxia would enhance intermittent glycolytic performance and repeated-sprint ability (RSA) is unclear. Our study evaluates the effects of repeated-sprint training in hypoxia or in normoxia on RSA.

Methods

50 trained subjects (35 ± 7 years, 75 ± 9 kg, VO2max 52 ± 1 ml kg min⁻¹) were assigned to three groups (hypoxic training H, normoxic training N, Control C). Training consisted in 8 cycling repeated-sprint sessions (3 x 5 x 10-s sprints) over 4 weeks in a normobaric hypoxic chamber (H at 3000 m and N at 485 m).

PRE and POST training, subjects performed 10-s isolated sprints, RSA until exhaustion (10-s sprints, rest 20 s) 30-s Wingate and 3-min all-out test on a cycle ergometer. Muscle biopsies were taken at rest and after RSA.

Results

Training increased significantly (p<0.01) the number of repeated sprints in H (9.4 ± 4.8 vs. 13 ± 6.2 sprints) but not in N (9.3 ± 4.2 vs. 8.9 ± 3.5) or in C (11.0 ± 7.1 vs. 10.3 ± 6.2). 10-s sprint and Wingate performances were improved (p<0.01) similarly in H and N. Lactate and 3-min all-out performance were unchanged (Table 1 and Figure 1).

mRNA gene expression of hypoxia inducible factor (HIF-1α, +55%, p<0.05), myoglobin (Mb, +16%, p=0.07) and carbonic anhydrase 3 (CA3, +35%, p<0.05) were upregulated in H only. Conversely, mitochondrial transcription factor A (TFAM) and peroxisome proliferator-activated receptor gamma coactivator 1α (PGC-1α) were downregulated in H only (-40% and -23% respectively, p<0.01).

Discussion

Repeated-sprint training in hypoxia allowed further enhancement of repeated-sprint performance than the same training in normoxia. Systemic glycolytic (Wingate) and alactic (10-s sprint) changes being similar in H and N, RSA improvement can only be due to molecular adaptations at the muscular level induced to a greater extent by HIT in hypoxia. The upregulation of genes involved in oxygen signaling (HIF-1α), oxygen carrying (Mb) and pH regulation (CA3) and the concomitant downregulation of genes implicated in mitochondrial biogenesis (TFAM and PGC-1α) suggest a shift from aerobic to anaerobic glycolytic activity in the muscle. Our findings confirm previous results indicating molecular muscular adaptations after HIT in hypoxia (2,4,5) but show for the first time large enough adaptations for further improvement in systemic RSA performance.

Table 1 Performance results PRE and POST training in the Hypoxic (H), Normoxic (N) and Control (C) groups

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<th>H (n=17)</th>
<th>N (n=17)</th>
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<td>Average power of all sprints during repeated-sprint ability</td>
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Figure 1 - Average power of all sprints during repeated-sprint ability test

Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.
Does whole body vibration influence motor unit recruitment and threshold?

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Whole body vibration (WBV) may improve muscular strength, power and balance (1). Improvements may be related to reflex muscular contractions (2,3) although this has never been verified.

Motor unit (MU) recruitment threshold of the vastus laterals was recorded in 7 healthy subjects (34 ± 15.4yr), using fine wire EMG, before and after 5 1min bouts of WBV separated by 30s (30Hz, 3mm peak to peak). Recruitment thresholds were recorded during ramp contractions of the knee extensors during which the force of contraction was recorded. Fine wire EMG was recorded during WBV, along with the vibration waveform by a strain gauge. From this the phase angle at which each MU fired during the vibration cycle was determined. In a further 8 subjects (29 ± 4.6yr) presynaptic inhibition of the soleus was assessed using the same WBV protocol. This was done by eliciting 15 unconditioned and 15 conditioned H-reflexes at a current producing H-reflexes at an amplitude of 30% Mmax. 32 single MUs were discriminated during WBV.

The phase of the vibration cycle at which each MU fired was not uniform (P<0.001 for all MUs), indicating that a single MU fired at a consistent phase of the vibration cycle, but the phase differed between units (Fig. 1).

There was no difference in MU recruitment threshold before and after WBV (P > 0.05). There was however a strong relationship (r = -0.68, P<0.001, Fig.2) between average recruitment threshold and change in threshold. Further analysis revealed that recruitment threshold of the lowest threshold units significantly increased (P=0.008) while the highest threshold decreased (P=0.031; Fig. 2) after WBV. Due to high levels of variability there was no change in the levels of presynaptic inhibition after WBV (P=0.93).

This is the first study to record activity from single MUs during WBV. The results indicate that MU firing is phase-locked to the vibration cycle and suggests that activity is reflexive. Variation in the phase at which each MU fired during WBV could be due to whether the MU was activated via mono- or poly-synaptic pathways. Overall no effect on recruitment threshold was found, although lower threshold MUs increased while higher threshold units decreased threshold after WBV. Lower threshold units are more likely to be influenced by mono-synaptic pathways, which are thought to be inhibited after vibration (4), while higher threshold units also have a polysynaptic component. If WBV elicits reflex activity similar to the tonic vibration reflex, which is controlled by mono- and polysynaptic pathways, this could explain the preferential effect on higher threshold MUs. This effect is not related to presynaptic inhibition, which was unchanged after WBV, however other presynaptic mechanisms such as post activation depression cannot be ruled out.

![Fig. 1.](image-url)

Rose plots of the phase angle recorded from 4 identified MUs during WBV. These MUs are representative of all 32 identified units.
Average MU recruitment threshold plotted against the change in threshold post WBV. MUs were split into 5 categories based on their initial threshold. Category 1 MUs increased (P=0.008) threshold while Category 5 decreased (P=0.031), no difference was observed in pre post thresholds in category 2, 3 and 4 (P>0.05).


We would like to thank Research into Ageing for funding this study.

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Effect of differential muscle-tendon unit length during dynamic resistance training on muscle function, architecture, morphology and detraining

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In human skeletal muscle, muscle size and strength are inextricably linked (1). A number of hypertrophy-inducing mechanisms are affected by the stress delivered to the working muscle (2), which in turn can be manipulated internally by joint-angle mechanics (i.e. altering moment arm of in-series elastic components). Simultaneously, joint-angle (and hence muscle length) will also determine the amount of stretch the muscle is undergoing. In vivo adaptations following training at different joint-angle specific ranges-of-motion are unknown.

Two training groups – Ex (aged 19±2.2 years; n=10) and Flx (21±3.4 years; n=11) undertook 8 weeks of resistance training of the quadriceps, with concentric/eccentric knee extension over either a relatively extended (Ex- i.e. 50degrees-0deg, where 0deg is a straight leg) or flexed (Flx- i.e. 90deg-40deg, where 90deg is a right angle) range of motion, followed by 4 weeks detraining. Quadriceps muscle strength, vastus lateralis architecture, size and subcutaneous fat were measured at weeks 0, 8, 10 and 12 using B-mode ultrasonography, surface electromyography and dynamometry. A control group (aged 23±2.4 years; n=10) was also monitored during this period. Following training, greater relative increases in fascicle length (proximal, central and distal [25%, 50% and 75% of femur length respectively] mean change Flx 29%±4 vs. Ex 14%±4%), strength, anatomical cross-sectional area (aCSA) (distal), and subcutaneous fat (central and distal) were present in Flx compared to Ex (p<0.05, see Figure 1). The relative decrease during detraining was greater in Ex than in Flx in strength, aCSA and fascicle length (p<0.05). To our knowledge, this is the first study to demonstrate through manipulating training mechanics, greater functional, architectural and morphological adaptations in Flx vs. Ex training. This effect is likely to be due to the different mechanical stress and stretch experienced by the muscles (3). In practice, where strength, lean mass and fascicle length adaptations could be advantageous in optimizing performance, exercise through a more flexed range-of-motion should be practiced.

Figure 1. Relative changes in (A) strength, and (B) distal aCSA and subcutaneous fat, in Flx and Ex groups following training (wk8) and detraining (wk10 and 12). * significant main effect of group (p<0.05).


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The ventilatory response to muscle metaboreflex stimulation during concurrent hypercapnia in humans: roles of central and peripheral chemoreception

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The role of skeletal muscle metaboreceptive afferents in human cardiorespiratory control can be examined using post exercise circulatory occlusion (PECO)( Alam and Smirk 1937, Coote et al 1971, Rowell 1976). We have shown that PECO produces ventilatory responses in humans, but only during concurrent hypercapnia (Bruce and White 2012). This is not due to local muscle hypercapnia as the effect is absent if only the active muscle, not the systemic circulation, is exposed to elevated arterial carbon dioxide. We now aim to determine the extent to which the ventilatory response to hypercapnia in PECO is due to an effect mediated by central or peripheral chemoreception. This was achieved by comparing ventilatory and cardiovascular responses to PECO during normoxic hypercapnia (5% carbon dioxide in air) with those seen during hyperoxic hypercapnia (5% carbon dioxide, 95% oxygen) which is expected to diminish markedly, peripheral chemoreflex activation (Dejours 1962).

With Ethical Committee approval, 10 males seated in an dynamometer performed the following protocol. During inhalation of the normoxic hypercapnic gas mixture a cuff was inflated to 200mmHg around the right thigh. Continuing to breathe this mixture subjects performed a sustained isometric contraction of their right plantarflexors for 1.5 min at 50% MVC, followed by PECO for 3.5min. At this point with continued PECO subjects were switched to inhale the hyperoxic hypercapnic mixture for a further 2 minutes after which time they reverted to the normoxic hypercapnic mixture for 2 more minutes. The thigh cuff was then deflated and after 3 more minutes of normoxic hypercapnia the subjects returned to breathing room air.

We found that PECO maintained blood pressure at exercising levels, suggesting activation of metaboreceptive afferents and as expected ventilation was maintained at exercising levels during normoxic hypercapnia (~6 l.min⁻¹). The switch to hyperoxic hypercapnia had no effect on this ventilatory response which was sustained until thigh cuff deflation.

These results suggest that ventilatory responses induced by muscle metaboreceptive afferent feedback during concurrent hypercapnia are primarily driven by central chemoreception.


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Focal adhesion kinase is required for contraction and IGF-1 induced muscle cell growth

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The focal adhesion complexes (or costameres) are macromolecular assemblies connecting the extracellular matrix to the cytoplasmic cytoskeleton. Focal adhesion kinase (FAK) is one such costameric component exhibiting important structural and non-receptor tyrosine kinase activities in response to integrin engagement and growth factor stimulation. Exemplifying its importance, overexpression of FAK stimulates muscle hypertrophy in vivo (1) and its expression is reciprocal to loading patterns (i.e. downregulated in atrophy/ upregulated in hypertrophy (2)). On this basis, we reasoned that reducing FAK expression by short hairpin (sh)RNA interference would restrict cell growth associated with insulin-like growth factor-1 (IGF-1) and contraction (mechanotransduction). Stably transfected C2C12 cells harbouring FAK targeted (pLKO.1-mFAK) or scrambled shRNA were developed using lentiviral transfection techniques (3). FAK and scrambled shRNA myotubes (4-5 d after differentiation) were incubated for 72 h with IGF-1 Long R3 (10 ng.ml-1 replenished daily) for measurement of total protein (μg/well by dissolution in 0.3 M NaOH). Immunoblotting was used to determine FAK levels and phosphorylation of ‘hypertrophy’ signalling targets (i.e. Akt-mammalian target of rapamycin (mTOR)) after 1 h IGF-1 incubation. In separate experiments, FAK and scrambled shRNA myotubes were contracted for 24 h (0.2 Hz, 5 V, 2 ms) using a C-pace system, before assay of total protein. Results were analysed by one-way ANOVA and Tukey’s post-hoc testing with the level of significance set at P<0.05. Data are presented as mean percentage differences ± standard error. FAK depletion was confirmed at the protein level (-90%: FAK vs. scrambled shRNA; P<0.00001). While IGF-1 treatment for 72 h elicited robust increases in total protein in scramble shRNA cells (+77±10%; P<0.001), this was markedly blunted in FAK shRNA cells (+34±14%; P=0.14). In response to 1 h IGF-1 treatment of scrambled shRNA cells, phosphorylated proteins of the Akt-mTOR pathway increased: p-Akt (Ser473: +180±9%; P<0.001), p-mTOR (Ser2448: +120±5%; P<0.001) and p-GSK3β (Ser9/21: 70±9%; P<0.001). However, in FAK shRNA cells, increases in phosphorylation were significantly attenuated at 1 h: (p-Akt, -31%; p-mTOR, -32%; p-GSK3β, -30% vs. IGF-1-treated scramble shRNA cells; all P<0.05). In contrast, IGF-1-induced increases in p-p70S6K (P<0.05) were not different (FAK shRNA Thr389: +41±13%; scrambled shRNA: +46±10%; P<0.01). Despite FAK shRNA cells displaying a contractile phenotype, increases in total protein in scrambled shRNA cells after 24 h contraction (+57±9%; P<0.01) were absent in FAK shRNA cells (+7±11% P=0.65). We have identified that FAK represents a key component of growth factor (IGF-1) and contraction (mechanotransduction)-mediated cell growth, possibly via modulation of Akt-mTOR signalling.


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Eccentric exercise is associated with marked impairment of maximal rates of mitochondrial ATP production in human skeletal muscle

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Unaccustomed eccentric exercise has been shown to disturb muscle high energy phosphates (Rodenburg et al. 1994), induce myofibre disruption (Feasson et al. 2002) and mitochondrial swelling (Fridén et al. 1983), and impair muscle glycogen storage (Asp et al. 1996) 24 to 48 h following exercise. However, contrary to what might be expected, respiration in skinned human muscle fibers in the presence of pyruvate and malate has been reported to be unchanged for up to 96 h following eccentric exercise (Walsh et al. 2001). The aim of this study was to determine the acute impact of eccentric exercise on mitochondrial ATP production rates, rather than oxygen consumption, in human muscle using a variety of mitochondrial substrates.

Eight untrained healthy male volunteers (age 26.8 ± 4.1 yrs, BMI 23.4 ± 1.2 kg.m-2) performed 30 min of running at a 0% (FR) or -15% (DR) gradient on a motorized treadmill at a speed equivalent to 80% maximal oxygen uptake (VO2max) determined using an incremental gradient protocol (Feasson et al. 2002). Exercise tests were separated by at least 2 weeks and were executed in a randomized order. Twenty-four hours after each bout of exercise, a vastus lateralis biopsy sample was obtained at rest. Maximal rates of ATP production (MRAP) in freshly isolated mitochondrial suspensions were measured in the presence of a variety of substrates using a bio-luminescence technique. The maximal activities of enzyme components of the electron transport chain (ETC) were also determined. Values represent mean ± SEM and statistical comparisons between interventions were performed using a paired Student’s t-test. Maximal rates of ATP production were markedly reduced following DR compared to FR in the presence of glutamate and succinate (8.0 ± 1.3 vs. 68.8 ± 8.1 nM min-1 mg-1 mitochondrial protein; P<0.001), glutamate and malate (6.3 ± 1.4 vs. 59.5 ± 8.2 nM min-1 mg-1 mitochondrial protein; P<0.001), palmitoyl-carnitine and malate (6.4 ± 1.2 vs 23.0 ± 3.4 nM min-1 mg-1 mitochondrial protein; P<0.05), and pyruvate and malate (3.5 ± 1.0 vs. 40.3 ± 5.8 nM min-1 mg-1 mitochondrial protein; P<0.001). No difference was observed between interventions when succinate was used as a substrate, and there were no differences in activities of components of the ETC (Data not shown).

Contrary to previous evidence from skinned muscle fibre preparations, it would appear that eccentric exercise is associated with a marked impairment of MRAP in humans. This may underpin at least part of the dysregulation of energy metabolism seen under these conditions.


This study was supported by funding from the BBSRC.

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The ergogenic impact of sustained high-dose short acting β2-agonist use during a six week training programme in healthy individuals

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There is little evidence available to demonstrate that inhaled short acting β2-agonists provide an ergogenic effect. However, the majority of research in this area has focused on acute doses of inhaled β2-agonists. At present there are no investigations examining the chronic use of short acting β2-agonist use during training. Ten healthy well-trained males (mean ± SD; age 20.4 ±2.1 years; height 178.1 ±8.8 cm; weight 71.2 ±11.3 kg) who had no history of asthma and presented with a negative indirect airway challenge, volunteered to participate in the study. Athletes were randomly assigned to one of two groups in a randomised double blind design; either placebo or 1600μg salbutamol (400μg (4x100μg inhalations) at 08:00h, 12:00h, 16:00h and 20:00h every day for 6 weeks). Baseline tests consisted of a VO2 peak assessment and a 3km time-trial. Strength assessments consisted of isokinetic dynamometry assessment for peak torque during maximal knee extension and flexion at slow (60°s-1) and fast (240°s-1) contracting speeds, alongside one repetition maximum (1RM) lifts for bench press and leg press, power was assessed via a vertical jump test. Subjects then underwent a 6 week training programme, which consisted of two resistance sessions and two endurance sessions per week, whilst inhaling either 1600μg salbutamol per day or placebo. Follow-up assessments for 3 km time-trial, 1RM bench and leg press, vertical jump heights, VO2 peak and isokinetic dynamometry were undertaken following 6 weeks of training. Mixed-model repeated measures ANOVA were used to compare baseline and 6 week assessments of endurance, strength and power between the salbutamol and placebo groups. There was a significant decrease in 3km completion time post training programme (983.5±183.8 vs. 945.6±186 s, p=0.05) with no difference between groups (salbutamol mean change 23.4±16.5 vs. placebo 52.5±37.1 s, p>0.05). There was no significant effect of the training programme on maximal isokinetic strength or jump height (p>0.05), nor was there a difference between groups (p>0.05). There was a significant increase in VO2 peak post-training (52.5±5.4 vs. 57.7±6.6 ml.kg.min⁻¹, p=0.01) with no difference between groups (p>0.05). There was a significant increase in 1RM leg strength post-training (218.3±45.5 vs. 272.8±48.9 kg, p<0.01) with a significant difference between groups (salbutamol mean change 35.2±24.7 vs. placebo 78.3±55.3 kg, p=0.04). In conclusion there were significant improvements in performance variables post-training, however these improvements were equal in both groups with no additive effect of inhaled salbutamol on any of the performance or physiological variables. The WADA guidelines that permit up to 1600 μg inhaled salbutamol are appropriate as there appears to be no significant performance enhancing effect of taking this dose on a daily basis.

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Cortical reorganization in elite athletes: lessons in plasticity for brain injury treatment

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Introduction. Muscles that habitually perform a bilateral activation receive a stronger bilateral corticospinal innervation. Athletes perfecting control of a movement that involves muscles acting bilaterally may alter the cortical representation of that muscle. With training a single cortical origin may provide a stronger bilateral control. Transcranial Magnetic Stimulation (TMS) provides a non-invasive means of mapping the cortical representation and characterizing corticospinal control of a muscle. Using TMS we demonstrate a pattern of cortical reorganization whereby ipsilateral projections from one hemisphere are strengthened in elite athletes, having implications both for training athletes and treating brain injury patients.

Methods. Canoe-polo players who had accumulated over 10,000 hours of training and represented their country in world-cup competition were compared to healthy controls. EMG was recorded from abdominal External Obliques, ipsilateral and contralateral to the side of cortical stimulation. Background activation at 10% of Maximum Voluntary Contraction was produced. Neuronavigation guided the TMS coil to points on a scalp grid 1cm apart, centred on the site of optimal stimulation (Hotspot) for the contralateral muscle. Each point in the surrounding cortex was then stimulated at 120% of the active motor threshold (aMT).

Analysis. EMG was rectified and Motor Evoked Potentials (MEP) were averaged for each grid point. MEPs were normalized to mean+1S.D. of background activity. Normalised responses for each grid coordinate expressed as mean %Background Activity were pooled for the two groups. The pooled maps were analysed by comparing with an unpaired t-test the mean response at sites 0cm, 1cm, 2cm and 3cm from the centre of the grid (i.e the contralateral hotspot) for ipsilateral and contralateral muscles.

Results. Bilateral MEP's were seen in all 20 subjects. Of note there was no significant difference between athletes and controls for contralateral muscle representation. This is important as it establishes that peripheral factors such as muscle size do not explain observed differences. In ipsilateral muscle the mean response at 1 cm from the hotspot was 246%+/-21(mean +/-SEM) for athletes and 107+/-11 for controls (p=<0.001), at 2 cm 266 +/-17 for athletes and 108 +/-7 for controls (p=<0.001) and at 3cm 219 +/-14 for athletes and 75+/-15 for controls (p=<0.001).

Discussion. We show that athletes have a cortical region surrounding the contralateral muscle representation that is able to produce ipsilateral responses. Athletes drive plasticity to increase the motor cortex map of ipsilateral muscles when bilateral axial muscle activation is important. This knowledge provides the athlete with a basis for alternative training strategies, and provides the neurologist with a therapeutic target in the intact hemisphere after brain injury.

Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.
Extracellular pH and buffer capacity does not influence time to exhaustion or reductions of intramuscular pH and maximal voluntary contraction force during exhaustive intermittent static exercise

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Intense exercise causes fatigue development and ultimately exhaustion within few minutes but the exact mechanisms protecting the myocyte from detrimental ATP depletion remains controversial. Here, the hypothesis that a reduced extracellular pH and HCO3- level would accelerate intramuscular metabolic acidosis and impair exercise performance was evaluated.

Seven healthy males (25±4 yrs; mean ±SD) participated in the double-blind placebo controlled experiment where intermittent isometric exercise was performed as 2 s contraction at 60–6 % of maximal voluntary contraction force (MVC) followed by 1 s rest until exhaustion. Each of three situations (oral intake of: 0.5 g × kg b.w.-1 NaHCO3; 0.3 g × kg b.w.-1 NH4Cl; 0.3 g × kg b.w.-1 CaCO3) was studied on two occasions. On one occasion intramuscular pH was monitored continuously using 31P-NMR and on the other muscle function was continuously monitored by conduction of MVC’s and induction of a twitch contraction by single pulse electrical stimulation every 30th second until exhaustion.

Prior to exercise capillary blood pH was higher (P<0.001) in NaHCO3 (7.48±0.02) than CaCO3 (7.41±0.01) which was higher (P<0.05) than in NH4Cl (7.30±0.07). Likewise, blood HCO3- was higher (P<0.001) in NaHCO3 (32.5±3.4 mM) than CaCO3 (24.5±1.8 mM) which was higher (P<0.05) than in NH4Cl (18.9±4.6 mM).

Time to exhaustion (TTE) was similar between trials. In the NMR experiment TTE was 6.3±1.4 min; 6.2±0.8 min, 6.1±1.3 min in NaHCO3, CaCO3, NH4Cl, respectively. In the muscle function experiment TTE was 4.0±2.2; 4.8±1.9; 5.5±4.5 min in NaHCO3, CaCO3, NH4Cl, respectively. Likewise, the reduction of intramuscular pH was similar between trials (Fig. 1) and so was the reduction in MVC (Fig. 2) and electrically induced twitch force (Data not shown).

This is the first study in which acid/base homeostasis is manipulated by intake of NaHCO3 and NH4Cl in vivo in combination with a high temporal resolution analysis of intramuscular metabolic acidosis as well as muscle function. The present findings demonstrate that manipulation of extracellular acid and base homeostasis has no effect on deterioration of m. tibialis anterior muscle function during exhaustive intermittent static exercise. Further, the findings indicate that myocyte outward H+ flux in conditions of metabolic stress cannot be accelerated by an increased H+ gradient and elevated extracellular buffer capacity. In a practical context, the finding questions the use of NaHCO3 as a performance enhancing agent, at least in sports engaging only a limited muscle mass.

Figure 1: Intramuscular pH (pHi) measured in m. tibialis anterior by 31P-NMR during intermittent static contractions lasting 2 s separated by 1 s rest until exhaustion. The arrow indicates an average of the last three measurements (~20 seconds). n=7. Mean±SD.

Figure 2: Maximal voluntary contraction force (MVC) determined every 30 s during intermittent static exercise consisting of 2 s contractions separated by 1 s rest until exhaustion. Measured values were normalized to the highest MVC recorded prior to exercise (% MVCpre). The arrow indicates an average of the last measurements before exhaustion. n=5. Mean±SD.

This study was supported by The Ministry of Culture Committee on Sports Research, Denmark.
Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.
Canonical Wnt-β-catenin signalling is active in primary human myoblasts

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It is now widely accepted that canonical Wnt signalling is essential for the formation of skeletal muscle during embryonic development (Münsterberg et al., 1995); yet far less is known about the role of this pathway in adult muscle. Mature skeletal muscle fibres are surrounded by a population of resident stem cells termed ‘satellite cells’ which are responsible for muscle repair and regeneration following exercise induced damage or injury. Recent research suggests that satellite cells are of embryonic origin which raises the possibility that these cells may be responsive to myogenic cues observed in the developing somite, such as Wnt proteins (Gros et al., 2005). Using primary human muscle cell culture as an in vitro model of human skeletal muscle regeneration and growth, the aim of the present study was to investigate whether the canonical Wnt signalling cascade was active and could be manipulated in these cells. Human satellite cells (myoblasts) were isolated from biopsy samples of the vastus lateralis of healthy young (23 ± 3.46 years) male volunteers (n = 3) following administration of a local anesthetic (2% lignocaine). Subsequent cultures were pre-plated to reduced fibroblast contamination and myogenic purity was assessed via desmin immunoreactivity (74-91% desmin+ve). Canonical Wnt signalling requires the nuclear import of dephosphorylated (active) β-catenin (Ser37 or Thr41) in order to activate Wnt target genes; thus the expression of this protein was measured by Western blotting. In proliferating myoblasts, dephosphorylated β-catenin was elevated in response to recombinant human Wnt-3A administration (200 ng/ml) at all time points studied when compared to untreated control cells; mean fold increases were 1.53 ± 0.25, 1.33 ± 0.14, 1.38 ± 0.06, 1.57 ± 0.47, 1.59 ± 0.29 at 1, 2, 4, 8 & 16 hours respectively; P <0.05). In addition, qPCR analysis revealed a significant increase in Axin-2 mRNA transcripts following Wnt-3A treatment at all time points except 1 hour (P <0.05). Mean fold increases in Axin-2 mRNA in Wnt treated cells compared to controls cells were: 2.17 ± 1.46, 5.94 ± 4.22, 8.72 ± 1.64, 8.45 ± 0.56 & 7.17 ± 1.24 at 1, 2, 4, 8 & 16 hours respectively. Axin-2 protein forms part of a multi-unit destruction complex which marks β-catenin for degradation in the absence of canonical Wnt ligands; thus the observed upregualtion of Axin-2 transcripts may represent a negative feedback loop reported for Wnt signalling in other animal cell types (Jho et al., 2002). Immunocytochemical analysis of β-catenin confirmed its cytoplasmic and nuclear expression in proliferating myoblasts. In conclusion this study has revealed an operative and perturbable canonical Wnt signalling pathway in primary human myoblasts.


Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.
Aging does not affect the profile of muscle deoxygenation during ramp incremental exercise in chronically endurance trained men

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The approximately linear relationship between whole body oxygen uptake (VO2) and cardiac output (Q) across a variety of exercising work rates (WR), predicts a hyperbolic relationship between VO2 (or WR) and whole body arterio-venous O2 content difference (a-vO2diff). Nevertheless, the dynamic adjustments of muscle blood flow (Qm) and O2 extraction during incremental exercise may be different in the periphery as a result of factors contributing to redistribution of blood flow and have recently been characterized as two linear components described by their slopes (m) and intercept (b). Whereas advanced aging has been associated with declining endothelial function and functional differences in the adjustment of blood flow to or within the working muscle, exposure to chronic endurance training may attenuate these declines. As a result, we examined a group of young, middle-age, and older chronically trained male cyclists to compare the dynamic adjustment of near-infrared spectroscopy (NIRS)-derived muscle deoxygenation ([HHb]) during ramp incremental exercise across the age-groups. Four young (Y; 27 ± 8 yrs.; mean ± SD), 5 middle-aged (M; 50 ± 5 yrs.), and 3 older (O; 66 ± 3 yrs.) males each completed a ramp (Y and M: 30 W/min; O: 20W/min) incremental cycling test to the limit of tolerance, during which breath-by-breath pulmonary VO2 (VO2p) and [HHb] were monitored continuously. After normalizing (0-100%) the [HHb] responses, individual profiles were plotted as a function of normalized WR (i.e., %WR) and were characterized by a piecewise ‘double-linear’ regression function to establish the slope of increase of deoxygenation (m1) and plateau as maximal exercise was approached (m2) and the break point (BP) between the increasing deoxygenation and its plateau. Maximal VO2 (VO2max) was lower in O (2.99 ± 0.31 L/min) compared to Y (4.40 ± 0.66 L/min) and M (4.38 ± 0.17 L/min) (p< 0.05) individuals. No differences were observed in the parameters describing the dynamic adjustment of [HHb] during ramp incremental exercise across the three groups (Y: m1 = 1.19 ± 0.26, y1 = -2.21 ± 2.09, m2 = 1.00 ± 0.34, y2 = 6.05 ± 31.29, break point (BP) = 58.16 ± 11.17; M: m1 = 1.57 ± 0.50, y1 = -23.41 ± 33.44, m2 = 0.81 ± 0.38, y2 = 22.93 ± 31.93, BP = 60.91 ± 23.84; O: m1 = 1.14 ± 0.58, y1 = -7.99 ± 6.41, m2 = 0.96 ± 0.75, y2 = 7.10 ± 72.81, BP = 55.06 ± 35.46). The similar m1 and BP among the Y, M and O suggest a similar rate of increase in muscle deoxygenation for a given increase in VO2 and hence, similar O2 delivery to accomplish a given VO2. Surprisingly, only half (6/12) of these individuals exhibited a characteristic ‘plateau’ in the [HHb] response at end exercise. These preliminary data suggest that aging does not affect the profile of normalized [HHb] in chronically endurance trained cyclists.

NSERC Canada.

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The impairment of glucose disposal following eccentric exercise is associated with increased muscle PDK4 protein expression and inhibition of PDC activation in humans

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A single bout of eccentric exercise has been shown to induce muscle inflammation (Chen et al. 2003), mitochondrial swelling (Friden et al. 1983) and impair muscle glycogen storage (Asp et al. 1996) 24 to 48 hr post exercise. This study aimed to determine whether eccentric exercise could impair glucose disposal under insulin clamp conditions, and if so, whether this was associated with up-regulation of muscle pyruvate dehydrogenase kinase isoform 4 (PDK4), which phosphorylates and thereby inactivates the pyruvate dehydrogenase complex (PDC), and impaired muscle PDC activation (PDCa), a rate limiting step in carbohydrate oxidation.

Eight untrained healthy male volunteers (age 26.8±4.1 yrs, BMI 23.4±1.2 kg.m⁻²) performed 30 min of running at a 0% (FR) or -15% (DR) gradient on a motorized treadmill at a speed equivalent to 80% maximal oxygen uptake (VO2max) determined using an incremental gradient protocol. Exercise tests were separated by at least 2 weeks and were executed in a random order. Twenty four hrs post-exercise, subjects underwent a 3 hr hyperinsulinaemic (~70 mU l⁻¹) euglycaemic (4.5 mmol l⁻¹) clamp to determine whole body glucose disposal, in combination with the infusion of mixed essential amino acids (18 g.hr⁻¹; to create a 'fed state'). Muscle biopsy samples were obtained from the vastus lateralis immediately before and after the hyperinsulinaemic clamp to determine PDCa and PDK4 protein expression levels. Statistical analyses were performed using ANOVA and a Wilcoxon non-parametric test. Data are expressed as mean±S.E.M.

Whole body glucose disposal was reduced by 17.4±4.9% following DR compared with FR (6.3±0.5 vs 7.5±0.3 mg min⁻¹ kg⁻¹; P<0.01). Furthermore, muscle PDK4 protein expression prior to the hyperinsulinaemic clamp was 2-fold greater following DR compared to FR (0.34±0.05 vs. 0.18±0.01 OD; P<0.05). Although muscle PDCa prior to the hyperinsulinaemic clamp was similar between interventions (0.27±0.07 vs. 0.40±0.06 mmol acetyl-CoA min⁻¹ kg⁻¹ wet muscle @ 37deg C, respectively; P=0.15), the increase in muscle PDCa during the clamp was blunted following DR compared with FR (Δ 0.10±0.05 vs. 0.23±0.08 mmol acetyl-CoA min⁻¹ kg⁻¹ wet muscle, respectively; P<0.01).

The impairment of glucose disposal following eccentric exercise was associated with increased muscle PDK4 protein expression and inhibition of PDCa in humans under hyperinsulinaemic clamp conditions and therefore is likely to be at least partly responsible for the reported development of whole body insulin resistance. Our observations are in line with the contention that muscle inflammation contributes to the dysregulation of muscle carbohydrate metabolism via modulation of muscle PDK4 and PDC (Crossland et al. 2008).

Chen YW et al. (2003). J Appl Physiol. 95, 2485-94.

This research was supported by the BBSRC and University of Nottingham

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Is there a time-delay in muscle oxygen uptake at the onset of contractions?

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Control of oxygen uptake (VO₂) kinetics in skeletal muscle is mediated through interactions among ADP feedback, oxygen and substrate delivery, and mitochondrial enzyme activity. At the onset of contractions VO₂ kinetics are well modelled by a mono-exponential function, consistent with a first-order control mechanism (1). Importantly, however, this model includes a time delay (\(\delta\)) to account for the transit delay between the muscle capillary and the downstream site of the VO₂ measurement (2). However, \(\delta\)VO₂ may also include the influence of an allosteric control mechanism(s) in activating VO₂ (2-4). In order to elucidate the nature of the VO₂ time delay we aimed to determine \(\delta\)VO₂ and the time delay in the change in deoxygenated hemoglobin and myoglobin (\(\delta[\Delta HHbMb]\)) during contractions in canine muscle with constant (pump) perfusion, before and after prior contractions to activate allosteric control processes.

Nine dogs were anaesthetised with 30 mg.kg⁻¹ pentobarbital (IV), and a deep surgical plane of anaesthesia was maintained with additional doses. Dogs were mechanically ventilated and the gastrocnemius and tendon were isolated and attached to a force transducer. A constant (high) blood flow was maintained by a pump during contractions before (S1) and after 3 min of priming contractions (PS1) separated by 2 min rest. Isometric tetanic contractions (50Hz; 200 ms duration) were elicited via supramaximal sciatic nerve stimulation at 0.33 Hz for 3 min. Muscle VO₂ was determined contraction-by-contraction using an ultrasonic flowmeter and inline venous oximetry. Muscle [\(\Delta HHbMb]\] was determined by near infrared spectroscopy. The kinetics of the fundamental VO₂ and [\(\Delta HHbMb]\] responses were modelled with a mono-exponential function.

Blood flow was 1.04±0.20 L.kg⁻¹.min⁻¹ (mean±SD) across the rest-exercise transition in both conditions. \(\delta[\Delta HHbMb]\) was greater in S1 (3.5±1.4 s) than PS1 (1.8±1.8 s; p=0.027). Similarly, \(\delta\)VO₂ was greater in S1 (7.1±2.2 s) than PS1 (3.2±1.7 s; p<0.001), which represented an apparent blood volume of 9.7±1.9 and 4.4±2.2 mL (p<0.001) in S1 and PS1, respectively. The VO₂ amplitude and mean response time were 91.5±33.8 vs. 85.7±20.1 mL.min⁻¹.kg⁻¹ (p>0.05) and 18.4±4.8 vs. 21.0±4.2 s (p>0.05) in S1 and PS1 respectively.

A first-order control mechanism suggests that, during constant blood flow, the time delay between the site of muscle gas exchange and the inline oximetry measurement should be constant. The reduction in \(\delta\)VO₂ following prior contractions is not consistent with first-order control – rather allosteric features in the control of VO₂ kinetics are implicated (4). That the \(\delta[\Delta HHbMb]\) was also reduced by prior contractions during constant perfusion supports this notion. In conclusion, a first-order control mechanism does not appear sufficient to explain the behaviour of the \(\delta\)VO₂ at the onset of stimulated contractions in situ.

Rossiter et al. (2002). J Physiol. 541, 991-1002.

Supported by BBSRC: BB/F019521/1

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Does central fatigue mediate the negative effect of prolonged mental exertion on subsequent endurance performance?

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Mental fatigue induced by prolonged and demanding cognitive tasks is well known to impair cognitive performance. A recent study (1) demonstrated that mental fatigue also reduces time to exhaustion during high-intensity cycling exercise. This negative effect of mental fatigue on endurance performance may be mediated by its effects on neuromuscular function. The first hypothesis is that prolonged mental exertion induces significant central fatigue defined as reduced ability of the central nervous system to fully recruit the active muscles during a maximal effort (2). The second hypothesis is that mental fatigue exacerbates the central fatigue induced by prolonged submaximal exercise and consequently reduces endurance performance. The aim of this study was to test these two hypotheses by assessing neuromuscular function in two different conditions: A) before and after a prolonged and demanding cognitive task known to induce mental fatigue (AX-CPT task); B) before and after an easy cognitive task (watching a movie). Both cognitive tasks were followed by a submaximal knee extensors contraction performed until exhaustion, and a third assessment of peripheral and central fatigue. Rating of perceived exertion was measured during the time to exhaustion test. Results are presented as means ± SD, and analysed by paired t-tests or two-way repeated-measure ANOVAs. Time to exhaustion was 13% shorter in the mental fatigue condition (230 ± 72 s) compared to control condition (266 ± 82 s) (P<0.01). However, prolonged and demanding cognitive activity did not have any significant effect on maximal voluntary contraction (MVC), voluntary activation level (VAL) and peripheral parameters of neuromuscular function. Neuromuscular alterations induced by the endurance exercise were in accordance with the literature (3). However, a similar decrease in MVC (mental fatigue 26.7 ± 17.9 %, control 27.6 ± 10.3 %) and VAL (mental fatigue - 10.6 ± 13.5 %, control - 11.2 ± 16.4 %) occurred in both conditions (Fig 1). Evolution of EMG indexes of vastus lateralis and rectus femoris muscles activation was similar to VAL values. Mentally fatigued subjects rated perception of effort higher during the time to exhaustion test compared to the control condition (P<0.05) (Fig 2). In conclusion, these findings provide the first experimental evidence that mental fatigue does not induce or exacerbate central or peripheral fatigue. Therefore, the reduction in endurance performance observed in mentally fatigued subjects cannot be mediated by a reduction in neuromuscular function. As suggested by the psychobiological model of endurance performance (4), the most likely mechanism for the negative effect of mental fatigue on time to exhaustion is the increase in perception of effort experienced by mentally fatigued subjects.

Fig 1. Evolution of knee extensors VAL. # Significant effect of time (P < 0.05). CT = cognitive task and ET = endurance task. Data are presented as means ± SD.

Fig 2. Evolution of perceived exertion (RPE) during the submaximal contraction of knee extensors until exhaustion. ### Significant effect of time (P < 0.001) and $ significant effect of condition (P < 0.05). Data are presented as means ± SD.


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Swimming improves the fibrinolytic activity in obese and non-obese rats

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The benefits of exercise in the prevention of cardiovascular diseases (CVDs) have been investigated for many years. With a growing evidence that coagulation-fibrinolysis imbalance could be contributory in the development of CVDs, the effects of exercise on fibrinolysis are to be perused. The aim of the present work is to study the effect of swimming on the fibrinolytic activity parameters in obese and non-obese rats. Sixty male Sprague-Dawley rats were divided into two groups (n= 30 for each). One group was fed on normal chow diet (Non-obese, wt = 180±5 g), and the other was fed on high-fat (53%) diet for induction of obesity (Obese, wt = 280±18 g). Each of the two groups was further subdivided into 3 subgroups (n = 10, each): Control group, Acute Exercise group (obliged to swim across a glass tank filled with tap water maintained at 35°C, until the signs of exhaustion appear on the animal), and Chronic Training group (obliged to perform the swimming exercise sessions, three times weekly for 12 weeks). In all groups, after completing the particular regime, retro-orbital blood samples were withdrawn, under ether (10%) anaesthesia, and the rats were then humanely killed, under terminal anaesthesia by cervical dislocation. Plasma was separated for the determination of various fibrinolysis parameters: tissue Plasminogen activator (t-PA) antigen, Plasminogen activator inhibitor-1 (PAI-1) antigen, D-dimmer of fibrin/fibrinogen degradation products (FDPs), and euglobulin clot lysis time (ECLT). Data are expressed as mean±S.D. and Significance (P<0.05) tested with ANOVA. Reduced fibrinolytic activity was detected in obese control rats, compared to their non-obese counterparts. This was elicited by increased PAI-1 level from 1.66±0.17 to 2.85±0.09 ng.ml⁻¹, decreased t-PA level from 1.06±0.11 to 0.85±0.08 ng.ml⁻¹, decreased FDPs from 1.85±0.05 to 1.53±0.09 ng.ml⁻¹, and prolongation of the ECLT from 62.6±2.67 to 73.8±2.61 min. Acute exercise caused similar significant activation of the fibrinolytic activity in both non-obese rats, and obese rats (decreased PAI-1 by 15% and by 13%, increased t-PA by 47% and by 53%, increased FDPs by 11% and by 6%, and shortened ECLT by 4% and by 8 %; respectively). In non-obese rats, chronic training, as compared to acute exercise, further improved the levels of t-PA (increased by 39%) and ECLT (shortened by 20%), without significant changes in PAI-1 and FDPs. However in obese rats, chronic training, as compared to acute exercise, was associated with better effects on fibrinolytic activity, featured by decreased PAI-1 by 36%, increased FDPs by 12%, and shortening in the ECLT by 19%. It could, therefore, be concluded that obesity produces significant inhibition in the fibrinolytic activity, which is corrected reasonably by acute exercise, and tremendously by frequent physical training. An advantageous consequence, that is valid also in absence of obesity.

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Reliability of loaded countermovement jump performance using the chronojump-boscosystem

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Countermovement jump test protocols are common measures of lower body performance in sport and exercise science settings. Various technology have been developed to facilitate reliable and faster measurements from vertical jump tests. In the recent decade, open source technology in sports performance have been introduced. In open source technology, the end user has access to the technology design and can distribute it to other people (González et al., 2003). One open source technology that can measure vertical jump characteristics is the Chronojump-Boscosystem (De Blas, 2011). The Chronojump-Boscosystem consists of a free software, open hardware and a contact mechanism. This study aimed to establish the reliability of a loaded countermovement jump performance using the Chronojump-Boscosystem utilizing two parallel (30.5 x 30.5 cm) home-made contact platforms as contact source. Researchers suggest that a percentage of coefficient of variation (%CV) within 10% is considered reliable (Duthie et al., 2006; Pyne, 2003). Also, the smallest clinically worthwhile change (SWC) is the smallest change that is of benefit to athletic performance. Variables are considered capable of detecting SWC if the typical error (TE) is ≤ SWC (Pyne, 2003). 15 sports science and physical education major students from the University of the Philippines - Diliman (age: 20.0 ± 2.4 yrs; height: 162.4 ± 27.3 cm; weight: 74.5 ± 28.6 kg) participated in two experimentation sessions separated by 1 day rest interval. Day 1 consisted of anthropometrics, a standardized warm-up involving a five-minute jogging and dynamic stretching exercises and 2 loaded countermovement jump trials. In Day 2, similar warm-up and 2 loaded countermovement jump trials were followed. The best trials from Day 1 (27.2 ± 4.8 cm) and Day 2 (24.5 ± 6.8 cm) were analyzed to derive TE, %CV and SWC. Results revealed a TE of 1.6, %CV of 2.3% and SWC 0.45. These data suggest that the loaded countermovement jump test using the Chronojump-Boscosystem is reliable. However, the same test is incapable of detecting the smallest worthwhile change from inter-test comparison.


The authors would like to thank the students from the College of Human Kinetics for their participation in the study.

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Bone traits benefits after long-term retirement from sports – a mean 39-years prospective controlled study in men


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Background: Physical activity during growth increases peak bone mass (BMD). BMD is also closely associated with fractures as it has been suggested that 1 standard deviation (SD) increased BMD reduces the fracture risk by 50%. But to recommend physical activity during youth as a strategy to reduce prevalence of osteoporosis, and fragility fractures, exercise-induced benefits must be retained at advanced ages.

Purpose: This study aimed primarily to prospectively evaluate BMD changes in male athletes, from activity into long-term retirement. Secondary aims were to evaluate other bone traits and fracture incidence during the follow-up period.

Methods: By single photon absorptiometry (SPA), this study evaluated whether exercise-associated high BMD in 46 active male athletes with a mean age of 22 years (range 15–40), that we have reported in the past, was retained a mean 39 years (range 38–40) later when the former athletes had been retired from sports for a mean 29 years (range 10–58). 24 non-athletic males of similar ages served as controls. BMD and bone structure were at follow-up also measured by dual energy X-ray absorptiometry (DXA), peripheral computed tomography (pQCT) and quantitative ultrasound (QUS). Both cohorts were normally distributed (Shapiro Wilk’s test). Data are presented as means with 95% confidence intervals (95% CI). Group differences were evaluated by Student’s t-test between means and chi-square test. Z scores, i.e. the number of SD above or below the age-predicted mean, were derived by linear regression using data from the controls.

Results: There was no group difference in anthropometry either at baseline or at follow-up. The active athletes (baseline) had BMD Z score of 1.0 (0.7, 1.4) in the femoral condyles (SPA). The former athletes (follow-up) had BMD Z score of 0.7 (0.2, 1.1) in the distal radius (SPA) and 1.2 (0.8, 1.7) in the legs (DXA) Figure 1). There were no changes in BMD Z scores during the follow-up period, neither when estimated by the same SPA apparatus but in different skeletal regions (delta Z score –0.3 (–0.8, 0.2)) nor when estimated in the same extremity but with SPA at baseline and DXA at follow-up (delta Z score 0.0 (–0.4, 0.4)) (Figure 1). Furthermore, tibial cortical area was larger in the former athletes (Z score 0.8 (0.5, 1.2)) as was tibial strength index (Z score 0.7 (0.4, 1.0)), and 10.9% of the former athletes had sustained fractures in comparison with 20.8% of the controls (no statistics done due to low power).

Conclusions: Exercise-associated BMD benefits seem to be retained even three decades after cessation from active training. In a wider perspective, this indicates that physical activity could be recommended in younger years as a feasible strategy to reduce osteoporosis in older ages.

Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.
Effect of a single set bench press on upper body power

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This study aimed to determine the existence of postactivation potentiation in the upper body from a single set bench press loading stimulus. 5 males from the UP College of Human Kinetics basketball intramurals team volunteered to participate in the study. 1 RM load was estimated from the 5 RM bench press test of the participants. On day 1, they were asked to perform 5 repetitions of bench press exercise at 40% 1 RM. Similar procedures were executed on day 2 but loading was at 80% 1 RM. Pre and post measures using the plyometric push-up test were analyzed to determine any difference in performance on both schemes. Paired T-test showed that there was no significant difference in the plyo push-up performance at both 40% 1 RM at t(4) = -0.47, p = 0.66 and 80% 1 RM at t(4) = -0.38, p = 0.73. In conclusion, performing a single set bench press exercise does not stimulate power production through postactivation potentiation at 40% 1 RM and 80% 1 RM load intensity levels.

| Table 1: Descriptive Statistics, 5 RM Load, 1 RM Load and Plyo Push-up Height Result |
|---|---|---|---|---|---|---|
| Age | Height (cm) | Weight (kg) | 5 RM Load (kg) | 1 RM Load (kg) | Plyo push-up height at 40% Load | Plyo push-up height at 80% Load |
| 19.2, 24 | 175.2, 66 | 79.6, 10.63 | 59.5, 12.7 | 60.2, 14.60 | 8.66, 1.34 | 8.65, 6.79 | 10.15, 10.59 |

Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.
Age-related changes in marathon running performances

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Older (or masters) endurance athletes are a positive example of exceptional aging and are a rich source of insight into a person’s ability to maintain peak physical performance and physiological function with advancing age (1, 2). Previous studies suggested that the performance of masters runners (> 40 yrs) during marathon running has improved (3). To date, it is not known whether masters athletes still improve their marathon performance or whether they have reached their limits. Accordingly, the purpose of this study was to examine the changes in participation and performance of masters athletes at the New-York City (NYC) marathon over the last 30 years (from 1980 to 2009). Averaged running time performances of the top 10 finishers of each age group (between 20 and 79 yrs of age) for both females and males were analyzed. Gender differences in performance times were also analysed for the top 10 male and female runners between 20 and 65 yrs of age. Over the three decades 1980-89, 1990-99 and 2000-09, the percent of finishers younger than 40 yrs significantly decreased (P < 0.05), while the percent of masters finishers significantly increased (P < 0.05), for both males and females. Over the 3 decades, male masters athletes represented 36%, 45% and 53% of total male finishers, respectively; while female masters athletes represented 24%, 34% and 40% of total female finishers, respectively. For males, mean finish times did not change over the three decades for age groups < 60-64 yrs. In contrast, running times significantly decreased (P < 0.01) over the three decades for age groups > 60-64 yr. Average running time of males within the 70-74 yrs age range significantly decreased (P < 0.01) by 13 min (4.9%) between 1980-89 and 1990-99, and by 4 min (1.6%) between 1990-99 and 2000-09. For females, mean finish times did not change over the three decades for age groups < 45-49 yrs, except the time of the 30-39 yrs group that was lower in 2000-09 decade compared to previous decades. Female running times significantly decreased (P <0.01) over the three decades for age groups > 45-49 yr. Average running times of females within the 60-64 yrs age range significantly decreased (P < 0.01) by 16 min (6.8%) between 1990-99 and 2000-09. These data suggest that male (> 65 yrs) and female (> 45 yrs) master runners have probably not yet reached their limits in marathon performance. The relative stability of gender differences in marathon running times across the different age groups over the last decade also suggests that age-related declines in physiological function do not differ between male and female marathoners. Literature on the masters athletes improvements in performance has already and, should still stimulate further research on the understanding of age related physiological changes and the potential slowing of some of the aging processes through athletic training.

Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.
Former male elite athletes have more osteoarthritis and arthroplasties in hip and knee than expected


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Background: Intense exercise has been reported as one risk factor for hip and knee osteoarthritis (OA).

Purpose: This study aimed to evaluate (i) whether former impact and non-impact athletes have more OA than expected, (ii) if former athletes have more hip or knee arthroplasties due to OA than expected, and (iii) if knee OA is driven by previous knee injuries.

Methods: The prevalence of OA and arthroplasty in the hip and knee were registered in 709 former male elite athletes with a median age of 70 years (range 50–93), retired from sports for a median 35 years (range 1–63), and compared with 1368 controls aged a median 70 years (range 51–93). Both cohorts were normally distributed (Shapiro Wilk’s test). Data are presented as means with 95% confidence intervals (95% CI). Group differences in anthropometrics and lifetime factors were evaluated by Student’s t-test between means and chi-square test. Age-adjusted odds ratios (OR) was estimated by logistic regression in different models adjusted for combinations of age, body mass index (BMI; kg/m²), occupational load, and previous soft tissue knee injury.

Results: The age adjusted risk of hip or knee OA was higher in former athletes (OR 1.9, 95% CI 1.5, 2.3), as was arthroplasty based on OA in either of these joints (OR 2.2, 95% CI 1.6, 3.1) (Table 1). The risk of hip OA was doubled (OR 2.0, 95% CI 1.5, 2.8) and hip arthroplasty 2.5 times higher (OR 2.5, 95% CI 1.6, 3.7) in former athletes than in controls, predominantly driven by a higher risk in former impact athletes. Also the risk of knee OA was higher (OR 1.6, 95% CI 1.3, 2.1), as was knee arthroplasty (OR 1.6, 95% CI 0.9, 2.7), driven by a higher risk in both former impact and non-impact athletes. The differences between the groups remained after adjustments for age, BMI, occupational load, and soft tissue knee injury, except for knee OA where impact sportmen no longer were at risk (OR 1.19, 95% CI 0.83, 1.71) when adjusted for previous knee soft tissue injury while the risk remained after adjustment in non-impact athletes (OR 3.19, 95% CI 1.47, 6.91).

Conclusions: Hip and knee OA and hip and knee arthroplasty are more commonly found in former male elite athletes than expected. A previous knee injury is associated with knee OA in former impact sportsmen, but not in non-impact athletes.

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Reproducibility of speed at fixed and modelled plasma lactate markers during treadmill running in humans

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Incremental exercise with blood sampling to characterise the lactate-workload relationship is commonplace in exercise physiology laboratories to determine exercise intensity and monitor training adaptation. Various lactate markers exist, yet the reproducibility of speed at fixed and modelled plasma lactate markers remains unclear during incremental treadmill running of different stage durations. Twenty-one healthy volunteers (male = 15; age 22±3 yr, height 175±8 cm, body mass 70±11 kg) performed repeat running trials of 4- and 8-min stages on a powered treadmill at a 1% gradient. Trials commenced at speeds of 1.94 and 2.22 m.s⁻¹ for females and males, respectively, with increments of 0.28 m.s⁻¹. Duplicate fingertip capillary blood samples were drawn into non-lysed tubes at rest and during the final 30 s of each stage and analysed for plasma lactate (2300 STAT Plus™, YSI Life Sciences, US). Lactate analysis software (1) employing 3rd degree polynomial fitting (r² = 0.983 ± 0.030) was used to determine running speed at the fixed markers: 2.0, 3.5, and 4.0 mmol.L⁻¹, the 1 mmol.L⁻¹ rise from baseline, and the modelled markers: deviation maximum (D_max), lactate threshold (LT) and log-log LT. D_max was the speed at the maximum perpendicular from a line connecting the first and the final lactate-speed points to the polynomial. LT method employs a ‘broken stick’ model (1) identifying the dividing point between two fitted regression lines as the corresponding speed, with log-log LT applying a log transformation (2). Reproducibility was assessed using Pearson’s correlation coefficients and limits of agreement (LoA) (3). With the exception of D_max for 8-min stages (P<0.05), marker running speed for repeat trials of 4- and 8-min stages were similar. Correlation coefficients for marker running speed between repeat trials of 4- and 8-min stages were: r = 0.57, 0.77 (2.0 mmol.L⁻¹); 0.58, 0.75 (3.5 mmol.L⁻¹); 0.65, 0.85 (4.0 mmol.L⁻¹); 0.70, 0.94 (1 mmol.L⁻¹ rise); 0.45, 0.62 (D_max); 0.57, 0.61 (LT) and 0.10, 0.43 (log-log LT), respectively. For 4-min stages, the LT recorded the lowest mean difference between trials (0.04 m.s⁻¹), yet the 1 mmol.L⁻¹ rise had the narrowest LoA (-0.84 to 0.71 m.s⁻¹). For 8-min stages, the 1 mmol.L⁻¹ rise demonstrated the lowest mean difference between trials (-0.05 m.s⁻¹) and the narrowest LoA (-0.35 to 0.26 m.s⁻¹). Agreement was greater for all lactate markers during 8-min stages, yet irrespective of stage duration, D_max and log-log LT were not reproducible. These findings suggest greater reproducibility of speed at fixed lactate markers as opposed to curve-modelled markers (with the exception of LT) for incremental treadmill running of 4- and 8-min stages. The application of summary markers from lactate-speed data for intensity prescription of treadmill running should take into account marker suitability and stage duration.


Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.
Exhaustive exercise and vitamins C and E modulate thyroid hormone levels at low and high altitudes

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Introduction: Thyroid hormones play an important role in cell growth and differentiation and regulation of oxygen consumption and thermogenesis. The effect of altitude and vitamin supplementation on thyroid hormone levels in animals or humans performing acute exhaustive exercise have not been investigated before. Therefore, we sought to test whether exhaustive exercise-induced stress with antioxidant supplementation was capable of modulating the level of thyroid hormones at different altitudes.

Methods: In both low and high altitude areas, native untrained rats were divided equally into three groups, each (N= 6); control group (non-stressed and untreated); stress group A (received normal saline); and stress group B, received a single intra-peritoneal dose of 25 mg/kg of vitamin E in combination to 20 mg/kg of Vitamin C orally for one hour before the beginning of the experimental procedure. Stress was achieved in groups A and B by forcing the rats to swim for a duration of 2.5 h in glass tanks (length 100cm, width 40 cm, depth 60 cm) containing tap water maintained at a temperature of 32 °C. The depth of water in the tank was 30 cm. At the end of the experimental procedure, rats were terminally anesthetized with light diethyl ether and blood samples were taken at the same time directly from the heart and used to measure serum levels of T3, T4 and TSH.

Results: Thyroid levels were significantly decreased in resting rats at HA compared to LA, and swimming exercise moderately increased T3 and TSH at both LA and HA, whereas T4 was markedly increased (62%) at LA compared to a moderate HA increase (28%). Co-administration of vitamins C and E augmented the observed forced swimming-induced thyroid release. However, the conversion of T4 to T3 was reduced in both altitude areas following swimming exercises and vitamin pre-treatment had no effect.

Conclusions: We conclude that acute stress induced thyroidal hormones in rats was augmented by antioxidant drugs in both LA and HA areas. These findings may play an important role in the human pathophysiology of thyroid gland at different altitudes.

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Acute caffeine ingestion results in increased vastus medialis muscle activation and improved muscle performance during short-term high intensity isokinetic exercise in trained men

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It is well established that acute caffeine ingestion enhances endurance performance in humans (Graham, 2001). However, the effect of caffeine on muscle strength performance has been less thoroughly examined and studies examining this issue have yielded equivocal findings (Astorino and Roberson, 2010). Some data suggest direct effects on muscle excitation-contraction coupling and motor unit recruitment, which are independent from those related to metabolic efficiency (Walton et al., 2003; Tarnopolsky and Cupido, 2000). These studies have focused on maximal isometric strength, whereas the effect of caffeine on neuromuscular function during dynamic contractions at different speeds has not been investigated thoroughly (Bazzuchi et al., 2001), thus limiting the conclusions that can be made across the torque-velocity relationship. The aim of this study was to examine the effect of caffeine ingestion on muscle torque production and muscle activity at different contraction speeds in trained men. Following ethics approval and informed consent, 10 strength trained males (Mean age ± SD = 22 ±1.1 years) volunteered to participate. A double-blind, randomised cross over design was used. Sixty minutes post ingestion of caffeine (6mg kg-1) or placebo diluted into 250ml artificially sweetened water, participants completed 6 repetitions of dominant knee extension on an isokinetic dynamometer (Cybex Norm, CSMi Solutions, Massachusetts, USA) at 3 angular velocities (30°sec, 150°sec, 300°sec, conducted in ascending order) with one minute rest between sets. Average torque for the knee extensors and electromyographical (EMG) activity of the vastus medialis were assessed at each velocity. Root mean square (RMS) EMG activity was normalised against a maximal voluntary isometric contraction at an angle of 15 degrees knee extension, established during habituation sessions. Results from repeated measures ANOVA indicated that muscle torque production was significantly higher (p=0.02) with caffeine (91.1±11.6nm) compared to placebo (84.5±12nm) and that muscle torque decreased as contraction speed increased (p=0.001). Mean torque ± SD was: 116.6±17.4nm, 86.2±9.9nm and 61.2±8.5nm for velocities of 30°sec, 150°sec, 300°sec respectively. A significant (p=0.02) substance by velocity interaction for muscle activity indicated significantly higher muscle activity in the presence of caffeine vs. placebo and that this difference was amplified as angular velocity increased (See Figure 1). Thus, this study demonstrates that acute caffeine ingestion improves muscle performance during short-duration maximal dynamic contractions. The concomitant increase in muscle activity also supports the hypothesis of an effect of caffeine on motor unit recruitment during, brief, high-intensity resistance exercise.


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Faster end-run speed and maintained glycaemia following pre-exercise ingestion of high molecular mass carbohydrate in type 1 diabetes

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Rationale: In healthy individuals ingestion of high molecular mass carbohydrates (HMM-CHO) empty from the stomach sooner, cause faster and greater increases in blood glucose and post-exercise muscle glycogen resynthesis and improve cycling performance compared with low molecular mass carbohydrates (LMM-CHO) (Leiper et al., 2000; Piehl Aulin et al., 2000, Stephens et al., 2008). Pre-exercise carbohydrate ingestion is an effective strategy for reducing the occurrence of hypoglycaemia during or after exercise in individuals with type 1 diabetes (T1DM) but the metabolic effects of ingestion of different carbohydrates in this group for glycaemic or performance gain has been under-researched. This study examined the metabolic and performance responses of pre-exercise ingestion of a low- and high-molecular mass CHO during running in T1DM.

Methods: With ethical approval, seven participants (34.3±5.5 years, 69.9±2 kg, HbA1c 76.6±6.5 mmol/mol) attended the laboratory on two occasions after preliminary testing. On each visit participants consumed 0.6 g.kg-1 BM of either LMM (Dextrose, DEX, MyProtein®, Cend ltd, UK) or HMM (Waxy Barley Starch; WBS, Vitargo®, Swecarb, Sweden) as a 9.2±0.1% solution, with 50% reduced rapid-acting exogenous insulin dose 2-h before exercise. Blood samples were taken over 2-h rest. Participants then completed a discontinuous incremental treadmill run which consisted of five 4 min stages at 31±2%, 41±2%, 53±3%, 69±4%, 80±4% VO2peak interspersed with 90s recovery. After 5 minute recovery, participants performed a 10 min performance run on a non-motorised treadmill (Curve, Woodway, Germany). Capillary blood samples were analysed for glucose (BG). Rates of CHO and lipid oxidation were determined using principles of indirect calorimetry. Data were expressed as mean±SEM and analysed using repeated measures ANOVA (P<0.05).

Results: Fasted BG reached similar peak values one hour after CHO ingestion. Immediate pre-exercise BG were comparable (WBS 15.9±1.7 vs. DEX 15.0±1.9 mM, P=0.47). Resting CHO oxidation was elevated under WBS (WBS 0.31±0.03 vs. DEX 0.19±0.04 g.min-1, P=0.024) and lipid oxidation lower in WBS (WBS 0.03±0.01 vs. DEX 0.06±0.01 g.min-1, P=0.017). BG increased similarly following performance running (WBS 0.9±0.2 vs. DEX 0.7±0.3 mM, P=0.45). There was a greater distance completed in the final quarter of the run in WBS (WBS 323±21 vs. DEX 301±20 m, P=0.02) equating to 0.5±0.2 km.h-1 greater speed during the last quarter of the test.

Conclusions: The results demonstrate maintained glycaemia and improved run performance following ingestion of a high molecular mass carbohydrate in individuals with type 1 diabetes.


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The effects of different recovery interventions following a repeated rugby union (sevens) game simulated protocol

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It has been established that rugby places extremely high physiological and psychological stresses upon players. It has also been demonstrated that the incidence of injury within rugby matches is higher than other contact sports (Gill et al. 2006). Additionally recovery methods within rugby have been under-researched, especially when compared to other sports (Duthie et al. 2003). The need for effective recovery interventions is essential to facilitate player revival and safety (Gill et al. 2006). The purpose of this study was to compare the effectiveness of four different recovery interventions following a rugby 7’s game simulated protocol and to determine whether any, or all, of the four recovery interventions were effective. Twelve undergraduate Premiership Level Rugby Union players completed a Wingate anaerobic cycle test (WAnT), Countermovement Jump test (CMJ), the Total Quality Recovery Questionnaire (TQR) and muscle soreness diagrams. Testing sessions were separated by one week. Subject’s completed the England Anaerobic Fitness Test (E-set), then received one of four different recovery interventions; Passive recovery (PR), Active Recovery (AR), Cold Water Immersion (CWI) or Combined Recovery (COMB). 24 hours later subjects repeated the pre-test assessments. A fully within groups’ factorial ANOVA was used to compare results. Perceptual responses revealed that PR was perceived to provide significantly lower levels of recovery compared to AR/CWI/COMB (14.0 (1.63) vs. 15.4 (1.43), 15.4 (1.71), 15.6 (2.91)): (p < .05). Performance variables; CMJ displayed no significant differences between PR vs. AR/CWI/COMB (p > .05). WAnT analysis produced no significant difference between PR vs. AR/CWI/COMB (p > .05). This study supports previous research that CWI provides an improved perceptual response of recovery compared to PR (Roswell et al. 2009). The study also supports previous studies in that no significant differences were found between PR, AR, CWI and COMB recovery protocols for performance measures (Kinugasa and Kilding 2009).

The current study lends support to the majority of recovery literature presently available, and despite the popularity of CWI, active recovery and combined recovery as recovery interventions, when compared to passive recovery their use remains unsubstantiated. During competition, turnaround times between games can be very short. The recovery intervention employed must be effective. Many coaches believe that something is better than nothing, however placing a greater demand upon the athlete’s time while implementing a non-effective recovery measure, may prove to be detrimental (Higgins et al. 2010).


I am very grateful to the Life University rugby team for participating in this study. In addition I would like the thank Dr Deloss Brubaker and Dr Jeffrey Lander for their continued support with this project.

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Influence of endurance training on the recovery of muscle metabolism and power output during repeated sprint exercise*

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Recovery of power output during repeated sprints has been found to be associated with phosphocreatine (PCr) resynthesis and endurance fitness (Bogdanis et al., 1996). The purpose of the present study was to examine whether an improvement of endurance fitness, following endurance training, can hasten power output restoration and PCr resynthesis during repeated bouts of high intensity exercise in female athletes. Fourteen female games players were divided into two groups: training (T; n=9) and control (C; n=5). The T group added endurance training consisting of 30 min running on a level treadmill at 85% of maximal oxygen uptake (VO2 max) 3 times per week, on top of their normal training programme for 6 weeks, while the C group followed their normal training programme. Before and after the 6 weeks training period, all participants performed two 30 s sprints separated by 2 min of passive rest on a non-motorized sprint treadmill. Power output was measured as the product of the horizontally applied force and running speed. Muscle biopsies were taken before and 10 s and 2 min after the first 30 s sprint only in the T group. Adenosine triphosphate (ATP), PCr, glycogen, lactate and glucose-6-phosphate (G6P) were measured in dry muscle. VO2 max and blood lactate responses during submaximal running were also measured before and after training to assess aerobic performance. The percentage of VO2max corresponding to a concentration of 4 mmol.l-1 lactate in the blood (%4mM) was used as an index of endurance fitness. Data were assessed using two-way analyses of variance, paired and independent t-tests. Values are presented as mean±SEM. The additional endurance training resulted in an improvement of %4mM by 8.3±2.8% in the T group, while there was no change in the C group (-1.5±2.5%)(p<0.05). VO2max remained unchanged in both groups. Power output restoration in the second compared with the first sprint was increased more in the T compared with the C group after training (7.4±1.4 vs. 2.1±1.1%, p<0.05). PCr concentration 10 s after the first 30 s sprint was 36.1±11.4% higher (p<0.01) following training in the T group (Table 1), while the rate of glycogen utilization and muscle lactate tended to be lower after training (p=0.054 and p=0.098, respectively; Table 1). In conclusion, this study showed that endurance training improved endurance fitness, and resulted in an enhanced power output restoration during repeated sprint exercise. This may be due to either a greater PCr resynthesis or a greater reliance on aerobic metabolism during sprint exercise.

Table 1. Muscle metabolites (mmol.kg dry muscle-1) in vastus lateralis before and after training for the training group (mean ± SEM, n = 9). ATP: adenosine triphosphate; PCr: phosphocreatine, G6P: glucose 6 phosphate.

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Baseline</th>
<th>10 s post</th>
<th>2 min post</th>
<th>Baseline</th>
<th>10 s post</th>
<th>2 min post</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP</td>
<td>19.6±0.7</td>
<td>17.2±1.0</td>
<td>18.6±0.8</td>
<td>18.3±1.1</td>
<td>18.1±0.9</td>
<td>19.0±0.9</td>
</tr>
<tr>
<td>PCr</td>
<td>82.6±2.3</td>
<td>23.3±1.5</td>
<td>59.3±3.5</td>
<td>84.0±2.1</td>
<td>30.4±2.1</td>
<td>66.0±3.2</td>
</tr>
<tr>
<td>Creatine</td>
<td>49.5±3.3</td>
<td>109.4±2.8</td>
<td>60.9±2.8</td>
<td>55.7±2.2</td>
<td>105.9±1.9</td>
<td>71.5±1.6</td>
</tr>
<tr>
<td>Glycogen</td>
<td>33.3±3.1</td>
<td>123±1.6</td>
<td>249±1.6</td>
<td>339±10</td>
<td>274±45</td>
<td>269±13</td>
</tr>
<tr>
<td>G6P</td>
<td>0.76±0.2</td>
<td>17.2±1.5</td>
<td>13.0±1.6</td>
<td>6.7±0.2</td>
<td>17.3±1.3</td>
<td>11.5±1.2</td>
</tr>
<tr>
<td>Lactate</td>
<td>0.1±1.7</td>
<td>88.9±1.0</td>
<td>69.1±1.5</td>
<td>6.6±1.4</td>
<td>78.9±4.3</td>
<td>43.7±4.2</td>
</tr>
</tbody>
</table>

Bogdanis G. et al. (1996) J App Phys 80, 876-884

*Supported by funded from the Greek State Scholarships Foundation.

Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.
The effects of selected resistance training on swimming records in female students

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The purpose of present study was to determine the effect of an eight-week of selected resistance training protocol on 25-50-75 and 100 meters swimming records in female physical education students.

Method: 18 people of 42 female physical education students of P.E. coaching (21±1.1 years old, 164.15±2.5 cm length and 56±8.6 kg weight) selected randomly and divided to Experimental (the swimming training program + selected resistance training) and Control (the swimming training program) groups. The selected resistance training (6session/week) included muscle adaptations, strength, and power with 85% intensity of 1RM. After 8 weeks (2session/week) of swimming training, Mean± standard deviation, K-S test (to examine the normality of the groups) and Dependent and independent t-test (to determine the differences between the two groups) were used for data analyzing (α= 0.05).

Results: The results indicated that the selected resistance training increased power in Scott in the leg, hand flexion), opposite stretch of hand, flexion and extension of femur motions significantly. No significant changes were seen in swimming performance between two groups [25 m (P=0/42), 50 m (P=0/30), 75 m (P=0/31) and 100 m (P=0/32) after 8-week of selected resistance training protocol but the results showed significant reduction in 25 m[E:( P=0/03) &C: (P=0/08)], 50 m [E : (P=0/003)], 75 m [E : (P=0/008) &C : (P=0/008)] and 100 m [E : (P=0/03) &C : (P=0/003)] and no significant decrease in 50 m (P=0/98) on control group swimming records after 8 weeks swimming training in both experimental and control groups.

Conclusion: Generally based on the results of this study it can be concluded that the most effect of the strength training was the last 25 meters of 100m speed swimming than other quartiles. This Distance in the most competition plays a decisive role in the fate of swimmers championship medals.

Table 1: results of t-test in experimental and control groups (pre and post test)

<table>
<thead>
<tr>
<th></th>
<th>Mean (pre)</th>
<th>Mean (post)</th>
<th>t-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>25m</td>
<td>E: 39.5</td>
<td>C: 40.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50m</td>
<td>E: 54.8</td>
<td>C: 55.0</td>
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<td></td>
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<tr>
<td>75m</td>
<td>E: 69.3</td>
<td>C: 70.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100m</td>
<td>E: 84.6</td>
<td>C: 85.0</td>
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</tbody>
</table>

*=significantly

P=0/05


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Kinematic asymmetries of the lower limbs during ergometer rowing

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Rowing injuries, particularly of the lumbar spine, are often attributed to poor technique. Rowing technique comprises a series of coordinated movements between the back, upper limbs and lower limbs, and abnormalities in these may lead to injury (Bull & McGregor, 2000). The primary aim of this study was to test the hypothesis that ergometer rowing is symmetrical with respect to lower limb motion, and that deviations from symmetry result from rowing experience, work rate, or stroke position. A secondary aim was to examine the relationship between lower limb asymmetries and lumbar-pelvic kinematics.

Twenty-two rowers in three levels of ability (8 elite, 8 club, 6 novice) participated in an indoor rowing step-test at four incremental work rates. A motion analysis system was used to record their lower limb and lumbar-pelvic kinematics including; bilateral knee joint, bilateral hip joint, lumbar-pelvic joint and pelvic twist angles. These kinematic parameters were analysed at four positions within a normalised rowing stroke. Hip and knee range of motion (ROM) through the stroke were calculated and assessed for asymmetry using the Symmetry Index (Robinson et al., 1987). The step test was performed on an instrumented rowing ergometer which incorporated load cells at the handle and seat so that performance measures such as peak handle force, stroke length, medio-lateral seat drift and stroke power could be derived.

Elite rowers exhibited the largest handle force and power (P < 0.01), and least medio-lateral seat drift (P < 0.01). All three groups exhibited lower limb asymmetries, with asymmetries at the hip significantly greater than at the knee (P < 0.01) (Figure 1). Regression analysis indicated that asymmetries in both hip and knee ROM were significant (P < 0.01) in predicting lumbar-pelvic flexion during the power producing phase of the rowing stroke. However, hip ROM asymmetry showed a better relationship with lumbar-pelvic flexion compared to knee ROM asymmetry, explaining a greater proportion of the variance in lumbar-pelvic kinematics. Six rowers exhibited a counter-clockwise pelvic twist, however, the direction of pelvic twist did not correspond with the direction of hip joint or knee joint asymmetry.

Bilateral asymmetries during the rowing stroke, particularly at the hips, can contribute to sub-optimal kinematics of the lumbar-pelvic region. Quantification of hip ROM asymmetries may therefore be a useful tool in predicting the development of low-back pain in rowers.

Sagittal plane joint angles over a normalised rowing stroke (average of ten strokes for a representative subject from elite (top row), club (middle row) and novice (bottom row) rowers). Solid line represents right side, dotted line represents left side.


This research was funded by the EPSRC and GB Rowing

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ACTN3 genotype and human fatigue-related neuromuscular performance

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Purpose: A common nonsense polymorphism (577XX) within the ACTN3 gene results in a total deficiency of the Alpha–actinin 3 (AA3) protein (North et al. 1999: Natural Genetics :21: 353-354) and has been purported to influence muscle performance capabilities during activities involving rapid production of high force (MacArthur et al., 2004: Bioessays :26: 786-795). Studies have shown the detrimental consequences of AA3 absence on post fatigue neuromuscular performance (MacArthur et al. 2008: HumMolGenetics 17:1076-1086) in animal models. There are no studies to date that have investigated fatigue-related neuromuscular performance in human populations in relation to ACTN3 polymorphisms.

Participants: 36 recreationally active European Caucasian males participated in the study.

Methods: Retrospective genotyping of whole blood via PCR and electrophoresis techniques confirmed that 27 participants possessed the R allele (expression of AA3 protein) and 9 participants were homzygotic for the X allele (AA3 deficient). Estimates of knee extensor volitional static peak force (PF), peak twitch force (PTF), rate of force development and twitch half relaxation time (THalf) were obtained prior to and following i) a fatigue intervention consisting of 4 bouts of 3 x 10 second maximal isometric contractions (45° knee flexion) separated by 5 seconds and ii) a control condition of equivalent duration consisting of no exercise. The control condition was performed first to minimise any carry over effects.

Results: Analysis of baseline performance revealed significant slower twitch half relaxation time in the AA3 deficient group (P<0.05) vs. AA3 expression group. Mixed-model repeated measures ANOVA revealed a significant exercise-related impairment in PF of a similar amount in both genotype groups (~18% vs. baseline, F[1, 34] = 33.9, p<0.001). No impairments to other indices of performance were observed following the fatiguing exercise.

Conclusions: THalf appears to be lengthened in AA3 deficient individuals, suggesting that AA3 deficiency is associated with the contractile properties of fast twitch muscle fibres (Vincent et al. 2007: Physiological Genomics: 32: 58-63). However, the lack of a differential change in PF and PTF throughout the fatigue protocol between the two groups suggests that more research is needed with larger cohorts to explore further the issues of fatigue-related muscular performance and ACTN3 genotype.


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**Plasma Hsp72 and Hsp27 during moderate and intense exercise to exhaustion in the heat**

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**MOTIVATION:** Thermostolerance during prolonged exercise in the heat is associated with adaptations linked to the heat shock response. This response confers transient thermal tolerance, in part due to the expression of heat shock proteins (Hsps). Hsp72 and Hsp27 are particularly responsive to physiological stress, including hyperthermia. During exercise-heat stress, extracellular levels of Hsp72 are proposed to increase in conjunction with the level of hyperthermia attained, regardless of the rate of heat storage. However, exercise performed to exhaustion at different intensities in the heat may elicit differing rates of Hsp expression related to the development of thermal and metabolic strain. In addition, the association between Hsp72 and Hsp27 expression in the extracellular milieu remains largely unexplored. Therefore, this study examined the influence of exercise at moderate and high intensities on plasma Hsp72 and Hsp27 expression.

**METHODS:** Sixteen male subjects cycled to exhaustion at 60% and 75% of maximal oxygen uptake (VO₂max) in hot conditions (40°C, 50% relative humidity). Core temperature, heart rate, oxidative stress, blood lactate and blood glucose levels were measured during exercise to determine the predictor variables associated with plasma Hsp72 and Hsp27 concentrations prior to exercise, on reaching exhaustion, and 24 h following exercise cessation. RESULTS: At exhaustion, heart rate exceeded 96% of maximum in both moderate and high intensity exercise conditions. A core temperature of 39.7 ± 0.4°C was reached in the 60% VO₂max trial after 58.9 ± 10.9 min of exercise, whereas 39.0 ± 0.5°C was attained in the 75% VO₂max trial after 27.2 ± 9.0 min (mean ± SD; P < 0.001). In the 75% VO₂max trial, the rate of rise in core temperature was 2.1 ± 1.4°C/h greater than in the 60% VO₂max trial (P < 0.001). A significant increase and correlation was observed between plasma Hsp72 and Hsp27 concentrations at exhaustion (P < 0.005). Plasma Hsp72 was highly correlated with the core temperature attained in the 60% VO₂max trial and the rate of increase in core temperature in the 75% VO₂max trial (P < 0.05). However, no common predictor variable was associated with the expression of both Hsps. CONCLUSION: The similarity in plasma Hsp72 and Hsp27 concentration during moderate and high intensity exercise may relate to the duration (i.e. core temperature attained) and intensity (i.e. rate of increase in core temperature) of exercise. As such, the expression of plasma Hsp72 and Hsp27 in response to exercise in the heat appears to be duration and intensity dependent.

Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.
Short-term increase in plasma IL-6 after downhill running is associated with increased core temperature during subsequent exercise-heat stress

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It remains unclear whether the acute inflammatory response that follows muscle damaging exercise (e.g. increase in circulating pyrogen interleukin (IL)-6), increases heat storage during subsequent exercise-heat stress, as proposed by Montain et al. (2000). As such, we tested the hypothesis that acute inflammation following exercise-induced muscle-damage (EIMD), using a downhill running model, increases core temperature during subsequent endurance exercise in the heat.

With informed consent, thirteen non-heat-acclimated healthy males (mean age ± SD, 20 ± 2 years) completed two, randomised and counterbalanced treadmill running trials separated by two-weeks. Participants performed a treatment which involved running for 60 min at 64%VO₂max in 20°C, 40% relative humidity (RH); on one occasion on a -10% gradient (EIMD), and another on a +1% gradient (CON). Following both treatments, participants rested for 30 min, timed to coincide with elevated circulating inflammatory mediators, and then performed an exercise heat-stress test (HS) which involved running for 40 min at 65% VO₂max on a +1% gradient in 33°C, 50% RH. Rectal core temperature (Tₑ) and oxygen uptake (VO₂) were measured throughout HS. Plasma IL-6 concentration was assessed prior to treatment (baseline), and immediately pre and post HS. Data were analysed using ANOVA, paired t-tests and Pearson’s correlations.

Plasma IL-6 concentration demonstrated a significant interaction (P < 0.001). There was no baseline difference between trials, but plasma IL-6 concentration was significantly greater on EIMD than CON immediately pre (P < 0.05), and post HS (P < 0.01, Fig. 1A). Despite similar Tₑ prior to HS (EIMD 37.8 ± 0.2, CON 37.6 ± 0.2°C, P > 0.05), ΔTₑ was significantly greater during HS on EIMD (P < 0.01, Fig. 1B) resulting in a higher final Tₑ on EIMD (EIMD 39.5 ± 0.4, CON 39.0 ± 0.4°C, P < 0.01). The acute inflammatory response after treatment, measured as the difference in plasma IL-6 response between EIMD and CON, correlated well with the difference between trials in the ΔTₑ during HS (r = 0.58, P < 0.05), and with the final Tₑ during HS (r = 0.67, P < 0.05). Mean VO₂ during HS was significantly greater on EIMD (3.0 ± 0.3 L/min) than CON (2.8 ± 0.3 L/min, P < 0.01). Despite this decreased economy, the difference in VO₂ between EIMD and CON did not correlate well with the difference between trials in the ΔTₑ during HS (r = 0.24, P = 0.43) or the final Tₑ during HS (r = 0.40, P = 0.17).

These data show that a bout of downhill running increases the short-term plasma IL-6 response to exercise and that this increase is associated with elevated Tₑ during subsequent endurance exercise in the heat. These results have practical relevance for athletes and soldiers undertaking multiple bouts of heavy exercise with an eccentric component in the heat.

Figure 1. A. Plasma IL-6 response at baseline, and pre and post exercise heat-stress (HS) which involved running for 40 min at 65% VO₂max in 33°C, 50% RH conducted 30 min after treatment which involved either exercise-induced muscle damage (-10% gradient running, EIMD) or +1% gradient running (CON). B. ΔTₑ during HS following EIMD and CON. Significantly different from baseline ** P < 0.01. EIMD significantly greater than CON # P < 0.05, ## P < 0.01. Data are mean ± SD. N = 13.


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The use of muscle dynamometer for correction of muscle imbalances in the area of deep stabilising spine system

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Dorsal pain caused by spine dysfunctions belongs to most frequent chronic illnesses in humans. Correct spine stabilisation is of immense importance for physiological body posture. The muscles of the deep stabilising spine system (DSSS) work as a single functional unit, where a dysfunction of only one muscle, causes dysfunction of the whole system. Non-invasive measurements of the condition of DSSS have been made possible by the construction of muscular dynamometer (MD) that is constantly being improved and its third version is currently available. The aim of our work has been the assessment of DSSS by diaphragm test and MD measurements. Based on an initial examination, a six-week intervention program is established including education on physiological body posture and correct basic body stabilisation for the given exercises and muscle strengthening. Consecutive measurements are then compared with the initial ones using pair t-test and the statistical program SIGMA STAT 3.1. Values with \( p < 0.05 \) are considered statistically significant. The initial measurements function also as control values. It was presumed that a smaller number of the tested subjects would be able to correctly activate the DSSS muscles before the intervention program as compared to after. A statistically significant change (\( p < 0.001 \)) could be shown between the initial and final measurements of the six-week intervention program (for 46 adolescents aged 12-16). A positive change has been found for 87% of the proband. It is clear that if a person actively approaches the program, then positive adaptation changes on the DSSS are seen after only six weeks. With the muscular dynamometer changes in diaphragm activation, increase of intra-abdominal pressure and condition of frontal spine stabilisation are recorded. The changes between the initial condition of a subject and states after some exercise or rehabilitation are especially noticeable. Also, the effect of given therapy or correct performance of the exercise can be followed and observed.


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Capillary blood pH in humans: a new possibility for monitoring the exercise?

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Evaluation of blood pH has been widely used in exercise physiology as an indirect way to assessing muscle acidosis. However, collecting venous or arterial blood in athletes in field condition is not an easy and secure method. Capillary blood is an alternate technique that is not so invasive and, in opposition to venipuncture, much more acceptable to athletes and coaches. So, the aim of this study was analyze the capillary blood pH alterations in response to incremental exercise on a cycle ergometer as an alternative method to traditional blood collection. In a pre-experimental design approved by the ethics committee of the University of Pernambuco/Recife-PE/Brazil, nine trained male cyclists (24±2 years; 71±7 kg; 170±4 cm; 47±9 mL/kg/min; 4.1±0.6 W/kg) underwent to an incremental exercise on a cycle ergometer, started at 10%Wmax (maximal output in Watts), determined by a previous (7-days before) peak power output test which included respiratory gas analysis (CPX, Cortex, Germany), which the load was increased incrementally by 10% each three minutes until volitional exhaustion. Capillary blood samples (25μL) from a finger were homogenized in 1% NaF (50μL) and the pH was immediately measured in a digital pH meter (Spear, USA) at rest, at the end of each 3-minute increment of exercise and at 3, 6, 9, 15, 30 and 60 minutes after exercise (passive recovery). A one-way repeated measures analysis of variance ANOVA (rest vs. exercise/recovery) and regression analysis were performed and the level of significance was set at p<0.05. The results showed that pH at rest was 7.7±0.08 and the lowest value was 7.08±0.22, obtained at third minute during recovery. Capillary pH showed a decrease in response to exercise, being significant different from resting values from the intensity equivalent to 70%Wmax, returning to baseline only after 60 minutes, F(15, 124)=14.710; p<0.001 (Fig. 1). The cubic trend was strong and statistically significant, where average capillary pH was correlated with the exercise intensity (%Wmax), (pH=7.752 – 0.076Wmax + 0.014Wmax² – 0.001Wmax³; F=117.9; R²=0.975; S.E.=0.023; p<0.001). In conclusion, the capillary blood pH of cyclists declined with increasing intensity of exercise on a cycle ergometer. The proposed model suggests that (i) pH determined in capillary blood is a sensitive method to evaluate exercise intensity (ii) and can be used as a low cost alternative for exercise intensity determination.

Fig. 1. Capillary blood pH of cyclists at rest (Basal), during exercise and at passive recovery. Data are represented as mean±SD (n=9). *Significantly different from rest point, p<0.05.

The authors acknowledge CAPES and PFA/UPE for financial support.

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Physiological determinants of repeated sprint ability in male team sport players

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The physiological determinants of repeated sprint ability (RSA) are an area of current debate (Bishop & Edge, 2006; Racinais et al., 2007; Billaut & Smith, 2010). The fatigue mechanisms that contribute to performance decrements in repetitive short-duration sprints remain elusive. To the author’s knowledge no studies have simultaneously tracked changes in heart dynamics, pulmonary gas exchange, muscle oxygenation kinetics and muscle activation to determine whether central or peripheral mechanisms lead to performance impairment.

Nine male team sport players (mean ± standard deviation: Age 21±2 yr; VO₂max 48.9±6.9 ml•kg⁻¹•min⁻¹) completed a test to maximal volitional exhaustion (Hopker et al., 2012), and on a separate day, a repeated sprint test on a cycle ergometer consisting of 10 x 6s maximal sprints interspersed by 30s of passive recovery. Prior to both maximal and RSE tests, participants undertook a 5 minute period of rest to facilitate baseline measurements and a 10 minute warm-up at 100W. Throughout the test, oxygen uptake was measured on a breath-by-breath basis, and tissue oxygen saturation index (TSI%) and total hemoglobin concentration (tHb) of the left vastus lateralis (VL) muscle was assessed using spatially resolved spectroscopy NIRS. Surface RMS EMG of the left VL muscle and power output were recorded for each sprint repetition. Cardiac output was assessed continuously throughout the test using the non-invasive thoracic electrical bioimpedance method (Charlouw et al., 2000). Data for each variable were analysed using a one-way ANOVA with repeated measures. A post hoc least significant difference test was used when necessary to determine where significant differences occurred. Values are mean ± standard deviation. Power output demonstrated a significant decline during the test (-209±83W, p<0.05). There was also a significant progressive decline in muscle TSI% (-15.0±7.5%, p<0.05) over the repeated sprints (see Figure 1). Regional muscle blood volume (as shown by changes in tHb) significantly declined at the first sprint (see Figure 1) compared to baseline (-8.1±6.8 μM•cm, p<0.05), and then remained consistent across the rest of the test. Muscle reoxygenation rate during recovery periods remained constant throughout the test (p>0.05), showing that availability of O₂ to the muscle was well preserved. The activation of the VL muscle (taking the 2nd and 3rd pedal revolution) changed significantly across the sprint repetitions (sprint 1: 0.36±0.12 mV vs. sprint 10: 0.30±0.09 mV, p<0.05).

VO₂ and cardiac output significantly increased across the RSE test (+11.4±5.9 ml•kg⁻¹•min⁻¹ and +6.9±5.0 L•min⁻¹ respectively, p<0.05), but were both significantly lower (p<0.05) than the values recorded during the maximal test. In conclusion, the results of this study suggest that short-duration RSA is determined by both muscle desaturation and changes in muscle activation.

![Figure 1: Changes in left vastus laterals TSI%, and tHb during repeated sprint exercise. Values are means for all subjects (N=9).](image)


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The reliability of heart rate variability measurement performed by an immediately repeated test-retest procedure

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At present, analysis of heart rate variability (HRV) is becoming widely used as a clinical or research tool. Additionally, HRV analysis represents an interesting possibility for controlling and managing the sport training process. Supported reliability studies for HRV measurement are, however, still limited. The test-retest procedure is often used for HRV reliability evaluation with repeating the retest after several hours or days. Since HRV can be influenced by a number of internal or external factors, it is more appropriate, in our opinion, to repeat the retest immediately without any interruption (even if this procedure also has limitations). The main purpose of the present study was to perform an assessment of the absolute and relative reliability of standard indexes of HRV from short-term laboratory recordings by means of orthoclinostatic stimulation (supine – standing – supine). The study group consisted of 60 participants (mean age (min - max): 22 (19 – 26) years; males). The second measurement was repeated immediately after the first measurement under the same conditions. Standard HRV indexes were computed: PT (total spectral power), PHF (high frequency spectral power; absolute units), PLF (low frequency spectral power; absolute and normalized units) and LF/HF. According to Pinna et al. (2007), absolute reliability was assessed by 95% limits of random variation; relative reliability was assessed by the intraclass correlation coefficient (ICC). There was also an estimate of the sample size needed to detect the mean difference ≥30 % of the between-subject S.D. A significant mean change was only determined in the standing position in the parameters: PT, PHF, PLF. The second measurement was, in individual subject, as high/low as 1.27/0.82 times (LFnu, standing position, best case) and 5.88/0.15 times (LF/HF, supine position, worst case) the first measurement, due to pure random variation. The ICC was > 0.7 for all the parameters (range 0.71 – 0.86). The estimated sample size ranged from 16 – 33 participants. In conclusion, a large random variation (within individuals) of short-term HRV parameters (both the supine and standing position) must be considered when the treatment effects are detected. Despite a low absolute reliability, the random error represents a limited portion of the total measurement variability amongst individuals, which indicates a solid relative reliability. Thus, differences between individuals are primarily caused by the true value of individuals.


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ACTN3 and ACE genotype affects muscular performance in response to high-speed power training in older Caucasian women

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The angiotensin-converting enzyme (ACE) and the alpha-actinin-3 (ACTN3) genes have been associated with power phenotypes and both have been suggested to influence skeletal muscle function in response to strength training (Lima et al. 2010). However, conclusions have been inconsistent across investigations. There is a paucity of research data concerning exercise training-induced adaptations in older population (Adams et al. 2000).

The purpose of this study was to investigate the possible associations between ACE I/D and ACTN3 (R/X) polymorphisms and maximum strength, power and muscle function in older Caucasian women (n=139; age= 62.5±8.1; ID:n=52; DD:n=52; II:n=35 and RX:n=54; RR:n=52; XX:n=33) and their adaptation during 12-weeks of high speed power training. Period of intervention consisted of 40% of one repetition maximum (1RM) to 75% and 3 sets 4–12 reps in countermovement jump (CMJ) (Pereira et al., 2012). Strength was measured dynamically in leg extension exercise (1RMLE), power was evaluated by CMJ and functional capacity was recorded by sit to stand test (STS). ACE I/D and ACTN3 R/X polymorphisms were determined by polymerase chain reaction. Significant differences were performed by ANOVA (means±SD). The trainingxgenotype effects were analyzed by repeated-measures ANOVA.

Whole body was independent of ACE and ACTN3 genotypes. At baseline no significant effects of both ACE and ACTN-3 genotype were found for all considered strength parameters. Over the 12-weeks training period, the subjects significantly increased maximum strength (62.9% in 1RMLE), lower limbs muscle power (30% in CMJ) and functional capacity (22.5% in STS test).

Genotype effect for ACE showed no statistically difference only in 1RMLE (P=0.187). But subjects with genotype DD had higher maximal strength than others after the high-speed power training. Although, genotype effect for ACTN3 showed significant effects for all measures: 1RMLE (p=0.011), CMJ (p=0.050) and STS (p=0.033). RR genotype exhibited a positive and a prevalence of strength (1RMLE: 33.1±6.4), power (CMJ: 0.164±0.02) and functional capacity (31.3±4.7) comparing to RX and XX genotypes.

The combined influence of ACE DD+ACTN3 RR vs. ACE II+ACTN3 XX polymorphisms was also studied. In response to high-speed power training, the results showed that the D-allele carriers and R-allele combination seems to induce significant increases but only for 1RMLE.

These data suggest that ACE genotype is not associated with muscle strength adaptation to high-speed power training. On the other hand, strength training response seems to be significantly affected by the presence of the ACTN-3 RR genotype alone or in combination with the ACE DD genotype in older Caucasian women's. The results provide a novel insight that these genetic variations may interact to determine muscle performance in older women.


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Left ventricular determinants of VO$_{2\max}$ in a heterogeneous group of healthy young males

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Aerobic training results in both increases in VO$_{2\max}$ as well as changes to cardiac structure and function. However, the degree to which changes in VO$_{2\max}$ are linked to changes in cardiac dimensions or to improvements in cardiac function is not clear. The purpose of this study was to establish which individual, or combination of, cardiac parameters best predict changes in VO$_{2\max}$. Seventy one young, healthy, male participants (22 ± 5 yrs; 1.79 ± 0.07 m; 79 ± 13 kg) volunteered to participate in the study, and represented a broad spectrum of cardiorespiratory fitness (athlete to sedentary). This facilitated the cross-sectional analysis of relationships between cardiorespiratory fitness with cardiac structure and function. Participants visited the laboratories on two separate occasions within 5 days. During the initial visit body composition, resting heart rate, resting blood pressure and echocardiographic examinations were performed. A maximal VO$_{2\max}$ ramp protocol was employed during a second visit. Echocardiographic assessment included structural measurements of interventricular septum wall thickness (IVS), left ventricular diameter (LVD) and posterior wall thickness (PW) in both systole and diastole, from M-mode images. Left ventricular (LV) function was assessed by transmitral Doppler, LV volumes, Ejection Fraction (EF), TDI and longitudinal peak strain, from the apical 4 chamber view. One tailed Pearson correlation coefficients were established between cardiac parameters and absolute VO$_{2\max}$, relative VO$_{2\max}$, and VO$_{2\max}$ relative to FFM. Four predictor variables were subsequently entered into a least-squares regression analysis. The strongest correlations with absolute VO$_{2\max}$ were for LVM ($r = 0.692$, $p < 0.01$) and SV ($r = 0.692$, $p < 0.01$) but the regression analysis found EDV, SV and LA to predict 65% of the variance in absolute VO$_{2\max}$ ($R = 0.81$, $F (3, 25) = 15.54$, $p < 0.01$). LVM/BM was the strongest correlate to relative VO$_{2\max}$ ($r = 0.684$, $p < 0.01$) and SV had the next strongest correlation ($r = 0.483$, $p < 0.01$), but was removed by the regression analyses due to collinearity, therefore LVM/BM was found to predict 47% of the variance in relative VO$_{2\max}$ ($R = 0.68$, $F (1, 28) = 24.67$, $p < 0.01$). SV was found to have the strongest correlation with VO$_{2\max}$ relative to FFM ($r = 0.605$, $p < 0.01$) and LVM/FFM had the next strongest correlation with VO$_{2\max}$ relative to FFM ($r = 0.598$, $p < 0.01$), however LVM/FFM was removed by the regression analyses finding SV and PWd to predict 52% of the variance in VO$_{2\max}$ relative to FFM ($R = 0.75$, $F (2, 22) = 13.76$, $p < 0.01$). The main findings of the current study were that cardiac structural measures were the strongest predictor of VO$_{2\max}$. These results suggest that increased cardiac size may make significant contributions to the concomitant increases in VO$_{2\max}$.

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Chronic intermittent hypoxia does not increase lipid peroxidation in rat diaphragm

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We have shown that chronic intermittent hypoxia – modelling human sleep apnoea – causes rat diaphragm muscle weakness and fatigue (1). Antioxidant treatment prevents the deleterious effects of CIH on diaphragm function. In the present study, we sought to determine if CIH causes oxidative stress in rat respiratory muscle.

Adult male Wistar rats were exposed to CIH consisting of 90s normoxia/90s hypoxia [5% oxygen at the nadir; SaO2 ~80%], for 8h/day or to sham treatment (air/air) for 2 weeks. Following gas treatments, we determined the concentration of thiobarbituric acid reactive substance (TBARS) in diaphragm homogenates as an index of oxidative stress using commercial kits. In addition, 10 μm transverse sections of diaphragm were prepared and immunohistochemically probed for the lipid peroxidation marker -4-HNE, using an indirect immunofluorescence approach. In separate sections, nuclei were tagged by Hoescht stain and probed for 8-OHgd – a sensitive marker of DNA oxidation.

CIH did not significantly increase diaphragm (1.8±0.6 vs. 3.7±1.4 μM MDA/mg protein; mean±SEM, n=6 for both groups, Student’s unpaired t test) or liver TBARS concentration. Moreover, there was no difference in 4-HNE labelling between sham and CIH diaphragm. Of interest, however, we noted evidence of DNA oxidation in 5 out of 6 CIH diaphragms.

Our previous studies suggest that the deleterious effect of CIH on respiratory muscle endurance is due to oxidative stress since antioxidants reverse CIH-induced respiratory muscle fatigue (1). The results of the present study indicate that there may be only mild oxidative stress in respiratory muscle in our model. Therefore, the mechanism underpinning the beneficial effects of antioxidant treatment warrants further investigation especially since we have shown that superoxide scavengers are powerful inotropic agents (2).


Supported by the Health Research Board (Ireland).

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Caffeine reduces perception of effort and movement-related cortical potential during submaximal isometric knee-extensor contractions

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The well-known ergogenic effect of caffeine on submaximal exercise performance is caused largely by a reduction in perception of effort (Doherty & Smith, 2005). However, the brain mechanisms underlying this effect are poorly understood. Perception of effort is thought to arise from corollary discharges of the central motor command to the active muscles (McCloskey et al., 1983), and there is evidence that movement-related cortical potential (MRCP) derived from the EEG is associated with central motor command (Siemionow et al., 2000; Jankelowitz & Colebatch, 2005), and perception of effort (Slobounov et al., 2004). Our aim was to investigate whether caffeine affects MRCP amplitude and rating of perceived effort (RPE) during submaximal isometric knee-extensor contractions, and whether caffeine affects brain activity related to movement planning and/or brain activity related to movement execution.

Twelve healthy, recreationally active women (age, mean 21 ± standard deviation 5 years, height 166 ± 7 cm, weight 67 ± 11 kg) performed 100 isometric knee-extensor contractions at 61 ± 5% of their maximal voluntary contraction torque 1.5 hours after caffeine (6 mg/kg) or alternative placebo ingestion, while RPE, vastus lateralis (VL) EMG, and MRCP were recorded. Conditions were presented in a randomly counterbalanced and double blind fashion. The effect of exercise duration was assessed by comparing the first 50 with the last 50 contractions. Two-way fully repeated measures ANOVAs (caffeine x exercise duration) showed that caffeine caused a significant reduction in RPE (caffeine 5.3 ± 1.7, placebo 5.9 ± 1.7; \( F_{1,11} = 8.51, p = 0.014, \eta^2_p = 0.44 \)), and that exercise duration caused a significant increase in RPE (first block 4.8 ± 1.7, second block 6.4 ± 1.6; \( F_{1,11} = 16.32, p = 0.002, \eta^2_p = 0.60 \)). Follow-up tests of the four-way fully repeated measures ANOVA (electrode x epoch x caffeine x exercise duration) for MRCP showed that MRCP amplitude at the vertex during the first second of movement execution was significantly decreased by caffeine (caffeine 12.1 ± 6.5 μV, placebo 16.2 ± 5.4 μV; \( t_{11} = -4.44, p = 0.001, \eta^2_p = 0.64 \)). A planned comparison showed a significant increase in the same MRCP component with exercise duration (first block 13.4 ± 6.0 μV, second block 14.9 ± 5.6 μV; \( t_{11} = 2.97, p = 0.013, \eta^2_p = 0.45 \)). Contraction torque and VL EMG were not affected by caffeine and exercise duration. These results demonstrate for the first time that the positive effect of caffeine on perception of effort is associated with a reduction in the central motor command required to produce the same motor neuron output and force output. Opposite effects were observed for exercise duration. Furthermore, we have shown that perception of effort is associated with brain activity related to movement execution, not movement planning.


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Effects of lactate on the voltage-gated sodium channels of skeletal muscle: modulating the current opinion

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Introduction: Muscle production of lactate and hydrogen ions increase during exercise. Major attention has been directed at elucidating the consequences of the latter (e.g. acidity) on muscle function and especially excitability (Pedersen et al., 2004). Conversely, few attempts were made to look for possible effects of lactate ion on muscle excitability. Voltage-gated sodium channels (Nav) initiate and convey the action potential on fibre membrane, thereby fine-tuning muscle excitability. We hypothesized that lactate could modulate the electrophysiological properties of muscle Nav.

Methods: The electrophysiological properties of muscle Nav were studied in the absence and in the presence of lactate by using the macro-patch-clamp method in dissociated fibres from rat Peroneus Longus (PL). Two different pipettes containing either a control or a lactate (10 mM) solution were sealed on the same area of PL fibers. Values are shown as means ± SEM.

Results & Discussion: Compared with control condition, lactate increases the maximal sodium current (14.5 ± 1.2 vs. 10.1 ± 1.4 A.mm-2, n = 18 fibres, p = 0.022, Student’s paired t-test), while the voltage-dependence of activation (normalized conductance) is shifted at its midpoint by 12.4 mV toward the hyperpolarized potentials (p = 0.0029, Student’s paired t-test). This indicates a more rapid depolarization, allowing an earlier recruitment of the muscle fiber. The voltage-dependence of Nav fast inactivation is shifted by lactate in a hyperpolarizing direction compared with control condition (-63.1 ± 3.7 vs. -52.1 ± 2.4 mV, respectively, n = 18 fibres, p = 0.015, Student’s paired t-test). This implies a more rapid membrane repolarisation which is crucial for the elicitation of a novel action potential. Lactate induces a leftward shift in the relationship between the kinetic parameters and the imposed potentials, resulting in an acceleration of Nav activation. The slow inactivation process is decreased by lactate compared with control condition as shown by the greater residual current INa min/INa max (0.67 ± 0.06 vs. 0.48 ± 0.05, respectively, n = 15 fibres, p = 0.0099, Student’s paired t-test), corresponding to an enhancement in the number of excitable Nav. When lactate was only added in the Petri dish (and not in the pipette), the electrophysiological properties of Nav were unaffected. Thus, the modifications of sodium current properties are observed when the pipette contains lactate, indicating an extra-cellular pathway.

Conclusion: Lactate ion modulates the electrophysiological properties of muscle Nav by an extra-cellular manner. These modifications of Nav characteristics are consistent with an increase in muscle excitability. This leads to preserve force production by reducing the muscle fatigability related to membrane excitability failure (Cairns et al., 2003; Karelis et al, 2004; Fitts, 1994).

Figure 1. Effects of lactate on muscle sodium currents

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Comparison of somatotypic components of Czech and Portuguese male and female students of Physical Education

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Somatotype is one of the basic classifications of physical characteristic and body type. Three components were identified in the classical anthropometric somatotype Method of Heat and Carter: relative fatness (endomorphy), musculoskeletal component (mesomorphy), and linearity (ectomorphy). As Grasgruber states (2008), the average somatotype of male European population is largely based on study of University students and is around 3.5-4.5-3.0. Pavlik’s measurements (2003) are not different; his average somatotype of students is 4.4-3. The aim of this study was to achieve a somatotypological comparison of male and female Physical Education students.

Physical parameters of students were measured using standardized anthropometrical methods and somatotypes were counted in line with the Heath-Carter Somatotype Method. A total 246 students (153 males and 93 females) participated in this study. Participants were students of the Faculty of Sport in the Czech Republic and Portugal. 63 Czech men (20-22 years old, 181.4±6.8cm, 77.3±9.4kg, BMI 23.9±2.7), 90 Portuguese men (18-21 years old, 176.1±6.2cm, 74.7±9.6kg, BMI 24.3±2.8), 58 Czech Women (20-22 years old, 169.0±6.7cm, 63.0±7.2kg, BMI 22.0±1.9), 35 Portuguese women (18-21 years old, 163.2±6.8cm, 55.3±8.5kg, BMI 20.6±2.2). To determine the differences in somatotype between the Czech and Portugal male and female students, Shapiro-Wilk W test was used. The level of significance was set to 0.05. Mean female Czech students somatotype was classified as Central type (Balanced somatotype) – (3.2±0.8-3.6±1.2-3.0±1.0), while a Balanced endomorph was registered for Portuguese female students (4.4±1.1-3.0±0.9-3.3±1.1). Mesomorphy component was higher in Czech female students than in Portuguese females students (p=0.037), endomorphy component was higher in Portuguese female than Czech female students (p=0.000) and ectomorphy component was statically non-significant between our two groups (p=0.162). Mean male Czech students somatotype was classified as ectomorphic mesomorph (2.2±0.8-4.7±1.2-3.1±0.9), while a mesomorph-endomorph was registered for Portuguese male students (3.9±1.4-4.2±1.2-3.1±0.9), while a mesomorph-endomorph was registered for Portuguese male students (3.9±1.4-4.2±1.2-3.1±0.9), while a mesomorph-endomorph was registered for Portuguese male students (3.9±1.4-4.2±1.2-3.1±0.9). Mesomorphy component was higher in Czech male students than in Portuguese males students (p<0.05), an endomorphy component was higher in Portuguese male than Czech male Students (p<0.05) and an ectomorphy component was higher in Czech male students than Portuguese students (p<0.05). Significant somatotypic differences between body structure of Czech and Portuguese male and female students of Faculties of Sport Studies. Whether these differences are caused by more theoretical bachelor programs, by differences in attitude towards sport in central and south-west Europe or by other influences.


Authors would like to thank students who participated in this study for their valuable contribution.

Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.
Effectiveness of strength (high repetition-moderate load) training program in normobaric intermittent hypoxia to increase cardiac reserve and performance


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Introduction: Intermittent hypoxic training (IHT) has been used as a method of high level trained athletes in an attempt to improve their sport performance through higher-red cell maintenance and other adaptive physiological mechanisms induced at peripheral level to transport nutrients and oxygen (Hoppeler, 2008). For repeated long-term endurance exercises the parasympathetic tone usually increases with a decrease in resting heart rate (HRrest) and maximum (HRmx), the latter being a constraint for maximum performance. This adaptation justifies greater efficiency and positive response unless the HRmx decreases. Hypoxic states induce an increase in sympathetic-adrenal activation of glycolysis (Calbet, 2009) and a possible increase in HRmx (Gonzalez, 1998) that would justify an increase in maximal oxygen uptake (VO2max).

Aim: To assess whether an IHT program is able to increase cardiac reserve and improve non-specific performance.

Material and Methods: 22 firefighters (age: 33.7 ± 5.5 years, height: 179 ± 4 cm, weight: 81.3 ± 4.2 kg) divided into 2 groups: hypoxia (H) (n = 11) and control group (C) (n = 11).

All of them underwent 4 sessions/week for two months, 60 minutes strength training (6x5 exercises of 30 repetitions; repetition rate 1/1 second) plus 30 minutes of interval exercise bike (3 minutes effort plus 2 minutes recover), for 8 weeks. The H group trained at 4000-5500m simulated altitude (first month: 4000-4500m; the second month: 5000-5500m) with normobaric hypoxia system (GO2Altitude) and group C trained in normoxia. Maximal test was performed in Concept-II rowing ergometer for 3 minutes before and after the intervention to obtain HRmx. The HRrest was measured lying in bed for 5 minutes at first time in the morning after waking and before breakfast. Cardiac reserve (CR = HRmx - HRrest) was measured before and after the intervention. Values are means ± S.E.M., compared by ANOVA.

Results: The CR increased in the H group (130 ± 3 ppm vs. 139 ± 5 ppm, p<0.022) compared with C group (132 ± 4 ppm vs. 135 ± 3 ppm, p=0.071) after the intervention. The H group improved performance in rowing ergometer (827.30 ± 8.25 m vs. 889.18 ± 9.79 m, p<0.019) compared with C group (868.11 ± 6.35 m vs. 879.30 ± 10.72 m, p=0.057).

Conclusions: A IHT training program of 8 weeks between 4000-5500m increases HRmx and CR and final meters made in 3 minutes rowing ergometer (non-specific test). Increased non-specific enhancement of athletic performance may be justified by the increase in HRmx and CR. These stimuli can be interesting to decrease HRmx in periods of high volume training in endurance athletes.


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Relationship between ventilatory function and age in master athletes and a sedentary reference population

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Background: Ageing is accompanied with a decline in respiratory function. It is hypothesised that this may be attenuated by high physical activity levels.

Methods: We performed spirometry in master athletes (71 women; 84 men; 35-86 years) and sedentary people (39 women; 45 men; 24-82 years) and calculated the predicted lung age (PLA).

Results: In Figure 1 it can be seen that the negative associations of age with forced expiratory volume in 1 s (FEV1) (34 mL/yr) and other ventilatory parameters were similar in controls and master athletes. FEV1pred was 9% higher (P < 0.005) and PLA 15% lower (P = 0.013) in athletes than controls. There were no significant differences between endurance and power athletes and sedentary people in maximal inspiratory and expiratory pressure. Neither age graded performance nor weekly training hours were significantly related to lung age.

Conclusion: Life-long exercise does not appear to attenuate the age-related decrease in ventilatory function. The better respiratory function in master athletes than age-matched sedentary people might be due to self-selection and attrition bias.

Figure 1: Individual data for Forced expiratory volume in 1 second (FEV1); — men: FEV1 = -0.034 * age (yrs) + 5.54; R² = 0.47; P < 0.001; — women: FEV1 = -0.033 * age (yrs) + 4.56; R² = 0.61; P < 0.001. Open squares: Female endurance; Open circles: Female Power; Open triangles: Female Control; Closed squares: Male Endurance; Closed circles: Male Power and Closed triangle: Male Control participants.

We appreciate the financial support from Strattec Company (Pforzheim, Germany) to perform oxygen diffusion measurements. We appreciate the support by Kurt Kaschke, Dieter Massin, Winston Thomas and Bridget Cushen as representatives from WMA, EVAA and BMAF. We are grateful to the participants – without their contribution this study would not have been possible.

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Translating systems biology of elite athletes from the laboratory to the sports clinic and arena

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In sports science, sports medicine and rehabilitation of sports injury, the nascent fields of exercise ‘omics’ (metabolomics and proteomics) encompass the identification, characterisation, and quantification of the metabolite and protein content of whole cells, tissues, or body fluids (1). The potential for ‘omics’ technology holds great promise for talent identification, optimising elite athlete training, avoiding training addiction and overuse injury and policing fair play. Beyond the currently characterised biochemical pathways of cardiac, muscle and kidney function lies the realm of neuroscience interfaced with the psychological aspects of optimised athletic output. Exploring this untapped systems biology hyperspace is limited by current analytical technologies and requires innovative research. This is driven by the ability to identify and quantify novel saliva, sweat, plasma and urine analytes that can function as biomarkers for sculpting elite sports performance. Toxicology evidence of licit enhancers (nutritional supplements and painkillers to mask injury) or illicit enhancers (doping) may simultaneously be monitored (1,2). However, there are many challenges in translating ‘omics’ from the sports research laboratory to the sporting clinic and arena, and relatively few novel biomarkers have successfully transitioned from discovery to routine use in training. Key barriers to this translation include the range and complexity of the biological samples, a preference for minimally invasive sampling during exercise, the need for “orthogonal” biomarkers (i.e., uncorrelated with existing markers), the presence of high abundance analytes in biological samples that hamper detection of novel low abundance analytes, false positive associations that occur with analysis of high dimensional datasets, lack of routine mobile biosensor devices and the limited understanding of the effects on performance of coaching, differential training regimes, age-related development and performance anxiety. State-of-the-art analytical technologies developed by us (1, 2) focusing on nuclear magnetic resonance (NMR) spectroscopy and associated strategies to overcome these challenges are discussed.


University of East London, School of Health, Sport and Bioscience PhD scholarship (AL)

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Inspiratory muscle fatigue as an exercise limiting factor

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Recent studies of cycling time trials (TT) suggest the existence of a threshold for quadriceps muscle fatigue being regulated via feedback from type 3 and 4 afferents of working muscles. In order to avoid surpassing this threshold of fatigue, feedback from working limbs is suggested to directly influence the regulation of exercise intensity (1). Since the diaphragm is also susceptible to fatigue during high-intensity whole-body exercise and owing to its vital role, one would expect it to be even more protected against excessive fatigue. Thus, we hypothesised that a threshold for diaphragm fatigue existed similar to that of the quadriceps muscle. To test this, we assessed diaphragm contractility via transdiaphragmatic twitch pressure (Pdi,tw) in response to cervical magnetic stimulation prior to and following 15- and 30-min running TTs. Additionally, we assessed mouth twitch pressures (Pm,tw) prior to and following exhaustive volitional normocapnic hyperpnoea in 7 well-trained healthy runners with normal lung function (age±SD: 31±5 yrs; VO2max: 65.9±3.9 ml/min/kg; FVC: 6.41±0.38 litre; FEV1: 4.83±0.33 litre). Well-trained endurance athletes were investigated for the reason that they are more likely to reach the potential limit of their respiratory system than healthy sedentary subjects due to the specificity of endurance training which mainly increases the capacity of the cardiovascular system and the skeletal muscles to transport and utilize oxygen with little effect on the functional capacity of the respiratory system (2). Contrary to the hypothesis, reductions in Pdi,tw and oesophageal twitch pressure (Poes,tw, a measure for global inspiratory muscle fatigue) were significantly larger (student’s t test, p<0.05) after the 15TT (Pdi,tw: -25.4±6.9%, Poes,tw: -25.8±7.5%) compared to the 30TT (-19.2±9.9%, -13.8±12.1%) thus not implicating a threshold of diaphragmatic or global inspiratory muscle fatigue during TTs. The observed difference in inspiratory muscle fatigue is likely the result of the significantly higher exercise intensity in the 15TT (91±1% VO2max) compared to the 30TT (86±4% VO2max) resulting in a higher ventilation during the 15TT (~9%, p<0.05) and thus greater inspiratory muscle work, i.e. inspiratory diaphragmatic and oesophageal pressure-time-product (PTPdi: ~23%, p<0.05; PTPoes: ~16%, p=0.07) and work of breathing (~26%, p<0.05). Moreover, global inspiratory muscle fatigue was significantly larger after exhaustive volitional hyperpnoea (decrease in Pm,tw: -34±9.4%) compared to Poes,tw after either TT. In summary, we conclude that the high level of ventilation achieved during whole-body exercise does not stress the inspiratory muscles to the point where a threshold of fatigue is reached. Thus, to which extent the level of inspiratory muscle fatigue contributes to exercise limitation, needs further investigation.


Support by Swiss Office of Sports (grant no. 11-11).

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Use of portable Near Infrared Spectroscopy to measure muscle oxygenation and haemodynamics during sports performance

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The recent development of reliable portable Near Infrared Spectroscopy (NIRS) devices presents the opportunity to non-invasively measure muscle oxygenation and blood flow in-vivo during simulated competition. In short-track speed skating, there is a high anaerobic component (greatly elevated post-race blood lactate concentrations [1]), and high degree of quadriceps deoxygenation [2, 3] due to the sustained nature of muscle contraction, and the ischemic effects of a high level of hip flexion. The local metabolic and haemodynamic response to a short-track race have previously been reported [4], but the effects at local muscle level of skating at different velocities, and using different techniques, are as yet unknown. Subjects were 10 elite short-track speed skaters (6 male, 4 female). Portable wireless NIRS devices (Portamon) were attached to the right and left vastus lateralis (VL) of each subject prior to completion of race simulation time trials (TT). Study design was a randomised crossover; each subject completed TTs over 3 distances: 500m (4.5 laps); 1000m (9 laps); and 1500m (1500m), separated by a minimum of 24 hours. During TT, data relating to Tissue Saturation Index (TSI), and changes in total concentration of haemoglobin (measure of blood volume; considered an indirect measure of blood flow) in the respective muscles was collected at a frequency of 10 Hz. Video recordings of each TT were synchronized with the NIRS-derived data, to permit analysis of blood volume and TSI% changes over the course of individual laps. Global oxygen consumption (VO2) and blood lactate concentration were also monitored. Post-1000m blood lactate was significantly higher than post-500m blood lactate (9.76 ± 0.54 mmol•l⁻¹ v 6.77 ± 0.61 mmol•l⁻¹, P=0.005) in males, but not in females. Peak VO2 was significantly higher during lap 1 of 500m than lap 1 of 1500m (49.04 ± 5.35 ml•kg⁻¹•min⁻¹ v 31.56 ± 5.24 ml•kg⁻¹•min⁻¹, P<0.05). Race distance did not affect magnitude of maximal TSI reduction, in either leg. However, the pattern of blood volume changes in both right and left VL during one lap was shown to be affected by race distance, and therefore mean velocity (Fig 1). Video analysis showed that the differences in the profile of blood volume changes across the three race distances were caused by different techniques employed during cornering when skating at different velocities. The data presented here show that portable NIRS technology has the required temporal resolution to accurately monitor changes in O2 and blood volume in the working muscle during dynamic exercise. This could have a wide range of uses within elite sport, by increasing understanding of the local metabolic effects of changes in technique and velocity.

Figure 1: NIRS-detected changes in blood volume in the right and left vastus lateralis of a representative subject during one lap (lap 3) of race simulations over three distances


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Effect of resistance training combined with normobaric hypoxia in elite athletes

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Low intensity (<50% 1RM) resistance training combined with vascular occlusion induces similar increases in muscle mass and strength as high intensity (>70% 1RM) resistance training (1-2). Muscle hypoxia and metabolite accumulation appear to be important antecedents to these adaptations (3). Hypoxic interventions may provide a similar stimulus without the risk of thrombosis and the limited limb movement range and velocity associated with vascular occlusion. Certainly, recent studies of the acute and short-term responses of resistance training in environmental hypoxia have elicited improved markers of muscle growth and enhanced strength development (4, 5). Therefore the aim of this study was to examine the effects of resistance training combined with normobaric hypoxia using a single-blind randomized controlled trial design. Highly trained male rowers (n = 21) were randomly assigned into either a hypoxic (H) or normoxic (N) group. Maximal strength (1RM Bench and Leg press) and indices of muscular power (counter movement jumps (CMJ) and squat jumps (SJ)) were assessed before and after a three-week resistance training programme. The resistance exercise programme consisted of five exercises (Squat, Bench, Leg and Shoulder Press, Deadlift). The final exercise and a further 60 min seated rest were completed inspiring hypoxic (FIO2 = 13%) or normoxic (FIO2 = 20.9%) air. Salivary and capillary blood samples were collected pre and for 4 x 15 min intervals post training. Saliva was analysed for free testosterone and cortisol concentration and capillary blood samples were analysed for lactate concentration. Maximal strength increased in both groups (Leg press H: 12.2±6.0%; N: 10.1±7.2%, Bench press H: 10.7±7.6 %; N: 8.5±8.0 %) after the training period (p<0.001) but no significant differences were found between the two groups for maximal strength or power. Trends emerged indicating increases in muscular power in H compared to decreases in N (CMJ H: 2.8±9.8 %; N: -0.7±3.1 %; SJ H: 6.2±13.1 %; N: -2.0±5.2 %). There were no differences in salivary testosterone, cortisol or blood lactate concentration in the post exercise period between hypoxic or normoxic groups. Both groups had a significant increase in testosterone:cortisol ratio by week 3. Contrary to previous research, these findings suggest that in well-trained athletes hypoxia does not enhance the resistance training induced gains in maximal strength or power; nor does there seem to be any augmentation of the metabolic or hormonal responses to training.

Fig 1. Testosterone:Cortisol Ratio measured Area Under the Curve. *=Significant difference between week 3 and week 1 in both groups (p<0.05)


We would like to acknowledge The Altitude Centre PLC for supplying the hypoxicators at no charge.

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Popliteal artery modifications to low load plantar flexion training with blood flow restriction

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In addition to the established increases in muscle size and strength, low load resistance training with blood flow restriction (BFR) may cause remodelling of the peripheral vasculature, as demonstrated by increases in conduit artery maximal diameter (Hunt et al. 2011) and reactive hyperaemic blood flow (Patterson & Ferguson, 2010). However, due to a lack of longitudinal measures it is unknown if functional changes precede these structural adaptations (Tinken et al. 2008). To gain insight we examined the effects of six weeks low load dynamic plantar flexion training with BFR on conduit artery function and structural capacity. With ethics committee approval six male participants (24 ± 4 yrs, 178.0 ± 3.2 cm, 78.7 ± 9.4 kg) performed 3 sets of unilateral plantar flexion exercise to volitional fatigue (3 days/week), at 30% of 1 repetition maximum, with a pneumatic cuff inflated at 110mmHg on their upper thigh. The contralateral leg (assigned in a counterbalanced manner to the dominant or non-dominant leg) was used as a non-exercised control (CON). The popliteal artery (PA), of both legs, was examined using Doppler ultrasound at 2-week intervals throughout the 6-week intervention. Artery diameter and flow velocity were measured at rest and following 5-mins of ischemia (peak diameter) and ischemic exercise (maximal diameter) to determine flow mediated dilation (FMD) and dilatory capacity (DC). Values are means ± SD, compared by repeated measures ANOVA. A priori sample contrasts with week 0 as the reference was used and cohen’s d effect size stated. There were no changes to resting PA parameters in either leg. In the BFR leg, FMD appeared to increase from baseline (6.1 ± 1.8%) at week 2 (8.0 ± 3.7%; t-test, P=0.068, d=1.14) before decreasing at week 4 & 6 (6.8 ± 2.5%, 6.3 ± 1.9%, respectively) but this was not significant (ANOVA, P=0.101). No change in FMD was observed in the CON leg (5.8 ± 1.4% vs. 5.7 ± 1.0% vs. 5.5 ± 1.0% vs. 5.9 ± 1.1%, week 0, 2, 4 and 6, respectively, ANOVA, P=0.567). Maximal diameter increased in the BFR but not the CON leg (Two-way ANOVA condition x time interaction, P=0.021). However, the increase in maximal diameter from baseline (5.95 ± 0.35mm) at week 2, 4 & 6 (6.04 ± 0.34mm, 6.04 ± 0.33mm, 6.19 ± 0.37mm, respectively) was not significant (ANOVA, P=0.068). There were no changes in peak diameter, shear rate stimulus and DC in either leg. Trends of enhanced FMD and maximal diameter in the BFR leg were masked by a heterogeneous response. Although these trends fit with established models of vascular adaptation (Tinken et al. 2008) a larger sample size is required to verify if dynamic low load resistance training with BFR induces complimentary adaptations in arterial function and structure.


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Separating central from peripheral determinants of muscle fatigue and exhaustion

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Muscle fatigue can be assessed as i) the reduction in the maximal voluntary contraction force (MVC) or ii) the inability to sustain a required level of force, i.e. endurance time (ET) of a sustained submaximal contraction until voluntary exhaustion. We tested the hypothesis that considering one or the other index may lead to different conclusions. In a first set of experiments fourteen men (28 ± 2 yrs) isometrically contracted their dominant leg knee extensor muscles at 20% of their MVC until voluntary exhaustion (ET1). At task failure, the knee extensors were electrically stimulated for 1 min with surface electrodes (40 Hz) aiming to develop the same 20% MVC target force that was not possible to develop voluntarily anymore. Potentiated doublets (100 Hz) were evoked by supramaximal stimulation of the femoral nerve before and immediately after ET1 to assess peripheral fatigue. Values are means ± S.E.M., compared by ANOVA. After task failure of ET1 (246 ± 18 s), all subjects developed 20% MVC under electrical stimulation. MVC was decreased (p<0.05) by 51 ± 3% after ET1. Potentiated peak doublet was impaired after ET1 (-37 ± 4%, p<0.001) and a trend (p=0.06) towards significant correlation was observed between this reduction and the MVC decrease. In another set of experiments, thirteen men (25 ± 2 yrs) sustained 50% MVC until voluntary exhaustion with four different muscle groups (knee extensors, plantar flexors, elbow flexors and thumb adductor, dominant side) on separate occasions and MVC was measured before and immediately after exercise. ETs varied significantly (knee extensors: 77 ± 25 s; plantar flexors: 221 ± 64 s; elbow flexors: 72 ± 14 s; thumb adductor: 114 ± 27 s, p<0.05) but MVC loss (~30-40%) was similar (p>0.05) for all muscle groups. Results of the first experiment suggest that although the large peripheral impairment seems to be associated with the reduction in maximal force generating capacity, the maximum duration of a sustained submaximal isometric knee extension performed at 20% MVC is not limited by extensor muscle force generating capacity but rather to mechanisms located above the neuromuscular junction, presumably involving descending motor drive. The results from the second experiment suggest that the MVC loss at task failure is independent of ET. Collectively, these results suggest that maximal voluntary force generating capacity and ET are two distinct indexes giving different insights into the process of muscle fatigue.

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Increase in maximal oxygen uptake in the fight against inactivity?

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Cross-sectional studies have previously shown that there is a positive association between maximal oxygen uptake (VO2max) and non-exercise activity thermogenesis (NEAT). However data on how an increase in VO2max affects NEAT or total daily energy expenditure in a sedentary population are scarce. The primary aim of this study was to investigate the effect of 6 weeks of high intensity interval training on VO2max and daily total energy expenditure in initially sedentary male subjects of normal weight. Thirty healthy sedentary males (39.1 ± 5.9 yrs and BMI 25.5 ± 2.6 kg/m²) performed aerobic endurance training on treadmills three times per week for a total of six weeks. The subjects were randomized to one of the following protocols: one interval (4 minutes) of high intensity training (HIIT-1) performed at 85-95% of maximal heart rate (HRmax), four intervals (each lasting 4 minutes) of high intensity training (HIIT-4) performed at 85-95% HRmax and a control group performing moderate continuous training (47 minutes) at 70% HRmax (MCT). Daily total energy expenditure (TEE), number of steps (Senswear, armband), VO2max and body composition were measured before and after the intervention. Values are Means±SD compared by ANCOVA for differences between groups and paired sample t test for within group differences. There was a significant increase in VO2max after HIIT-1 (from 44.5 ± 6.6 mLxkg⁻¹xmin⁻¹ to 47.7 ± 7.7 mLxkg⁻¹xmin⁻¹, p<0.05) and HIIT-4 (from 43.1 ± 5.4 mLxkg⁻¹xmin⁻¹ to 47.1 ± 5.7 mLxkg⁻¹xmin⁻¹,p<0.05) with no change in the MCT group. No change in daily energy expenditure or number of steps was observed after HIIT-1 and HIIT-4. However, daily total energy expenditure was increased by 14% after MCT (from 2557 ± 312 calories to 2921 ± 394 calories). Six weeks of HIIT-4 induced a significant decrease in body fat (from 22.4 ± 8.4 to 20.7 ± 8.4 after, p< 0.05). In the initial phase of structured exercise training, an increase in VO2max has no effect on daily activity level in untrained men. Importantly, this study shows that short bouts of high intensity interval training may be just as effective as longer interval training to increase VO2max for sedentary middle-aged men.

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Elevations of tendon-related markers after different velocities of lengthening contractions in rat

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Although muscle strain injury is one of most important issues in sports medicine, there is little known about the molecular events on regeneration process and embryonic formation in vivo injured muscle. The purpose of this study was to examine the effects of lengthening contractions (LCs) on tendon-related markers signaling pathways. We hypothesized that tendon-related markers are important role for the recovery from muscle injury. We employed our originally developed device with two LC modes to modulate the intensity in rat gastrocnemius muscle (1, 2). Since we reported one bout of LCs causes decreases in muscle torque and increase protein degradation signaling pathway and myostatin (3), we hypothesized that LCs cause an increase in fibrosis concomitant with activations of tendon-related markers. Male Wistar rats (n = 18) were randomly divided into fast velocity LCs group (FAST, 180°/s, n = 6), slow LCs group (SLOW, 30°/s, n = 6), and control group (control, n = 6). The FAST and SLOW rats were anesthetized with isoflurane (gas flow rate, 450ml/min, concentration, 2.0%). The triceps surae muscle of the right hindlimb was then electrically stimulated with forced isokinetic dorsi-flexion (30°/s and from 0 to 45°). Tissue contents and localizations of tenomodulin, scleraxis, and myostatin were measured by western blotting and immunochemistry. The mRNA expression of type I collagen alpha 2 (col1a2) and mohawk was evaluated using real time reverse transcriptase-polymerase chain reaction. One-way ANOVA was used to compare the body mass, muscle mass, protein and mRNA analysis. No significant changes were observed in both body mass and hindlimb muscles between three groups. The torque was significantly lower in FAST than in SLOW (day2; 59.9±17.2 vs. 101.5±22.9 mNm, P < 0.01). Tenomodulin and myostatin and col1a2 mRNA showed significantly enhanced expression in FAST than in the other two groups (tenomodulin; ∼2.5 fold, P < 0.01, myostatin; ∼3.8 fold, P < 0.01, col1a2; ∼8.5 fold, P < 0.05). Immunohistochemical staining in FAST, but not in SLOW, was mainly localized in connective tissues between muscle fibers. On the other hands, scleraxis and mohawk mRNA in SLOW was significantly higher than that in control (scleraxis; ∼2.7 fold, P < 0.01, mohawk; ∼6.7 fold, P < 0.05). We conclude that fast LCs cause an increase in connective tissue fibrosis through the activated myostatin signaling pathway. In addition, the present results suggest that the severity of LCs-induced damage cause different expressions of tendon-related markers.


This study was supported by a Grant-in-Aid for Young Scientists (B; 21700663) from KAKENHI.

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Effects of exercise-induced hyperthermia and neck cooling on cognitive performance

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Information on the effects of heat stress on cognitive function remains largely equivocal despite substantial number of studies conducted in the area (Gaoua, 2010). Recent evidence has shown that cooling the neck region can enhance endurance performance (Tyler et al., 2010; Tyler and Sunderland, 2011). There is however limited information about the efficacy of cooling the neck region on cognitive performance. The objectives of this study were to assess the effect of exercise-induced hyperthermia and the efficacy of a neck cooling collar on cognitive performance. Prior to the experimental trials, 12 healthy males (age: 24±2 (mean±SD) years; peak oxygen uptake: 59±5 ml/min/kg) undertook three practices of a battery of five cognitive tests (symbol digit matching, search and memory, digit span, choice reaction time and psychomotor vigilance) at different days and a full familiarisation trial. Following which they completed two experimental trials (no-collar: NC and cooling collar: CC) separated by at least a week using a counter-balanced design. On each of these trials, they were required to run on a treadmill at 70% of their peak oxygen uptake for 75 min under a warm and humid condition (dry bulb temperature: 30.2±0.3°C, relative humidity: 71±2%). Cognitive performance was assessed before and after the exercise. Body core temperature, neck and mean skin temperatures (chest, arm, thigh and calf), heart rate, rating of perceived exertion and thermal sensation were measured. Body core temperature was substantially elevated at the end of the runs (NC: 39.5±0.4 vs. CC: 39.6±0.3°C, p>0.05). Neck temperature was lower in the CC trial (26.0±3.6°C) than in the NC trial (36.0±6.0°C, p<0.001). Neck cooling had no effects on other physiological and subjective responses (p>0.05). Exercise-induced hyperthermia improved mean reaction time in the Symbol Digit Matching test (pre: 1624±79 vs. post: 1490±232 ms, p<0.05). After the run, maximum span was increased in the Digit Span test (pre: 10±2 vs. post: 11±2, p=0.05) and median reaction time was improved in the Psychomotor Vigilance test (pre: 262±55 vs. post: 244±46 ms, p<0.05). Exercise-induced hyperthermia had no effects on the Search and Memory and Reaction Time Choice tests (p>0.05). Application of a neck cooling collar reduced the number of search errors made in level 3 of the Search and Memory test (NC: 0±1 vs. CC:-1±2; p<0.05). Wearing a cooling collar had no effects on the other tests (p>0.05). These results suggest that, with prior practices of cognitive tests, exercise-induced hyperthermia can improve memory and behavioural alertness. The benefits of wearing a neck cooling collar may only occur during tasks of higher complexity.


The authors express their gratitude to all participants in this study. We would also like to thank Ms Teo Ya Shi, Ms Jacinta Yeo, Ms Amanda Nio and Mr David Fun for their roles in data collection.

Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.
Effect of four weeks of calcium supplementation on plasma non-esterified fatty acids during a twenty five mile cycling time trial

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High calcium diets have shown to markedly inhibit lipogenesis (Xue et al., 1998) and accelerate lipolysis (Zemel et al., 2000). An in-vitro model suggesting the role of cAMP and phosphodiesterase 3B (Xue et al., 2001) has been implicated in the relationship between calcium and lipolysis. Recently the role of calcium sensing receptor in adipocytes has been shown to impact lipolysis via a similar mechanism (Youghan, et al., 2011). Increased lipolysis during endurance exercise may improve the availability of fat as an energy substrate, thus sparing glycogen.

Therefore the objective of the current research was to investigate the effect of calcium supplementation on the availability of non-esterified fatty acids (NEFA) during a cycling time trial.

Ten male well-trained cyclists (mean ± SD; age 35.8 ± 11.3 yrs, stature 176 ± 7.1 cm, body mass 73.8 ± 9.3 kg, O2peak 4.59 ± 0.8 l/min, Wpeak 350 ± 42 W) were recruited. Participants were tested using a randomised, single blind, test-retest intervention trial design. Each participant was given 1000 mg/d of elemental calcium (citrate) in tablet form for 4 weeks. They undertook a 25 mile bicycle ergometer time trial test at baseline (25TTB) and end of the intervention period (25TTC). A stationary electromagnetically braked ergometer (SRM ergometer; Schoberer Rad Messtechnik, Jülich, Germany) was used. Plasma NEFA concentration was determined using a NEFA C test kit (WAKO Chemicals GmbH, Neuss, Germany). Plasma NEFA was measured at rest, start and thereafter every 20 minutes till the end of the exercise. Descriptive data and all related analysis of significance were generated using PASW (Predictive Analytics SoftWare) Statistics Version 17 (SPSS Inc., Illinois, USA). Significance level was set at 95% confidence intervals (CI) (p < 0.05). Data are represented as measures of centrality and spread (mean ± standard deviation (SD). Main effect for time, main effect for trials and interaction between trials*time was used to detect statistical significance at each time point and across each trial respectively. Magnitudes of inferences were calculated using Cohen’s d.

There was no statistically significant difference in appearance of NEFA in plasma between the two trials. However, the effect size of the changes in the plasma NEFA was large at the start of the exercise (d = 0.70) and moderate at rest (d = 0.46), 20 (d = 0.31), 40 (d = 0.30) and 60 (d = 0.47) min during exercise and small at the finish of the time trial (d = 0.07). The results indicate that calcium supplementation in well trained athletes may effect lipolysis during endurance exercise. A larger sample is needed to detect any statistically significant changes in the appearance of NEFA in the plasma if a recommendation to use calcium as an ergogenic aid in endurance sport is to be made.

![Graph showing Mean (± SD) NEFA during the twenty five mile time trial before (25TTC1) and after (25TTC2) calcium supplementation (n = 10).](image_url)


Youghan He. et al. (2011) Biochemical and Biophysical research communication, 404 (1): 393-399.


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Acute oral tyrosine administration does not improve exercise performance in the heat in man

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Acute oral administration of the catecholamine precursor tyrosine is associated with increased exercise capacity in the heat. It is unclear whether exercise performance in the heat is improved following acute tyrosine administration. To explore this possibility, seven male endurance-trained volunteers [median age, 20 (range 19 - 45) years; mean stature, 1.82 ± 0.05 (SD) m; body mass, 77.9 ± 11.7 kg; VO2peak, 4.6 ± 0.6 l min⁻¹], unacclimated to exercise in the heat, performed two tests in a randomised crossover fashion, separated by at least 7 days. Following familiarisation, subjects were assigned in a double-blind fashion, 500 ml of sugar-free lemon and lime flavoured water with 150 mg kg body mass⁻¹ tyrosine (TYR), or the same volume of sugar-free flavoured water with an isocaloric quantity of hydrolysed whey powder (Whey). After 1 h subjects cycled at a constant exercise intensity (57 ± 4% VO2peak) for 60 min then performed a simulated cycling time trial (TT), requiring the completion of an individualised set work amount (393.1 ± 39.8 kJ) as quickly as possible, in 30°C and 60% relative humidity. Normally distributed data were analysed using repeated measures (time × trial) analysis of variance (ANOVA), and post hoc paired Student’s t-tests with the Bonferroni correction. Data not normally distributed were analysed using Friedman’s test and post hoc Wilcoxon matched-pairs tests. The plasma ratio of tyrosine + phenylalanine:Σ(free tryptophan, leucine, isoleucine, valine, methionine, threonine, lysine) exhibited an interaction (P < 0.001; ANOVA, n = 7), had increased over 2.5-fold at pre-exercise compared to rest with TYR (P < 0.001; n = 7), and remained elevated from rest throughout 60 min of submaximal exercise and at end of TT (P < 0.001 in all cases; n = 7), whereas it had declined in Whey at pre-exercise (P = 0.004; ANOVA, n = 7). The plasma ratio of free tryptophan:Σ(phenylalanine, tyrosine, leucine, isoleucine, valine, methionine, threonine, lysine) had declined at pre-exercise in TYR (P < 0.01; ANOVA, n = 7) but was unchanged in Whey at any timepoint (P > 0.05; ANOVA, n = 7). Mean power output throughout TT was 198 ± 41 W in TYR and 191 ± 46 W in Whey (P = 0.869; ANOVA, n = 7), therefore time to complete TT (P = 0.4167; paired Student’s t-test, n = 7) was similar in both trials (34.8 ± 6.5 min and 35.2 ± 8.3 min in TYR and Whey respectively). There was no difference between trials in RPE (P > 0.05; Friedman’s test, n = 7), thermal sensation (P > 0.05; Friedman’s test, n = 7), core temperature (P = 0.860; ANOVA, n = 7), skin temperature (P = 0.683; ANOVA, n = 7), or heart rate (P = 0.314; ANOVA, n = 7). These data indicate that acute tyrosine administration does not improve simulated TT performance in the heat. The lack of effect compared to a capacity trial to exhaustion may be related to the inherent self-paced nature of TT.

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Antioxidants do not improve force recovery following induction of fatigue in single mouse muscle fibres
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Reactive oxygen and nitrogen species have been implicated in the delayed recovery of force following muscle fatigue. The purpose here was to investigate whether two different antioxidants, or a nitric oxide synthase inhibitor, could improve the recovery of force following repetitive brief tetani produced at physiological temperature. Mechanically-dissected intact single fibers from flexor digitorum brevis muscles of C57 mice, killed by cervical dislocation, were superfused with Tyrode solution (~32°C). Fibres were injected with indo-1 to assess free myoplasmic [Ca^{2+}] ([Ca^{2+}]_i) changes. In unfatigued conditions and after induction of fatigue, FDB fibres were electrically stimulated at various frequencies and the resultant force and [Ca^{2+}] were measured. Fatigue was induced with brief 150 ms, 70 Hz tetani given every 1 s for a total of 60 contractions. For 20 min before the start of fatigue until 30 min after fatigue, fibres were superfused with either a general antioxidant [N-acetylcysteine (NAC) 1mM, n = 8], a mitochondrial-targeting antioxidant (SS-31 200nM, n = 10), a nitric oxide synthase inhibitor [N^G-nitro-L-arginine methyl ester (L-NAME) 200μM, n = 9], or a control Tyrode solution (n = 13). Data are reported as means ± SD, and statistical significance was determined at p<0.05 with ANOVA. In control fibres at the end of fatigue induction, 70Hz force was reduced to 53 ± 34% of its initial value. NAC and SS-31 as well as L-NAME had no noticeable effect in preventing the fatigue-induced force loss. In control fibers at 5 min after fatigue, the decrease in 30 Hz force (35 ± 29%) was greater than the decrease in force at 120 Hz (74 ± 22%), whereas relative values of [Ca^{2+}], at the respective frequencies (88 ± 23%) and (76 ± 19%) were similar. During the 30 min recovery period, prolonged low-frequency force depression was evident with greater relative reductions in force at 30 Hz compared with 120 Hz, with no differences in the extent of force depression between control, NAC, SS-31, or L-NAME conditions. Under physiologically-relevant conditions, the recovery of force after fatigue could not be improved with antioxidants or with a nitric oxide synthase inhibitor.

Supported by The Swedish Research Council

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Influence of ACE and ACTN-3 polymorphisms on times of performance in World’s off-road Triathlon race

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Triathlon is a 3-event endurance sport composed by swimming, cycling and running that are performed by athletes subsequently. The three events are separated each other by a transition period of only few seconds. A scarce number of studies have examined the influence of genetic factors on final results during an international competition.

The aim of this study was to identify the single and combined influence of two polymorphisms (ACE and ACTN-3) on times of performance in a off-road Triathlon race.

A simple random sample of twenty-six male athletes were selected from the two-hundred-sixty participants at the world stage of the “X TERRA World Championship”. All selected athletes have completed the World’s off-road triathlon race and the Swim Time (S), the Bike Time (B), the Run Time (R), the Transition Time between S and B (T1) and Transition Time between the B and R (T2) were recorded for each athlete using the SYNOPSIS GPS system (T4 model). Correlation between genotypic and phenotypic factors was computed. Forward stepwise multiple regression models were used to explore the predictive role of each genotypic (ACE and ACTN-3) and phenotypic (S, B, R, T1, T2) variables for the final race time (FT).

The ranges of the time for each event were the follows: $S_{\text{min}}=18-21$; $B_{\text{min}}=86-103$; $R=35-45$; $T1_{\text{s}}=26-48$; $T2_{\text{s}}=25-53$; $FT_{\text{h}}=2.24-2.49$. We found a significant correlation between polymorphisms and S (ACE, $r=0.65$; ACTN-3, $r=0.60$) and T2 (ACE, $r=0.71$; ACTN-3, $r=0.79$). The athletes with ACTN-3 R577X and ACE I/D genotypes have performed better time in both S and T2. The 85% of the FT variability was explained by the B ($R2_{\text{adj}}=0.8566$, $F=143.44$, df=1, $p<0.01$). The second most important variable was the R ($R2_{\text{adj}}=0.9780$, $F=557.10$, df=2, $p<0.01$), followed by the S ($R2_{\text{adj}}=0.9996$, $F=22531.30$, df=3, $p<0.01$). The combined influence of genotypic traits explained only the 0.03% of the FT variability.

Our results suggest that the ACE and ACTN-3 polymorphisms have a significant influence on time spent swimming during off-road triathlon competition. Nevertheless, our data also highlight that the best predictor of the final race time was the time spent cycling. On the bases of our data, we conclude that the ACE and ACTN-3 polymorphisms are not determinant factors for the success in a off-road triathlon race.

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Development of a compact dynamometer to evaluate propulsive power on the wheelchair

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The methods and instruments used to determine kinetic parameters about the manual wheelchair propulsion, as well as power output (PO), have disregarded interactions related to wheelchair-user system (subject and wheelchair), besides their excessive size and complex utilization. Therefore, the aim of this study was to develop a new dynamometer to evaluate PO on the wheelchair, and determine the specificity of the electromechanical system. The mechanical portion of the prototype consists of three parallel cylindrical rollers mounted on rotating axes (Easy Scroll, Brazil). In the central roller are attached rotation sensors. Between the two kits is a calibration system to calculate the dynamic moment of inertia. The PO was determined by measurement of the inertia and rotations (RPM) of cylinders. Data about RPM are acquired by magnetic sensors connected to Arduino board converter (Sparkfun, USA) and this linked to a computer. The converter is programmed to read the signs at each rotation for own algorithms providing two software environments: the first for calibration system and the second to determine PO. The data are sent to a computer and converted in graphics (PO versus RPM) by PLX-DAQ (Parallax, USA) and Microsoft Excel 2007. This study was approved by the ethics committee of the University of Pernambuco (Recife/PE/Brazil). Non-wheelchair users (men, 21±2 years, n=5) were tested on the same basketball wheelchair in four different styles of propulsion selected and randomized in advance: pumping, single-looping, double-loop and semicircular. The subjects were instructed to keep pace and thrust comfortable at all times. Independent investigators counted the number of propulsions executed by the subject to determine the freely chosen cycle frequency (FCF: 100%) to each volunteer at the end of last minute familiarization. The resting heart rate was measured in the seated position after 10 minutes of rest. The experimental protocol for each style consisted of a continuous effort of 5 minutes at 120% FCF. Push frequency was controlled by audio metronome (M&M Systems, Germany). Overall and local (active muscles) rating perceived exertion and heart rate (Polar FS1, Finland) were collected at the end of each minute. Subjects started the following style after heart rate recovery at baseline. The selected variables were analyzed using the Kruskal-Wallis and the level of significance set at p<0.05. There were no significant differences in the heart rate and overall and specific rating perceived exertion (Figure 2, p>0.05). This result suggests one basic characteristic of dynamometry and a new possibility to evaluate performance on the wheelchairs in opposition to methods of measuring the amount of work done.

Figure 1 – Dynamometer compact mounted: top view of the complete system (A); calibration system in two moments, (B) stabilization and (C) calibration.

Figure 2 - Heart rate (A), overall (B) and local (C) perceived exertion in the different styles of propulsion in the continuous exercise protocol (120%FCF).

The authors acknowledge CAPES and PFA/UPE for financial support.

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The effects of testosterone on molecular markers of hypertrophy in C2C12 skeletal muscle cells

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Resistance exercise acutely elevates endogenous levels of circulating testosterone (Ratamess et al. 2005), eliciting an anabolic effect, and leading to increases in muscle strength and fibre hypertrophy. The molecular mechanisms by which testosterone (T) contributes to the anabolic process during hypertrophy in skeletal muscle remains poorly understood. Various molecular pathways to promote muscle growth and hypertrophy have been suggested, including directly via the androgen receptor (AR) or indirectly through insulin-like growth factor-I (IGF-I). However results still remain inconclusive. Therefore we investigated the effect of testosterone on molecular markers of hypertrophy. A confluent monolayer culture of mouse C2C12 skeletal muscle cells was exposed to standard low serum conditions. Treatment consisted of a vehicle control, testosterone (50nM and 500nM) or IGF-I (10ng/ml) for 3 days’ (early) and 6 days’ (late) muscle differentiation. For both time points, reverse transcriptase-polymerase chain reaction (RT-PCR) analysis was performed for AR, IGF-I and myogenin (terminal marker of differentiation) messenger RNA (mRNA) and immunocytochemistry to determine myotube width (μm), fusion index (%) etc. The experiment was performed in triplicate, with 3 separate repeats (n=3). Values are means ± S.D, compared by ANOVA. After 3 days, myogenin mRNA expression significantly increased with exogenous T treatment (50nM T 1 ± 0.3; 500nM T 1.1 ± 0.4 (p<0.01 respectively)). No further changes occurred in myogenin mRNA levels after 6 days exposure in any of the treatments. As for AR mRNA expression, there were no significant changes between testosterone doses at either time points, whereas exogenous IGF-I significantly increased AR mRNA expression after 3 days exposure (1.55 ± 0.82, p<0.05). Finally, following 6 days exposure, both doses of testosterone significantly increased myotube width (50nM T 21.8 ± 5.9 μm; 500nM T 21.1 ± 5.5 μm (p<0.05 respectively)) compared to control (18.08 ± 3.6 μm) and IGF-I (17.8 ± 4.3 μm) treatment. The present study supports testosterone’s role in myogenic differentiation. Interestingly, the 50nM dose exerted a greater effect in this cellular process. An observation potentially explained by testosterone-androgen receptor interactions and the reported (Altuwaijri et al. 2004) low levels of AR in C2C12 cells. This data also supports in vivo results demonstrating increased fibre diameter during testosterone supplementation (Hartgens et al, 2002). Furthermore varying levels of AR may have been observed between muscle groups (Kadi et al. 2000), optimising training regimes for such muscle groups may be important to maximise testosterone hypertrophic effect on skeletal muscle.


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Rapid component of oxygen uptake kinetics is associated to rowing performance in competitive young rowers


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Rowing performance is strongly influenced by rapid response to a step transition from rest to constant high intensity exercise. It has been suggested that the time constant of oxygen uptake (VO2) increase is closely reflected by the time constant for O2 utilization in the exercising muscles (1), suggesting that it could be related with performance (2). The aim of this work was to study the VO2 kinetics with non-linear analysis and its relation with rowing performance. Eleven elite young male rowers (16.1 ± 1.4 years) were included in the present study. First, VO2max and maximal aerobic power was determined by gas analysis (MetaLyzer 3-B, Cortex Leipzig, Germany) in an incremental test on rowing ergometer (Concept II- model C, Nottingham, UK) and, then, the VO2 kinetics was registered during a constant test at maximal aerobic power intensity (MAP). A mathematical model of VO2 max kinetics was processed in MATLAB software v7.12 (Natick, MA, USA), utilizing wavelet toolbox and optimization. The physiological signals were filtered through a De-noise of wavelet (daubechies 6) applied in first order. A triple exponential model of VO2 kinetic was calculated by equation proposed in previous studies (3) and adjusted through least-squared method. The time constant of II component (τON) stands for the time constant of the fast component the time to reach 63% of the plateau of this phase during which physiological adaptations adjust to meet the increased metabolic demand. The rowing performance was determined in water conditions, during a 2000m test. The results were expressed as mean± standard deviation (SD)and pearson correlation was applied. The cardio-dynamic phase was not analyzed and slow component was not found in the VO2 signals. The τON values observed were 24.09± 11.2 with amplitude of 53.1± 6.7 mL/kg/min during a limit time test at PAM. We found a significant correlation between velocity during a 2000m race and τON of II component (r=0.9155 p <0.0001). Moreover, we observed the negative correlation between τON with VO2 max (r= -0.8165 p <0.005). Our finding suggests, for first time, that τON of rapid component of VO2 kinetics can be related with the aerobic capacity and performance of young rowers. This index may be useful for talent identification and training control.


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Introduction: Orientation of ground reaction force (GRF) vector of runner is related with acceleration ability (Kugler and Janshen, 2010; Morin et al., 2011), but the orientation of force three-dimensionally is unclear. Therefore, this study clarified that both magnitude and orientation of the force three-dimensionally between sprinter and novice in starting block clearance. Methods: Twelve male sprinters (S) and twelve healthy male students (N) were participated in this study. Movement of whole body and GRF data were captured as each subject was sprinting from pushing starting blocks to 2nd step with 16 high speed cameras (Raptor-E digital 590-1097-RE1; Motion Analysis Corporation, Santa Rosa, CA) and 10 force plates (TF-4060-B; Tech-Gihan, Inc., Japan). Horizontal velocity of center of mass from pushing starting blocks to take-off moment in 2nd step (HV) was utilized as start dash performance. Results: Higher HV of S than N (2.91 ± 0.13 m/s vs. 2.76 ± 0.15 m/s; p < 0.01) was caused by both larger step length (1.05 ± 0.09 m vs. 0.96 ± 0.09 m; p < 0.05) and step frequency (2.25 ± 0.14 Hz vs. 2.04 ± 0.21 Hz; p < 0.05) from pushing starting blocks to 1st step. From take-off of front foot to landing of 1st step, step width of S was larger than that of N (0.27 ± 0.05 m vs. 0.20 ± 0.07 m; p < 0.001), and air-borne time of S was larger than that of N (0.083 ± 0.023 s vs. 0.060 ± 0.028 s; p < 0.05). In pushing starting blocks, mean horizontal (0.92 ± 0.08 N/bw vs. 0.76 ± 0.08 N/bw; p < 0.001), lateral (0.05 ± 0.02 N/bw vs. 0.02 ± 0.03 N/bw; p < 0.01) and vertical forces (1.03 ± 0.06 N/bw vs. 0.95 ± 0.07 N/bw; p < 0.01) of S were larger than those of N. Kugler and Janshen (2010)’s study of physical education students, it was reported that orientation of force was correlated with degree of leaning body forward (r = 0.93). Orientation of the force in sagittal plane at maximal resultant force of S was smaller than that of N during pushing starting blocks (41.9 ± 4.0° vs. 47.4 ± 4.4°; p < 0.01). These suggest that S facilitates greater forward leans of the body than N during pushing starting blocks. In contrast, no significant differences of orientation of force in frontal and transverse planes at maximal resultant force were found between S and N during pushing starting blocks. Conclusion: Our findings are summarized as follows: 1) Enough air-borne time to increase step length and horizontal velocity after pushing starting blocks can be achieved by larger magnitude of force; developed leg extension muscles, even if runner leans body forward. 2) Magnitude of lateral force is determinant of larger step width but orientation of the force at pushing blocks from crouching start. Therefore, larger medio-lateral movement is generated naturally during faster sprinting, and sprinter’s orientation of force does not always direct closer to running direction.

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Skeletal muscle fatigue: effects of age, sex and pattern of muscle activation

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Skeletal muscle fatigue can limit athletic performance and the activities of daily living and is mainly determined by the balance between rates of energy use and replenishment. Differences between young and older men and women in contractile speed and aerobic capacity and variation in the duty cycle of contraction and relaxation are likely to influence development of fatigue. In the present study 37 young and older (mean age 22 and 70 yrs) men and women completed assessments of knee extensor contractile properties and two different fatigue tests. In one fatigue test a sustained isometric voluntary contraction was held at 50% of maximal force until task failure, giving no opportunity for metabolic recovery during the task. The other test consisted of a series of electrically evoked 30 Hz stimuli of 1 s contraction and 1 s rest intervals, giving brief periods between contractions for metabolic recovery. Fatigue in this latter test was presented as a fatigue index (FI), being the force after 2 minutes expressed as a percentage of the initial force. Data were examined using ANOVA and are presented as mean ± SD. Results showed that young men had the fastest contractile properties, followed by young women, and that older men and women were slowest, evidenced as a leftward shift in the torque-frequency relationship and slowing of rates of relaxation. The older subjects were able to hold the sustained contraction for around 35% longer than young (71.8±13.5 s vs 97.5±26 s; P<0.0005), irrespective of sex. In all groups task failure was almost entirely due to peripheral muscle fatigue, as assessed by superimposed electrical stimulation. However, during the intermittent contractions, which more closely resemble the recruitment patterns during activities like walking or running, there was no difference between young and old in fatigue resistance (FI: 59%±10 vs 59%±9 in young and old, respectively), but men showed 16% greater loss of force compared with women (FI: 65%±9 vs 54%±7 in women and men, respectively; P=0.001). It is concluded that women fatigue less than men during intermittent contractions but not during prolonged isometric contractions and that older muscles are more resistant to fatigue when performing sustained isometric contractions. The most likely explanation for age differences is that with ageing there is a shift towards a slower phenotype with reduced energy requirements during prolonged isometric contractions. However, during brief intermittent contractions the energetic advantage of older muscle was no longer apparent. This may be in part due to reduced aerobic capacity and ability to regenerate ATP during the recovery intervals in the older muscle and in part due to the slower, older muscles being less efficient during the initial phase of internal shortening against series compliance that occurs at the start of brief isometric contractions.

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Heat acclimatization in elite professional Australian football players

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This study aimed to determine the benefit of a heat acclimatization camp on physical performance in both neutral and hot environments in 18 elite professional Australian Football (AF) players.

Players participated in a two-week pre-season acclimatization camp (environmental temperature 31-33°C, humidity 34-50%). At the beginning and end of the camp, all players performed a heat-response test in a hot environment (24 min walk at 5 km.h⁻¹ + 24 min seated; 44°C, 44% RH) and a YoYo Intermittent Recovery Test level 2 (YoYoIR2) in a neutral environment (22°C). In addition, the total distance and the amount of high speed running (>14.4 km.h⁻¹) were quantified during a standard small-sided game (SSG) in a hot environment (32°C) at the start and the end the camp (average of two SSG at each period).

There was an increase in the distance reached during the YoYoIR2 in neutral environment following the camp (+44%, p<0.001). Both the total distance and the amount of high speed running performed by the players during the free-paced SSG in hot environment were also higher at the end than at the beginning of the camp (distance +4.7%, high speed running +10.3%, p<0.045).

Results from the heat-response tests showed significant improvement in some (skin temperature -0.5°C, sweat sodium concentration -26%, p<0.01) but not all (core temperature -0.02°C, sweat rate +2%, p>0.65) physiological markers of heat acclimatization over the 2 weeks. Plasma volume calculated by CO rebreathing increased from 4.4 to 4.7 L (p=0.003) and players enhanced their hydration level (specific urine gravity 1.018 vs. 1.013, p=0.003).

There were no significant differences in haematocrit levels measured at the end of the heat-response test between at the beginning and end of the camp (42.0% vs. 42.4%, p=0.44). However, the individual changes in haematocrit during the 2 week camp were correlated with the increases in both distance covered (r=0.50) and in the amount of high speed running (r=0.55) during SSG (p<0.05). There was no correlation between the improvement in the YoYoIR2 performance in neutral environment and the increases in physical performance during the SSG in hot environment.

In conclusion, the two-week heat acclimatization camp resulted in significant performance enhancement in elite AF players. We observed an increase in the physical capacity tested in neutral environment (YoYoIR2) as well as in the physical performance during competitive situations in hot environment (SSG). However, the correlations analyses suggest that improvement of performance in neutral and hot environments are not directly related and, therefore, may depend on different physiological mechanisms.

Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.
The effects of fatigue on maximal cadence, muscle recruitment and power output do not limit exercise tolerance in humans

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It is traditionally assumed that exhaustion during high-intensity aerobic exercise occurs because the fatigued neuromuscular system is not able to maintain the power output (PO) required by the task despite maximal levels of voluntary effort (1). Marcora & Staiano (2) challenged this assumption by showing that exhausted subjects can produce a PO much higher than the PO required by the task. Criticisms were raised for failing to account for the power-velocity relationship due to ergometer set up (3).

In the present study, after a maximal incremental test on a cyclosimulator (Velotron, Racer Mate®) to obtain the peak PO (PPO), the volunteers [n=18; PPO=273 (29)W] performed a constant workload test (CONST) until exhaustion defined as cadence < 90 rpm despite strong verbal encouragement. Each subject had his respective gear ratio defined so that, at the cadence of 90 rpm, the PO of CONST would equal 80% PPO and any increase in the CAD would result in an increased PO due the fixed length of the crank arm and gear ratio. Upon exhaustion, subjects were required to quickly increase their cadence maximally over 10 s (MAXCAD). This all-out effort was unknown by volunteers prior to testing. During CONST, electromyography of the quadriceps muscle (VL+VM+RF) (QEMG) was recorded. The rating of perceived exertion (RPE) was obtained every minute. Repeated measures ANOVA with Bonferroni’s post hoc test was used to analyze the data (p<0.05).

CONST lasted 498 (213)s, with high RPE [20 (0.5)] at the end of the test. Albeit lower than MAXCAD before const, PO, cadence, and QEMG during MAXCAD after CONST were significantly higher than PO, cadence, and QEMG during CONST (Table 1).

These results show that, albeit significant, the effects of fatigue on the ability to recruit (QEMG) and shorten the locomotor muscles against a given load (cadence) cannot explain why subjects terminate high-intensity aerobic exercise. Indeed, immediately after exhaustion, all subjects were able to achieve during MAXCAD a PO higher than the PO required by the CONST. Therefore, as previously suggested, neuromuscular fatigue does not seem to cause task failure during high-intensity aerobic exercise. High perception of effort appears to be the key factor limiting exercise tolerance in well-motivated subjects.

Table 1 - Mean (SD) of the variables obtained before, during, and after CONST

<table>
<thead>
<tr>
<th>Variable</th>
<th>Before Const</th>
<th>During Const</th>
<th>After Const</th>
</tr>
</thead>
<tbody>
<tr>
<td>Power Output (W)</td>
<td></td>
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<td></td>
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<tr>
<td>Cadence (rpm)</td>
<td></td>
<td></td>
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<tr>
<td>EMG (μV)</td>
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</table>

* Significantly different from previous tests (p<0.001). ** Significantly different from MAXCAD before CONST (p<0.05).


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Distinct profiles of neuromuscular fatigue during muscle contractions below and above the critical torque in humans

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Fatigue during exercise is usually defined as a reversible decline in maximal muscle force or torque generating capacity (Gandevia, 2001; Allen et al., 2008). During submaximal tasks, fatigue processes during “low intensity” contractions differ from those during “high-intensity” contractions. However, whether the transition in fatigue processes occurs gradually or occurs suddenly at some identifiable “threshold” torque is unclear. This study tested the hypothesis that distinct profiles of central and peripheral fatigue would occur below and above the critical torque (CT). Following institutional ethics committee approval, nine healthy men gave written informed consent to perform seven experimental trials. Each trial involved intermittent isometric contractions of the quadriceps femoris (3 s contraction, 2 s rest; Biodex System 3 dynamometer) continued to task failure or for up to 60 min (whichever occurred the sooner), with maximal voluntary contractions (MVCs) performed at the end of each minute. Five trials were performed above CT (~35-55% MVC, denoted S1-S5 in ascending order), and two trials were performed below CT (denoted CT–20% and CT–10%). Dynamometer torque and the electromyogram (EMG) of the right vastus lateralis were sampled continuously. Peripheral and central fatigue was determined from the fall in potentiated doublet torque and voluntary activation, respectively. Above CT, contractions progressed to task failure in ~3-18 min, at which point the MVC did not differ from the target torque (Mean ± SEM: S1 target, 88.7 ± 4.3 N.m vs. MVC, 89.3 ± 8.8 N.m, P = 0.94), the average rectified EMG amplitude was not different from that measured during a MVC (S1, 76 ± 9 %MVC vs. 76 ± 8 %MVC, P = 0.97), and the potentiated doublet had fallen significantly in all trials (from 96.5 ± 5.0 to 62.5 ± 4.4 N.m, P < 0.001). Voluntary activation was also reduced in trials S1-S3, but not trials S4 and S5. During contractions below CT, contractions could be sustained for 60 min in 17 out of 18 occasions. Although the fall in MVC was significant (CT–20 from 212.4 ± 16.9 N.m to 148.3 ± 11.5 N.m, P = 0.001), a substantial reserve in MVC torque and muscle activity was evident at the end of the tasks. Potentiated doublet torque and voluntary activation were both reduced, i.e. central and peripheral fatigue developed. The rate of development of global and peripheral fatigue was 4-5 times greater during S1 than during CT–10% (e.g. ΔMVC/Δt S1 vs. CT–10%: –7.2 ± 1.4 vs. –1.5 ± 0.4 N.m.min⁻¹). The results of the present study demonstrate that the rate of neuromuscular fatigue development does not scale as a simple linear function of the target torque, but is substantially accelerated above the CT.


The author thanks Andrew M. Jones and Anni Vanhatalo for conceptual discussions that contributed to the interpretation of these data.

*Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.*
Six weeks of 10 x 1 minute of high intensity interval training increase plasma volume and hemoglobin mass in untrained obese subjects


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Endurance trained athletes are reported to have higher total blood volume and hemoglobin mass (Hb-mass) than untrained individuals. It is unknown if a short period of endurance training increase total blood volume and Hb-mass in inactive obese subjects. The primary aim of the study was to investigate the effect of 6 weeks of high intensity interval training (HIIT) (4x4 and 10x1 minutes at 90-95% of Maximal Heart rate (HRmax) and moderate continuous training (MCT) at 75 % HRmax on total blood- and plasma volume and Hb-mass in inactive obese subjects. Thirty obese inactive men and women were randomized 1:1:1 to 3 training groups and supervised for 6 weeks with 3 weekly treadmill training sessions. Training was performed as either 1) 4 x 4 minutes HIIT. 2) 10 x 1 minutes of HIIT 3) 47 minutes of MCT. The improved carbon monoxide-rebreathing method was used to measure total blood volume and Hb-mass before and after the training intervention. Measurements were done in the morning in the fasted state, with 500 ml of water consumed minimum 1 hour before testing. Maximal oxygen uptake test, a performance test, measurement of endothelial function was performed, and blood and muscle biopsy samples collected before and after training intervention. Twenty-one subjects completed the training intervention with a complete blood volume measurement, a total of 6, 7 and 8 subjects in the 4x4, 10x1 and MCT group respectively (age 41 ± 9 years, height 173 ± 8 cm, weight 91 ± 11 kg, BMI 30 ± 4, VO2max 35.79 ± 7.25). At baseline, total blood volume was 5.80 ± 0.62, 5.68 ± 1.07 and 5.95 ± 1.22 L in the 4x4, 10x1 and MCT groups respectively, with no significant change after training. At baseline, total plasma volume was 3.53 ± 0.34, 3.50 ± 0.61 and 3.64 ± 0.72 in the 4x4, 10x1 and MCT groups respectively. After training total plasma volume was increased by 4% to 3.65 ± 0.50 L (p ≤ 0.05) in the 10 x 1 minute interval training group with no change in the other groups. Baseline total Hb-mass was 758 ± 126, 729 ± 759 and 788 ± 192 grams in the 4x4, 10x1 and MCT groups respectively. After training the 10x1 group increased total Hb-mass by 4 % to 760 ± 155 g (p ≤ 0.05), with no change in any of the other groups. When values are reported per kg body weight, blood volume, plasma volume and Hb-mass was significantly increase from before to after training in the 10x1 group (p ≤ 0.05), with no change in any of the other groups. Body weight remained unchanged from before to after training in all group. These data suggest that 6 weeks of 10x1 minutes HIIT significantly increase plasma volume and total Hb-mass in obese untrained subjects.

We acknowledge the participation of the subjects in the study and the Regional Health Authorities of Central Norway for funding the equipment used in the study.

Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.
Pacing strategy and role of expertise in broken 800’s workout regarding the 800-m run

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Introduction

It’s been described the existence of different pacing strategies in 800-m races. When racing the clock (i.e. improving Season Best [SB]), athletes describe a Positive Pacing (PP) where speed gradually declines throughout the race. When tactical running (i.e. qualifying races), there is a more Variable Pacing [VP], or even a parabolic pacing (1). On the other hand, coaches use speed endurance workouts (i.e. Broken 800’s [B8]) to develop the athletes’ ability to tolerate fatigue while running at the race pace (2-5). However, little is known about this workout’s influence on pacing. Therefore, our purpose was to analyze athletes’ pacing in B8 regarding to that in the 800-m run; and to investigate whether this pacing is an age-related ability.

Methods

19 male 800-m runners, belonging to either Under23 & Senior (GA; n=10; SB= 113.75±4.08 s) or Juvenile & Junior category (GB; n=9; SB= 120.92±3.33 s), performed a B8 consisting in 2x(4x200-m) at 102% (SB), recovering 30 s between repetitions and 15 min between sets. Time for 200-m segments (T200) was obtained by recording with a CASIO HS EX-FH100 camera (420Hz) placed in the centre of the track, and later video analysis (Kinovea-0.8.7). A two-way Repeated Measures ANOVA was conducted, with 1 intra-subject factor: Pacing (T200 differences), and 1 grouping factor: age category (GA vs. GB). Further Bonferroni adjustments were conducted. The study, approved by University of Valencia Ethics Committee, followed the standards, controlling for athletes’ previous tapering, rest, nutrition and hydration.

Results

Univariate contrast showed significant T200 differences both on Pacing (p<0.001) and on the interaction Age-category*Pacing (p<0.05). Figure 1 reflexes Bonferroni adjustments (see numbers and letters for T200 significant differences on Pacing; Stars for T200 performance differences between categories). Regarding performance, GA ran always significantly quicker except for B81. Regarding pacing*category, GA maintained a slight and progressive velocity impairment, thus PP, although neither linearly nor significantly for intermediate consecutive T200. In fact, after 15 minutes of recovery, GA ran B8a even quicker than B81, although with no significance. GB ran a VP first set, followed by a second PP set.

Conclusions

B8 workout is more related to the PP in 800-m racing the clock events than to tactical pacing variations. However, lack of within significance in intermediate T200, and category significant differences, suggest a certain possibility to pacing variation and choice learning. Pacing confirms to be an age-related factor.

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Figure 1. Broken 800’s pacing analysis: Under23 & Senior athletes (GA) vs. Juvenile & Junior category (GB). T200 (mean time in 200-m segments). Significant differences on Pacing (p<0.005): numbers = B8 1st set, letters =B8 2nd set. Significant differences on Performance between categories: black Stars (p<0.005); grey stars (p<0.05)

Pulmonary blood flow, its reserve, and distribution in highly trained endurance athletes and healthy control subjects

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¹PET Centre, University of Turku, Turku, Finland and ²Turku University Hospital, Turku, Finland

Pulmonary blood flow is an important factor in endurance sports performance, but studies investigating possible differences between athletes and matched healthy subjects are scarce. In the present study we measured pulmonary blood flow, its reserve and distribution in highly trained male endurance athletes (n=10, age=25±4 years, mostly cross-country skiers whose bicycle VO2max was 62±5 ml/kg/min) and untrained but fit healthy controls (n=10, age=26±4 years, VO2max=46±3 ml/kg/min) with [15O]water positron emission tomography (1-2) at rest and during adenosine infusion (140 mg/kg/min I.V.), a protocol commonly used in cardiac flow reserve studies. The athletes had been training on a regular basis for 12±4 years, 8.7±1.4 times and 12.8±1.5 hours a week primarily in endurance type of exercise at various intensities. They had started sport training at the age of 12.5±2.4, and competed in endurance sports for 15.6±4.5 years at high national and international level. The control men, on the other hand, had been performing physical activity only occasionally, less than three times per week. Our results indicate that pulmonary blood flow at rest and during adenosine stimulation was similar in both groups (in athletes, 213±55 ml/100ml/min in rest and 563±138 ml/100ml/min during adenosine infusion and in controls, 206±83 ml/100ml/min and 473±212 ml/100ml/min, respectively, p=NS). Although the absolute pulmonary blood flow reserve was unchanged in athletes, blood flow heterogeneity was reduced from rest to adenosine infusion (from 84±18 % to 70±19 %, p<0.01) while remaining unchanged in healthy controls (77±16 % to 85±33 %, respectively, p=NS). Moreover, there was a marked gravitational influence of supine body posture on general pulmonary blood flow distribution so that clear dorsal dominance was observed both at rest (in athletes, 284±64 ml/100ml/min dorsally vs. 102±48 ml/100ml/min ventrally, p<0.001, and in controls, 290±115 ml/100ml/min vs. 102±59 ml/100ml/min respectively, p<0.001) and during adenosine infusion (in athletes, 677±134 ml/100ml/min vs. 375±165 ml/100ml/min, p<0.001, and in controls, 642±179 ml/100ml/min vs. 320±200 ml/100ml/min, p<0.01), thus, training status did not have effect on flow distribution. Blood flow heterogeneity values were also markedly lower in the high perfusion dorsal areas at rest (in athletes, 63±17 % vs. 86±24 %, p<0.05, and in controls 53±18 % vs. 81±21 %, p<0.01) and during adenosine infusion (in athletes, 52±18 % vs. 97±22 %, p<0.001 and in controls 54±22 % vs. 110±38 %, p<0.001). In conclusion, although highly trained endurance athletes appear not to have supranormal absolute pulmonary blood flow during adenosine stimulation, reduced blood flow heterogeneity may be an indication of capillary reserve which is more extensively recruitable in athletes than in matched healthy control subjects.


Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.
Predictors of 2000-metre performance in world-class lightweight rowers

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Rowers compete over a 2000-m distance lasting 6-7 min at international level with an estimated anaerobic (ANA) and aerobic (AER) energy contribution of ~25 and ~75% (1). Studies on elite rowers suggest that maximal oxygen uptake (VO2 max) is an important physiological variable to predict 2000m performance (2,3), but little is known about the influence from ANA power and capacity and the association between AER and ANA performance parameters.

Twenty-two world-class male lightweight rowers (VO2 max 5.5±0.3 L/min, 75.3±1.8 kg, avg±SD) – 13 of whom have won medals at the World championships and Olympics - were tested on a rowing ergometer (Concept II) for establishment of the power-duration curve (100-m~15s, 1-min, 2000-m~6min, 6000-m~20-min, 60-min max rowing) and a 6-min max test to determine VO2 max and peak lactate (Lac) in blood collected from a fingertip 1, 3 and 5 min after the test. Pearson correlation coefficients were calculated.

The power-duration curve is shown in Figure 1, and Table 1 shows the average values (±SD) for the different tests as well as the correlation coefficients between the variables. ANA power (100-m max) and capacity (Lac & 1-min max) were not correlated with any measure of AER power (VO2 max) or capacity (6000m & 60min max rowing). ANA power and capacity determined as 100-m and 1-min max rowing respectively were correlated with 2000-m performance (r²=0.29 & 0.43), and the 1 min max test actually showed a stronger relationship to 2000-m performance than VO2 max (r²=0.34).

In conclusion, ANA and AER performance parameters are not opposites in top level rowers. For example, the lack of correlation between 100-m and 60-min max rowing shows that an athlete can have both a high ANA power and high AER capacity. Furthermore, a high ANA power (100-m max) and capacity (1-min max test) appears to be as important for 2000-m performance as AER power (VO2 max). Despite large parts of training in rowing traditionally focusing on improving AER power and capacity (4,5) the present study indicates that ANA training may be beneficial for 2000-m performance.

Average values (± SD) for 22 world class lightweight rowers in different performance tests (average power shown), and the correlation coefficient (r²) between the various variables. *P<0.05, **P<0.01, ***P<0.001

<table>
<thead>
<tr>
<th>Test Duration</th>
<th>Average Power (W)</th>
<th>ANA Power (100-m max)</th>
<th>ANA Capacity (1-min max)</th>
<th>AER Power (VO2 max)</th>
<th>AER Capacity (6000m &amp; 60-min max rowing)</th>
<th>r²</th>
</tr>
</thead>
<tbody>
<tr>
<td>100-m</td>
<td>350 ± 20</td>
<td>420 ± 10</td>
<td>350 ± 10</td>
<td>390 ± 15</td>
<td>420 ± 10</td>
<td></td>
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<tr>
<td>1-min</td>
<td>250 ± 15</td>
<td>300 ± 10</td>
<td>250 ± 10</td>
<td>290 ± 15</td>
<td>300 ± 10</td>
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<tr>
<td>2000-m</td>
<td>900 ± 30</td>
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<td>950 ± 40</td>
<td>1000 ± 20</td>
<td></td>
</tr>
<tr>
<td>6000-m</td>
<td>900 ± 30</td>
<td>1000 ± 20</td>
<td>900 ± 30</td>
<td>950 ± 40</td>
<td>1000 ± 20</td>
<td></td>
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<tr>
<td>60-min max rowing</td>
<td>300 ± 15</td>
<td>350 ± 20</td>
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</tbody>
</table>

In conclusion, ANA and AER performance parameters are not opposites in top level rowers.

Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.
Effect of hypoxia severity on quadriceps fatigability during exhaustive intermittent cycling

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Increasing hypoxia severity has been shown to exacerbate peripheral fatigue, which affects exercise performance during intermittent, maximal and submaximal isolated contractions. To date, how hypoxia severity modifies the neural versus muscular determinants of high-intensity, whole-body intermittent exercise to exhaustion is however unknown. The aim of this study was to examine the effects of hypoxia severity on quadriceps fatigability during exhaustive intermittent cycling. Fifteen well-trained cyclists performed an intermittent cycling exercise until exhaustion at supra-maximal intensity - 15 s at 30% of the anaerobic power reserve (609±23 W) with a fixed pedaling frequency of 110 rpm, interspersed with 45 s of passive rest - in normoxia (simulated altitude/end-exercise arterial O2 saturation = 0 m/96%), moderate (2200 m/90%) and severe hypoxia (4200 m/79%). Neuromuscular tests including electrical femoral nerve and transcranial magnetic stimulations during brief (5-s) and sustained (30-s) maximal isometric voluntary contractions (MVC) of the knee extensors were performed at baseline and 7 min post-exhaustion. Exercise performance differed (P<0.001) among the three conditions of oxygenation (39 ± 8, 22 ± 3 and 13 ± 2 sprint repetitions in normoxia, moderate and severe hypoxia, respectively). At exhaustion, the reduction in peak twitch amplitude (P<0.001) in response to supra-maximal, unpotentiated single (-53.2%) and potentiated paired (-33.1%) electrical stimuli was identical across conditions. Compared with baseline, strength loss during brief MVC was similar at exhaustion in normoxia and moderate hypoxia (-9.3% and -9.9%; both P<0.01), while a smaller (-6.3%; P=0.136) force decline occurred with severe hypoxia. When contraction was prolonged, exercise-induced decreases in force were consistent across conditions (-22.0% from the beginning to the end of the 30-s sustained MVC; all conditions compounded, P<0.01). This was accompanied by lower (P<0.05) end-exercise voluntary activation values obtained from both motor nerve and motor cortex stimulations during brief (-1.9% and -4.6%, respectively; all conditions compounded) and sustained (-2.1% and -9.7%, respectively; all conditions compounded) MVCs, with no hypoxia severity effect. Maximal M-wave amplitude (at rest and during MVCs) and maximal RMS activity of vastus lateralis and rectus femoris muscles obtained during MVCs did not change from pre- to post-exercise in all conditions. Despite earlier exercise cessation with increasing hypoxia severity, end-exercise reductions in quadriceps twitch force were similar across conditions. These results indicate the existence of a critical threshold of peripheral fatigue with intermittent cycling to exhaustion, independently of the hypoxia level. Another novel finding was that a suboptimal output from the motor cortex may also contribute to exhaustion.


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Senescence, but not in vivo ageing, is associated with delayed differentiation, increased DNA damage and elevates TGF-β release in human primary myoblasts

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Reparative senescence of cells in culture has been used as a model to study the ageing process in human muscle (Bigot et al., 2008). However, the relevance of this approach to in vivo ageing remains unclear. The aim of this study was to investigate if in vivo muscle ageing differs from in vitro muscle ageing by studying primary human muscle cells obtained from young and elderly people, before and after they reached reparative senescence. Several parameters associated with ageing process were compared: ability to undergo differentiation; presence of DNA damage; and production of TGF-β. Muscle biopsy samples were taken under local anaesthesia from the vastus laterals muscles of 5 young (aged, 23-25) and 4 healthy elderly (aged, 67-82) subjects. Cells were cultured in a skeletal muscle cell growth medium until they reached senescence. The proportion of muscle cells (positive for desmin expression), differentiating cells and cells with double strand DNA breaks (positive for γ-H2AX) was assessed by immunocytochemistry, whereas TGF-β production was assessed in conditioned medium using a Luminex based assay. Cells were plated in growth medium in 96 well dishes at densities of 2000 (desmin, γ-H2AX) and 7000 (myogenin, MHC, TGF-β) cells/well. Cells were fixed 24 hours after plating for assessment of desmin and γ-H2AX or had their medium replaced with a serum free medium to induce differentiation. Marked heterogeneity between the different myoblast cultures was observed during multiple passaging. Several populations of cells maintained their initial desmin content over time (50-94%) and underwent 1-8 mean population doublings. However, others lost their desmin positive cells over time in culture (50-95% starting, 0% at senescence) and underwent 15-22 MPDs. Populations that did not maintain desmin expression were discarded from further analysis after the initial characterisation. The main finding of the study is that there is no difference in any of the parameters measured between the cells obtained from old and young people that had not undergone replicative senescence. However, the senescent cells had a decrease in the expression levels of myogenin (50 ± 3% young, 49 ± 3% old and 6 ± 1% senescent, P<0.001) after three days of differentiation and MHC (71 ± 2% young, 70 ± 1.4% old and 15 ± 1% senescent, P<0.001) after five days of differentiation, higher expression of DNA damage markers γ-H2AX (7 ± 1% young, 8 ± 1% old and 90 ± 4% senescent, P<0.001) and increased TGF-β secretion (111 ± 13pg/ml young, 115 ± 19 pg/ml old and 268 ± 11pg/ml senescent, P<0.001). This questions the use of senescence in culture as a model of in vivo ageing. The findings also point to a potential link between DNA damage, TGF-β production and inhibition of differentiation that should be further investigated.


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Comparison of youth football teams using Unifittest 6-60 at ages U11 SK Dynamo Ceske Budejovice, AC Sparta Praha, 1. FK Pribram, Bohemians Praha 1905, SK Tochovice

P. Pozarek

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Purpose of the thesis is to determine and compare the level of motor skills using a standardized test of five youth football teams U11. A series of exercises using Unifittest 6-60, tests the player in four different exercises focused on the individual physical abilities. All teams will complete tests by the end of training, at which time they should exhibit the highest degree of fitness. We personally perform all testing. The aim of our work was to measure and compare four league football teams and one district team using a test battery Unifittest 6-60 and to find out the level of motor abilities and skills. For this testing we used certified Unifittest 6-60, which we have slightly rearranged. The test included four disciplines. Players underwent scaphoid run of 4 x 10 m, then long jump from the place, sit-ups for 1 minute and finally we tested their endurance in pull-up. We got some somatic data from the players – this data included their height, their weight, the leg they naturally use for playing and the hand they use for writing.

All measured data was processed and evaluated using both total and partial arithmetic mean. Standard divergence was also used. When creating graphs and boxplots we used Microsoft Office Excel and statistical program called R. For collecting data we used standardized methodology which provides high precision measurements and the possibility to compare the results with norms for that age group. For processing and interpreting data commonly available statistical methods were used.

In mutual comparison the winner was AC Sparta Praha. The players showed on average the best results and with the exception of the discipline sit-up for 1 minute, they controlled the rest of remaining three disciplines and they deserve appreciation for the winners of our testing. As the weakest was for obvious reasons team SK Tochovice. In comparison of older and younger players in all four exercises, older players won but not always with a great distance. In two disciplines the results were very close, but for players born in the first half of the year were a bit better.

This thesis has accomplished our aims. We enjoyed testing and work with promising footballers. We found out the difference between the league and the district team, which of the four major league clubs have the best conditions for training, base and how they hold up in our tests. We have to say that physical fitness and preparedness is very good.

For evaluation we used the statistical program R and Microsoft Excel. We used the program R for Box plots illustrating the range of outcomes, then 25% and 75% quartiles, which enclose 50% of the results and median, which is depicted by a thick line. Microsoft Office Excel helped us to create tables and graphs.

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Fromel (2002) Compendium of writing and publishing in kinanthropology

Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.
Many human movements involve stretch-shortening-cycle (SSC) actions of lower limb muscle-tendon-units (MTU), whereby an eccentric muscle action is immediately followed by a concentric action. Tendons comprise the majority of eccentric MTU actions, particularly as movement frequencies increase (1), which optimises concentric muscle force output. However, this hypothesis has not been tested during conditions which replicate functional performance. Hopping involves SSC muscle actions and provides a reliable model for examining mechanical properties of lower limb joints, such as joint stiffness, which correlates positively both with movement frequency and functional performance (2). Six well-trained males performed three, single-leg hopping trials (each lasting for 15 seconds) on an inclined sledge apparatus, at two frequencies (1.5 and 2.5 Hz). For each trial, five consecutive hops within ±5% of the prescribed frequency were analysed further. Three-dimensional motion analysis, ultrasonography of medial gastrocnemius (MG) and ground reaction forces were simultaneously and synchronously collected. Sagittal plane joint angles and joint moments were determined via a combination of motion data, force data and inverse dynamics. Ankle joint stiffness was calculated as the ratio of peak joint moment (relative to body mass) to peak joint angular displacement during the eccentric phase of the hops (3). MG MTU length was determined as a function of shank segment length and joint angle data (4). MG muscle length was calculated as MG fascicle length multiplied by the cosine of the pennation angle (Figure 1) and MG tendon length was determined by subtracting MG muscle length from MG MTU length (5). Dependent t-tests were used to compare mean differences between variables measured at both frequencies (p<0.05). Data represents the mean ± S.E.M. of three trials performed at both frequencies. Ankle joint stiffness doubled (0.08±0.01 vs. 0.16±0.02 Nm/kg/deg, p<0.05) and ground contact times shortened (329±0.21 vs. 254±0.01 ms, p<0.05) as hopping frequency increased from 1.5 to 2.5 Hz. MG muscle lengthening was either slightly less or non-existent (-3.67±2.86 vs. 7.15±1.66 mm, p<0.05) at 2.5 Hz compared to 1.5 Hz, whereas MG tendon lengthening was much greater (18.31±3.34 vs. 6.98±2.26 mm, p<0.05). MG muscle shortening velocity was similar at both frequencies (48.3±9.5 vs. 48.3±17.0 mm/s, p=1.00), however, MG tendon shortening velocity was far greater at 2.5 Hz (143.3±16.7 vs. 85.0±14.6 mm/s, p<0.05). Results suggest that single-leg hopping performed with increased ankle joint stiffness resulted in more efficient use of the SSC, as greater tendon lengthening and subsequent rapid recoiling allowed the MG muscle to operate within a more optimal range for maximising concentric force output, due to muscle force-velocity and length-tension relationships.

Figure 1 - Instantaneous medial gastrocnemius (MG) muscle length was determined by multiplying MG fascicle length (dashed white line) by the cosine of the pennation angle (θ), as measured between the superficial (A) and deep MG aponeuroses (B).


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The musculoskeletal ultrasound in critical care: longitudinal evaluation (UK-MUSCLE) study: severity of acute critical illness determines the degree of muscle wasting

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Acute muscle wasting in critical illness is a major cause of disability amongst intensive care survivors. There are limited data detailing sequential loss of muscle mass and less data characterising histological muscle changes. We hypothesised that loss of Rectus Femoris Cross-Sectional Area (RFCSA) would be determined by the severity of acute critical illness and paralleled by a reduction in myofibre cross-sectional area determined from analysis of muscle biopsy samples.

Critically ill patients were recruited from two hospitals within the first 24 hours of admission. Inclusion criteria were: likely to (i) remain intubated ≥ 48 hours and (ii) remain in critical care ≥ 7 days. Patients were excluded if they i) were pregnant ii) had disseminated malignancy or iii) suffered from a primary neuromuscular disease. Serial RFCSA measurements were taken using B-mode ultrasound, on days 1, 3, 7 and 10 of admission. Bedside physiological data was collected, for stratification of illness severity by numbers of failed organ systems, defined by the Sequential Organ Failure Assessment score. Patients were retrospectively excluded if they failed to maintain inclusion/exclusion criteria for the length of the study. Serial vastus lateralis muscle biopsies were performed in 50% of patients.

91 patients were recruited, of which 63 were included for final analysis. 1 patient could not undergo RFCSA due to morbid obesity (BMI 67kg/m2). The greatest RFCSA reduction was observed in patients with ≥ 2 organ failure; 21.5±10.5% in ≥ 2 organ failure vs.7.2± 9.7% in 1 organ failure; p <0.0001 (Fig.1). Greater loss of RFCSA was observed at day 3 in patients with multi-organ failure compared with single organ failure patients (8.7±16.3% in ≥ 2 organ Failure vs. 1.8± 9.6% in 1 organ failure; p<0.01). Significant differences were observed between those with 2-3 organ failure compared with ≥ 4 organ failure by days 7 and 10 (19.5±9.4% vs. 26.3± 12.0%; p<0.01). Histological analysis showed a reduction in type 1 fibre cross sectional area (4.3±3.1% loss per day in single organ failure and 2.9±3.3% loss per day in multi-organ failure; p =0.24). The change in type 2 fibres was more variable.

The data show that changes in rectus femoris cross-sectional area can be detected early in critical illness by ultrasound. Using this approach, it was possible to discriminate between patients with single organ and multi-organ failure in regard to muscle loss using this technique. It was not possible to discriminate between patient groups on the basis of fibre size changes anlaysed from biopsy samples.

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The effect of caffeine ingestion on skill maintenance and fatigue in épée fencers

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Scientific literature discussing caffeine supplementation is extensive. The ergogenic effect of caffeine through peripheral and central mechanisms on sports performance focuses predominantly on endurance sports [1,2]. Fencing is a high-intensity, intermittent sport demonstrating strong associations between the physical, perceptual and psychological demands of sport required to prevent central and peripheral fatigue [3]. This study aimed to explore the effect of caffeine ingestion on skill maintenance and perceived fatigue following exercise simulating the demands of a fencing competition. Seven elite fencers participated (3 female; 4 male; age 32 ± 6.2 years). Following a maximal test to exhaustion, fencers completed two further trials assessing accuracy and reaction times pre and post a fatiguing protocol designed to simulate the demands of the first round of a fencing competition. The reaction test used was the Stroop test. Skill testing involved 30 lunge-touché movements to hit a target replicating competitive attacks. 500ml placebo or 3mg/kg caffeine supplemented drink was administered after the initial reaction and skill tests in a single-blind crossover design. The fatiguing protocol involved the fencer performing a series of bouncing movements (8 seconds on 9 seconds off) with a standardised number of retreats, arm extensions and lunges. 6 fights were completed with a 6-minute rest between each fight. Capillary blood glucose and lactate levels were measured throughout the protocol and fencers rated their perceived exertion (arms, leg, whole body) using the Borg scale. Statistical analysis was a two-way (Treatment X Time) ANOVA for repeated measures (Minitab 15). There was no overall effect of caffeine on skill maintenance (F(1,24)=0.04; p=0.84), however there were significantly fewer misses during the skill test (F(1, 24)=1.37;p=0.04) in the caffeine trial. No effect of caffeine on the Stroop Test was demonstrated. A significant difference in perceived fatigue for the whole body was observed (F(1,96)= 15.33; p= 0.00) in the caffeine trial with a reduction in Borg scores being reported with caffeine. These results provide some support for maintenance of skill and reduction in perceived fatigue during high intensity intermittent sports. Central mediated mechanisms of caffeine appear to be supported from the results as a primary mechanism. Fencers need to continue to make individual decisions regarding caffeine supplements remaining aware that its use in sport continues to be monitored by WADA.


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Cardiorespiratory fitness does not affect pacing during a 30-min running time trial in humans

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Pacing is defined as the distribution of work during an exercise task (Abbiss & Laursen, 2008), and it is thought to be an important determinant of endurance performance. Previous studies have investigated the effects of duration/distance of the event, environmental cues, feedback and prior experience of the task on pacing during endurance exercise. Although it is well known that cardiorespiratory fitness (CF) has a strong influence on endurance performance (Bassett & Howley, 1997), its effect on pacing during endurance exercise is poorly understood. The aim of this study was to test the hypothesis that CF has a significant effect on pacing during a 30-min running time trial. (Lima-Silva et al., 2010)

Thirty recreational and amateur runners were divided into three different groups (high, mid, and low CF) according to their VO²max (61.5 ± 2.1, 54.8 ± 1.8, 49.2 ± 2.1 ml min⁻¹ Kg⁻¹ respectively, p < 0.001) measured during a preliminary incremental treadmill test. Afterwards, these runners performed a time trial on a motorized treadmill of which they could control the speed. Subjects were instructed to run as far as possible over 30 min, and were informed of the elapsed time. Speed, ratings of perceived exertion (RPE), and heart rate were recorded every three minutes throughout the time trial. ANOVA showed a higher performance for the High CF group (7301 ± 376 m, p<0.05) compared to Mid and Low CF groups ( 6967 ± 824, 6479 ± 667 m respectively). Two way repeated measures ANOVAs did not detect significant group x time interactions for speed (p =0.463), RPE (p =0.333), and heart rate (p =0.780). Significant main effects of time were found for speed, RPE, and heart rate (all p values < 0.001), but the only significant main effect of group was found for speed (p = 0.030) (Fig 1). Follow up tests show that all runners adopted a negative pacing strategy with a significant increase in speed at the end of the time trial. Both RPE and heart rate increased linearly during the time trial. These findings confirm that that athletes with high VO₂max achieve better running performance compared to those with lower levels of CF. However, contrary to the findings of Lima-Silva et al. (Lima-Silva et al., 2010), CF does not influence the distribution of work during the time trial (pacing). The identical time course of RPE suggests that runners of different CF levels choose different average speeds in order to avoid reaching maximal RPE and, thus, exhaustion, before the end of the time trial.


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Intramyocellular and extramyocellular lipids of human vastus lateralis muscle: effect of age, whole body adiposity, and muscle size

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It is known that the excess fat stored within muscle tissues in two forms; i.e. intramyocellular (IMCL) and extramyocellular lipids (EMCL) (1). Some studies reported that both IMCL and EMCL contents increase with total body fat mass and ageing (2). In contrast, it is paradoxically reported that high IMCL content is observed in endurance athletes (3). The precise physiological role of IMCL and EMCL remains uncertain. PURPOSE: The aim of this study was to confirm the interrelationship between age, whole body adiposity, and muscle size on the concentration of IMCL and EMCL. METHODS: Two hundred sixty-four male subjects participated (age range 17-82 years) in the study. Body mass index ranged from 16.4 to 33.3 kg/m2 (means ± SD, 23.1 ± 2.8 kg/m2). The present study was approved by the research ethics committee involving living human participants in Ritsumeikan University. Written informed consent was obtained from all subjects. For separate quantification of IMCL and EMCL, noninvasive localized proton magnetic resonance spectroscopy (1H-MRS) measurement of the right vastus lateralis was performed on a 1.5T MR system (Signa HDxt, GE Medical Systems). Multi-slice T1-weighted spin-echo images were acquired to guide the positioning of the volume of interest, and used for measuring the muscle CSA. Thereafter, the single voxel MRS measurements were performed using a PRESS sequence (TR/TE 2000/35 ms, 20 × 20 × 20 mm3, 32 acqs). The estimation of the muscle lipid concentration was accomplished by the LCModel software with customized calculation reported by Weis et al. (2009) (4). Whole body fat mass (%fat) was measured by dual-energy X-ray absorptiometry (Lunar Prodigy, GE Medical Systems). The measurement was performed after an overnight fast. Simple linear regression was applied to examine the relationships between age, %fat, muscle size and each of absolute concentration of IMCL and EMCL. Pearson’s regression coefficient was calculated. Multiple linear regression models with age, %fat and muscle CSA as independent variables were used to calculate the dependent variables. RESULTS: The concentrations of IMCL and EMCL were 9.5 ± 5.2 mmol/kg and 21.4 ± 20.7 mmol/kg (means ± SD), respectively. The EMCL level positively correlated with age (r=0.58, p<0.05) and %fat (r=0.51, p<0.05), and negatively correlated with muscle CSA (r=-0.52, p<0.05). The partial coefficient of correlation between %fat and EMCL was still significant when adjusted for age and muscle CSA (r=0.34, p<0.05). There was no significant correlation between IMCL and either age, %fat and muscle CSA. CONCLUSION: These results indicate that the amount of EMCL in the vastus lateralis is associated with the whole body adiposity, while IMCL is independent of whole body adiposity.


This work was supported by Grants-in-Aid from the Nakatomi Foundation (2011).

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Gene expression signature of Gamma hydroxybutyric acid (GHB) exposure in Human monocytic leukaemia THP-1 cells

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Gamma hydroxybutyric acid (GHB) is a natural neurotransmitter found in the brain in low concentrations. GHB has been used in general anaesthesia and is currently used to treat narcolepsy and alcoholism. The abuse of GHB, especially in date rape sexual assaults, has increased in recent years. GHB has a rapid rate of metabolism causing it to disappear quickly and criminal cases are often difficult to prosecute. This study is aimed at extending the window of detection of GHB beyond 12 hours by measuring the GHB-dependent changes in gene expression and finding robust surrogate markers of GHB exposure. Human monocytic leukaemia THP-1 cells were treated with 10 μM and 900 μM concentrations of GHB and gene expression after 24h exposure to GHB was evaluated using Agilent SurePrint G3 Human gene expression 8x60K arrays. Microarray data were analyzed by GeneSpring GX software and differentially expressed genes were identified. The results show that 900 μM GHB induces alteration in 2380 genes using P<0.05 and a fold change of >2 as criteria of significance and this number is reduced to 587 genes using P<0.001 and >2 fold change. Gene ontology (GO) enrichment analysis of the gene lists found in the GO cellular component the largest numbers of the altered genes were in the intracellular and membrane parts of the cell. In terms of GO molecular functions, the majority of altered genes coded for proteins and nucleotide binding sites and some of the altered genes affect the catalytic activity of steroid dehydrogenase enzyme. Quantitative real-time PCR analysis was carried out on the genes affecting steroid dehydrogenase activity to validate the microarray findings. The results show that GHB induces changes in gene expression in blood THP-1 cells and this information may be useful in finding markers for GHB exposure in forensic toxicology.


My appreciation is to Iraqi ministry of higher education (study sponsor) and my supervisor Dr. Elizabeth Ellis. Also, I would like to thank Dr Rothwelle Tate for his help.

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Heart rate complexity following high-intensity interval training is impaired in junior but not in senior athletes

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Introduction

Highly-trained runners need to endure workouts at intensities very close or above their Personal Best (PB) to induce adaptive changes and improve their performance. Unfortunately, these high-intensity trainings could also trigger undesirable negative stress effects, what increases the importance of thoroughly quantifying their training load (TL). Previous research have suggested that post-exercise Heart Rate (HR) dynamics may offer objective information on TL (1, 2). Therefore, our purpose was to investigate the effect of a high-intensity speed-endurance workout for 800-m runners (i.e. the “Broken 800’s”) on HR complexity, comparing senior to junior athletes.

Methods

19 male 800m runners, belonging to either Senior (n=10; PB800-m: 112.61±3.87 s) or Junior category (n=9; PB800-m: 120.92±3.33 s) participated in the study. Subjects performed 2x4x200-m, with a recovery period of 30 s between bouts and 15 min between the sets. Intensity was established for every subject at the 102% of his 800-m running velocity (i.e., according to his previous season best performance). Total time required to complete each 4x200-m was retained as a measure of performance. Heart interbeat intervals were recorded during 5 min using a Polar RS800 in a seated position before the warm-up (PRE) and 5 min following the first (POST1) and the second set (POST2) of the interval training. HR complexity was quantified by means of Sample Entropy algorithm (SampEn), utilizing Kubios HRV software. A repeated measures ANOVA followed by a Bonferroni post-hoc, with one within factor (‘fatigue’: PRE, POST1, POST2) and one between factor (‘category’: SENIOR vs JUNIOR), was employed to elucidate the effect of “Broken 800’s” on SampEn. The same statistical approach was used to analyze possible differences in performance.

Results

A significant effect was found for ‘fatigue’ factor and the interaction ‘category x fatigue’ on SampEn. Regarding fatigue factor, further Bonferroni pairwise comparisons showed that SampEn was higher at PRE compared to Post1 and Post2 (1.30±0.35; 0.90±0.32 & 0.96 ± 0.30; p<0.05); however, post-hoc analysis of the interaction between category and fatigue revealed that these differences remained significant only for junior athletes (p<0.05). Similarly, whereas junior’s performance was significantly worse in the second 4x200 (119.7±3.1 s vs. 122.6±3.3 s; p<0.05), there were no differences for senior athletes.

Conclusions

Our results showed that “Broken 800’s” have a greater disturbing effect on HR complexity of junior athletes (i.e., compared to senior athletes), despite individualizing running velocity. This is consistent with a significant performance decay in juniors during the second set of repetitions, unlike senior athletes. Therefore, this type of maximal speed-endurance workout should be used with caution on junior runners.

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Resting vagal tone is related to sympathetic activation and workload during a 600-m running test

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Introduction
Nunan et al. (1) found no association between resting Heart Rate Variability (HRV) and the increase in Heart Rate (HR) during a maximal exercise. Hynynen et al. (2), however, have pointed to a Cardiac Autonomic Resource Hypothesis, by which a greater resting vagal tone may allow a further sympathetic activation during a cognitive task (i.e., a higher reduction in vagally-mediated HRV indices); enabling, in turn, a better performance. Therefore, our purpose was to investigate whether resting vagal activity is related or not to sympathetic activation and workload during a 600-m running test.

Methods
19 male 800-m runners, belonging to either Senior [n=10; Season Best (SB800-m) 113.75 ± 4.08 s] or Junior category (n=9; SB800-m: 120.92 ± 3.33 s) performed a maximal 600-m run. Heart interbeat intervals (RR) were recorded during 10 min using a Polar RS800 in a seated position before the warm-up and during a 600-m running test. Afterwards, an artifact-free 5-min epoch from resting recording was analyzed using Kubios HRV software. Root-mean-square difference of successive RR intervals (RMSSD) and high-frequency power of RR intervals (HF) were examined as measures of vagal activity, and ratio of low-frequency to high-frequency power (LF/HF) retained as an index of sympathovagal balance (3). Meanwhile, according to Leeper et al. (4), difference between resting and peak HR achieved during the test was employed as a measure of sympathetic activation (∆HR). Mean velocity during the test was expressed as a percentage of each athlete’s SB800-m (%SB800-m) to obtain a relative measure of workload, inasmuch as the sample consisted of athletes of different age categories. A partial correlation, controlling for resting HR, was conducted to analyze possible relationships between RMSSD, HF, LF/HF, ∆HR and %SB800-m. Previously, HRV measures were logarithmically transformed to allow parametric statistical analysis.

Results
An inverse significant relationship was found between ∆HR and resting LF/HF (r=-0.53; p<0.05). Furthermore, %SB800-m displayed a significant positive association with resting HF (r=0.48; p<0.05) and a tendency towards significance with RMSSD (r=0.43; p=0.77).

Conclusions
Our results showed that those athletes with a greater dominance of vagal activity over sympathetic drive (i.e., lower LF/HF) before beginning the training, were capable afterwards of managing a further sympathetic activation during the workout. Moreover, those runners with a greater resting vagal tone (higher HF and RMSSD) achieved a higher relative workload during the 600-m running test. Thereby, current results confirm previous assumptions from Hynynen et al. (2); and as a foremost conclusion, it may be suggested that analysis of HRV prior to high-intensity workouts may provide coaches with a useful information in relation to athletes’ subsequent responses.

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Dynamic response in intramyocellular lipid after the localized exercise in human: a proton magnetic resonance spectroscopy study

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[Introduction] The modern lifestyle increases body fat in both obese and non-obese individuals. An increase in adipose tissue and decrease in muscle mass may further cause to decrease in peripheral uptake of glucose and insulin resistance. It is well known that exercises are efficient in reducing body fat and preserving fat-free body mass. There are a great number of studies with acute metabolic responses in whole body exercises. However, the metabolic responses especially in lipid to exercised muscle after the localized exercise have seldom been studied. In theory, a dynamic response of lipid oxidation in exercised muscle can be higher as compared to non-exercised muscle because exercising muscle needs more energy from within-the same muscle in order to maintain a level of ATP quickly. Nevertheless, there is no information available how the metabolic response occurs in intracellular lipid of exercised muscle after the localized repetitive muscle contractions. Magnetic resonance spectroscopy (MRS) offers to assess metabolism non-invasively. The purpose of this study was to use single voxel proton MRS to assess the lipid metabolic changes of the triceps muscle in young women before and after elbow extension exercise. Such knowledge will contribute to our understanding of local muscle metabolism in exercised muscle, which may provide new information to prevent metabolic disorders. [Methods] Twenty-one young healthy women volunteered for this study. The upper arm was assessed before and immediately after dynamic elbow extension exercise by a 1.5-Tesla Signa HDx MR scanner (GE Medical Systems). The entire upper arm was encased in a receive-only 4-channel shoulder coil. By using a water and lipid-suppressed double-spin echo point resolved spectroscopy (PRESS) sequence, intramyocellular lipid (IMCL) and extramyocellular lipid (EMCL) values of the triceps muscle were calculated. Subject performed dynamic elbow extension without carrying any load for 30 minutes long and paced at over 60 contractions per minute. Subject positioned at lying in the prone and forearm of the exercise arm was hanging down from the bed. Also, blood glucose and lactate were measured before and after exercise. [Results] After dynamic exercises, IMCL values of triceps muscle were calculated. Subject performed dynamic elbow extension without carrying any load for 30 minutes long and paced at over 60 contractions per minute. Subject positioned at lying in the prone and forearm of the exercise arm was hanging down from the bed. Also, blood glucose and lactate were measured before and after exercise. [Discussion] Changes in IMCL value of triceps and blood glucose after dynamic exercise of upper arm may suggest some local metabolic responses in exercised muscle. Further studies require the possibility of local lipid metabolic response in the exercised muscles. Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.
Upper limb muscle-bone asymmetries in elite junior tennis players

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Studies of athletes in sports favouring one limb (tennis, jumping, etc.) have shown large side-to-side differences in bone strength, muscle cross-sectional area (CSA) and strength in favour of the dominant limb. In sub-elite tennis players, bone strength differences correlated with grip strength and muscle CSA differences (1), supporting the idea of a strong influence of the muscle on bone. To examine an elite population, forty-one national-level tennis players (26m, 15f, mean age 13.4±1.7yrs) were recruited. Bone strength parameters were examined in both arms from pQCT scans at 4% (R4) and 60% (R60) distal-proximal radial length and 35% (H35) distal-proximal humeral length. Muscle CSA (MuscA) was also examined at R60 and H35. Peak force (F_{peak}) and power (P_{peak}) during a power press-up on a force platform and grip strength (GS) in both arms was measured, along with details of participant’s training history. Data were examined using paired T-tests to locate side differences, univariate ANOVA to examine age/gender effects and linear regression to examine the muscle-bone relationship - data shown as mean +/- SD. Large side differences (in favour of the racquet arm; P < 0.001) were found in MuscA at R60 (20.2±6.6%) and H35 (10.7±5.3%). At R4, total CSAs (Ar.tot) of both radius and ulna were greater (23.3±13.4% and 13.6±28.3% respectively; P < 0.001) in the racquet arm. Radial and ulnar bone mineral density (vBMD.tot) was also greater (15.9±10.8% and 9.1±14.3%) in the racquet arm. These size and density differences resulted in higher racquet arm total bone mineral content (vBMC.tot) in both radius and ulna (39.6±20.5% and 23.7±34.5%; P < 0.001). However, cortical BMC (vBMC.ct) side differences at R60 radius and ulna (19.2±8.8% and 13.8±7.3%) and H35 humerus (39.2±12.9%) were made up almost entirely of a greater cortical bone CSA (Ar.ct) (radius 18.9±8.1%, ulna 15.1±9.0% and humerus 39.8±13.5%) - all P < 0.001, with no significant difference in BMD. Compared to age-matched reference data (2) R60 Ar.tot was 18.3±8.7% greater and MuscA 14.1±18.3% greater than average in the racquet arm (P < 0.01), values in the non-racquet arm were not significantly different than average. Racquet arm muscle-bone ratio was lower in the ulna (3.2±9.0%; P < 0.01), radius (5.6±9.6%; P < 0.001) and humerus (20.2±7.5%; P < 0.001). There were strong correlations between MuscA and Ar.ct in both forearms and upper arms (Figure 1) (P < 0.001). F_{peak} (13.0±11.4%), F_{peak} (4.9±7.1%) and GS (24.2±26.9%) were all higher in the racquet arm (all P < 0.001). These results show an association of participation in elite-level tennis with side differences in bone strength, muscle size and force/power production. Whilst both arms showed a strong muscle-bone relationship, side differences in these relationships show that other factors aside from muscle size dictate exercise-induced bone adaptation.

Figure 1. Muscle-bone relationships in racquet and non-racquet arms.


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A comparison of age-predicted and measured maximal heart rates in humans exposed to acute normobaric hypoxia

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Athletes may incorporate periods of training at altitude (normobaric and hypobaric) in their annual training programmes. Training intensities are often controlled or prescribed using a percentage of an athlete’s maximal heart rate (HR_{max}). However, the direct measurement of HR_{max} may not be available, leading athletes and coaches to estimate it using age-related predictive equations with the two most commonly used being: 1: 220-age (1) and 2: 208-0.7*age (2). Research examining the response of HR_{max} to acute hypoxia while inconclusive; suggest HR_{max} may be altered suggesting that the use of predictive equations in hypoxia may not be valid. The purpose of the present study was to examine the accuracy of these two equations for predicting HR_{max} in adults exposed to acute hypoxia. Fifteen healthy volunteers (7 women; age 22±2 years; height 176±10 cm; body mass 73±14 kg; body mass index 23±3 kg.m²; VO₂max 45±7 ml.kg⁻¹.min⁻¹) participated in the study. Participants performed incremental cycle ergometer exercise tests (women: 20 W.min⁻¹, men: 25 W.min⁻¹) to exhaustion at sea level (SL) and in normobaric hypoxia replicating four altitudes (1000, 2000, 3000 and 4000 m) in a single-blind manner. Heart rate (Polar F1, Polar Electro) was measured continuously and the peak 10s rate taken as HR_{max}, oxygen saturation (S_{O₂}) was also recorded. Data were analysed with repeated measures ANOVA and paired t-tests. Measured HR_{max} was similar in all conditions including SL (182±13, 178±11, 177±9, 178±9, 176±11 b.min⁻¹) despite a reduction in maximal S_{O₂} with increasing altitude (95±5; 95±2; 92±2; 88±3; 82±4%; P<0.05). The HR_{max} predicted with both equations (Equation 1: 198±2 b.min⁻¹, Equation 2: 192±1 b.min⁻¹) was higher than measured for all conditions except SL (Equation 1: 9, 11, 12, 11, 13%; Equation 2: 6, 8, 9, 8, 10%; P<0.05). Commonly used age-related predictive equations overestimated HR_{max}, which could lead to the inaccurate prescription of training intensities. Such overestimations at altitude may result in a training programme that is unachievable for the athlete, effecting their motivation and exposing them to an increased risk of overtraining or acute mountain sickness (3). It is recommended that a direct altitude or hypoxia specific measure of HR_{max} is used for prescribing training intensities at altitude in the absence of a valid predictive equation.


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Differences in heart-specific genes expression and cardiac function in physically active rats after repeated administration of testosterone

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The effects of long-term treatment with testosterone on cardiac function are controversial. Inappropriate administration may result in the development of cardiac hypertrophy resulting in cardiac injury and failure. On the other hand clinical studies suggested beneficial effects of testosterone therapy on heart failure symptoms associated with increase of exercise capacity. Therefore, we tested the effects of testosterone, alone or in combination with voluntary running physical activity, on heart function, exercise capacity and expression of heart-specific genes. Testosterone isobutyrate (100 mg/kg, s.c.) was administered weekly Wistar rats (male, n=7-10 per groups, 330-450g). Physical activity was provided by freewheel running during eight weeks. Ventricular mass to body weight ratio was significantly increased by 15% in simple trained rats (2.81 ± 0.08 mg/kg vs sedentary controls (2.43 ± 0.27 mg/kg; p≤0.05). Application of steroid increases relative cardiac weight by 33% in sedentary (3.24 ± 0.12mg/kg) and 39% physically active rats (3.38 ± 0.05mg/kg) in all cases p<0.05 vs. sedentary controls). Neither running (137.71 ± 4.77mmHg nor testosterone (131.13 ± 5.25mmHg) showed effect on sBP (control 127.42 ± 4.97mmHg) were determined using left ventricular catheterization under 2,2,2-tribromoethanol anaesthesia/analgesia (15ul/g 2,5% solution i.p.). Interestingly, their combination (139.62 ± 3.17mmHg) slightly (13%) but significantly increased arterial dBP (p≤0.05). Running itself increased parameters of left ventricular function and the simultaneous application of testosterone accentuated this increase (LVP by 12%, δP/δtmax by 31% and δP/δtmin by 33%, p≤0.05 vs. sedentary controls) at stable heart rate. Testosterone-treated rats ran a significantly longer total distance compared to non-treated group (228 km vs. 81 km). The QRSmax were significantly (p≤0.05) higher in the testosterone treated and physically active rat (1.46 ± 0.09mV) compared to controls (0.94 ± 0.17mV) and physically active rats (1.22 ± 0.06mV). These functional changes were independent to the expression of analysed genes (Cx43, ryanodine receptors, actin, as well as of myosin regulating genes) remained stable in cardiac tissue. Only, cardiac expression of STAT3 gene was significantly increased after testosterone treatment (+45% and +67%, resp., p≤0.05 vs. sedentary controls) independently to physical activity of rat. Taken together, testosterone showed potential to induce hyperdynamic cardiac function. It could be useful in therapy of cardiac failure but other hand testosterone also increase blood pressure what might be later responsible for development of cardiac hypertrophy and associated with increase of cardiovascular risk.

Supported by VEGA 1/0503/11 and 1/0786/11

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The effect of exercise on executive function – investigating an alternative explanation for “runner’s high”

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The exercise induced transient hypofrontality theory (eTHT) has been proposed as an alternative neurophysiological explanation for the mood and cognition enhancing effect of aerobic exercise (1), popularly known as “the runner’s high”. In light of the computational demands of movement, eTHT hypothesises that hypoactivity occurs, particularly in the prefrontal cortex during exercise. The prefrontal cortex is involved in executive function (2). Connor’s Continuous Performance Test (CCPT) was used to investigate changes in attention and executive control during and post treadmill running exercise in young healthy volunteers (n=30, 15 male, 15 female). Subjects performed a VO2max test to assess aerobic capacity and maximum heart rate (MHR). In separate sessions, for which the order was randomised subjects performed CCPTs at rest and during low intensity (LI; 63% MHR) and moderate intensity (MI; 75% MHR) treadmill running exercise. In a second phase of the study subjects performed isocalorically matched exercise bouts, in separate sessions in a randomised order, of LI, MI and high intensity interval training (HIT). The HIT session consisted of 4x4 intervals: 4x4 min 90% MHR with 3 min recovery at 60-70% MHR. For the statistical analysis repeated measure ANOVAs were done for both the during exercise and the post exercise tests.

Preliminary statistical analyses of the CCPT results gave the following results. Values are means ± S.E.M., compared by ANOVA. Choice reaction time (HitRT) increased significantly during exercise (LI 323.2 ± 34.1 ms; MI 324.1 ± 34.4 ms) compared to at rest (309.1 ± 39.4 ms; p≤0.005). Commission errors were not significantly different during exercise, compared to at rest but showed a trend to increase during exercise compared to baseline (p=0.08).

The findings for the post exercise CCPTs showed that choice reaction time (HitRT) decreased significantly from rest (309.1 ± 39.4 ms) to post exercise levels (LI 282 ± 36 ms; MI 276.6 ± 33.95 ms; HI 275 ± 30.9 ms) in an exercise intensity dependent, linear fashion (p≤0.0001). Commission errors were not significantly different post exercise, compared to at rest but showed a linear increasing trend with intensity (p=0.075).

The main preliminary findings of the current study are that volunteers made the same level of commission errors during exercise and at rest, regardless of exercise intensity. The lack of change in impulsive errors during exercise paired with increasing reaction times during running may imply reduced activity in the prefrontal cortex during exercise, consistent with eTHT. According to the post exercise CCPT results HitRT was decreased post exercise compared to rest, with a linear trend for exercise intensity (p≤0.0001). This may imply prefrontal cortex hyperactivity post exercise and thus has implications for elite performance in a variety of sports.


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Reliability and symmetry of Myoton-Pro measurements of viscoelastic properties of biceps brachii in young adult males

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Maladaptation of skeletal muscle may occur in response to chronic training overloading. Reliable quantification of changes in viscoelastic properties of muscle in a sports setting is challenging. The Myoton-Pro offers a portable, handheld method of measuring viscoelastic properties of muscle based on dampened oscillations of muscle tissue. The intra-rater reliability of Myoton-Pro measurements and symmetry of the viscoelastic properties of the biceps brachii (BB) were recorded in a sample of convenience of healthy, young adult males aged ≥18 to ≤35 years (n=21) who are right-hand dominant. The Myoton-Pro was used to measure frequency (Hz), decrement and stiffness (Nm) of dominant and non-dominant BB on two testing sessions, seven days apart, by the same rater. Within-day and between-day reliability was assessed using intraclass correlation coefficients (ICC3,1), the standard error of measurement (SEM), the minimal detectable change (MDC) and Bland-Altman graphs. Paired t-test (α level =0.05) compared symmetry between body sides. Within-day intra-rater reliability was very high (ICC 0.90–0.99) for frequency, decrement and stiffness. Between-day comparisons were moderate-high (ICC= 0.68-0.81) for frequency; low-moderate ICs (0.39-0.59) for decrement; high-very high (ICCs 0.88-0.94) for stiffness. No significant difference was found between sides. (p=0.80-0.93). SEM =0.1-0.5Hz; 0.04 0.09 decrement, 3.2-8.7Nm. MDC =0.4-1.3Hz, 0.1-0.25decrement, 8.8-24.1Nm. These results conclude that the Myoton-Pro offers an objective, non-invasive and reliable measurement of frequency and stiffness of biceps brachii in young adult males. Measures of decrement failed to meet the commonly accepted threshold of reliability required for valid use of human assessment tools (ICCs 0.7). Coaches could use the Myoton-Pro to identify true change in muscle to detect abnormalities and aid injury prevention.

We thank Dr Aleko Peipsi of Muomeetria Ltd for training in use of the Myoton Pro, Dr. Peter Nicholls for statistical support and study participants.

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Effect of hypoxia severity on peripheral fatigue development and subsequent exercise performance during exhaustive intermittent cycling

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Under acute severe hypoxia, central nervous system fatigue may limit performance during high-intensity continuous exercise, while also curtailing the extent of peripheral fatigue development. Whether this scenario occurs during whole body high-intensity, intermittent exercise is unknown. The aim of this study was to examine the effect of hypoxia severity on peripheral fatigue development and subsequent exercise performance during exhaustive intermittent cycling. Eleven well-trained cyclists performed an intermittent cycling exercise until exhaustion in different levels of hypoxia (set 1) followed 30 min later by the same exercise to exhaustion in normoxia (set 2). Each set consisted of the maximum number of sprint bouts – 15 s at 30% of the anaerobic power reserve (643 ± 38 W) with a fixed pedaling frequency of 110 rpm. Set 1 was performed under normoxic (simulated altitude/end-exercise arterial O2 saturation = 0 m/96%), moderate (2200 m/90%) and severe hypoxic (4200 m/79%) conditions, in a counterbalanced order. Maximal isometric voluntary contractions of the knee extensors (MVC torque) and mechanical responses to supra-maximal femoral nerve stimulations (peak twitch torque) were obtained at rest and 7 min after each set. During set 1, performance was dependent on hypoxia severity (23 ± 9, 16 ± 6 and 10 ± 3 sprint repetitions in normoxia, moderate and severe hypoxia, respectively; p<0.001), whereas the number of sprint bouts completed did not differ during set 2 (12 ± 6, 14 ± 8, 18 ± 15; p>0.49). Compared with baseline, reductions in peak twitch amplitude post-set 1 were of similar magnitude among all conditions (-53.0%, all conditions compounded; p<0.001), without any further changes following set 2. At exhaustion following set 1, strength loss compared to baseline was similar among the normoxic and moderate hypoxic conditions (-10.8% and -11.4%; both p<0.05), while it tended to be smaller in severe hypoxia (-8.7% p=0.057). However, voluntary strength capacity was further decreased in all conditions after set 2 (-5.7%, all conditions compounded; p<0.05). Despite performance being hypoxia severity-dependent during the first set, there was no significant performance difference in a subsequent normoxic exercise of the same nature. At task failure, peripheral fatigue was substantial compared with baseline but similar across conditions, which was associated with rather consistent decreases in voluntary strength. After set 2, voluntary strength was further decreased in all conditions despite no additional peripheral fatigue development. These results suggest that an end-exercise arterial O2 saturation value of 79% or above is probably not severe enough to elicit a shift from a predominantly peripheral origin of fatigue to a hypoxia-sensitive source of inhibition within the central nervous system.


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Effect of hypoxia on thermal hyperemia in humans

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Local heating of the skin produces a well established bi-phasic (initial peak, IP; plateau phase, PP) response in skin blood flow that has been suggested to achieve maximal values (BF$_{sk_{max}}$) when temperatures up to 44°C are used (Cracowski et al. 2006). It is reported that sensory nerve fibres and endothelial derived nitric oxide predominantly mediate IP and PP respectively (Minson, 2010). Therefore, thermal hyperemia provides a tool to examine the mechanisms of vascular reactivity under different experimental conditions. Hypoxemia is known to increase cutaneous blood flow (Simmons et al. 2007), however, its effect on the phases of thermal hyperemia are unknown.

With local ethical approval and written informed consent, 12 subjects (9 males and 4 females) were exposed to normoxia (NO, 21% O$_2$) and hypoxia (HY, 12% O$_2$) for 9 hours in a temperature controlled environmental chamber. Skin blood flow (BF$_{sk}$), oxygen saturation (SpO$_2$), heart rate (HR) and mean arterial blood pressure (MAP) were obtained at 1 and 9 hours during both exposures. To obtain an index of BF$_{sk}$ a laser Doppler probe was attached to the volar aspect of the forearm while subjects were supine. Subsequent to obtaining baseline BF$_{sk}$ (clamped at 33°C), thermal hyperemia was induced by increasing skin temperature (T$_{sk}$) via a heating unit at a rate of 0.5°C every 5 seconds up to 42°C and held constant for 30 minutes. Thereafter, T$_{sk}$ was increased to 44°C for 10 minutes to achieve BF$_{sk_{max}}$. BF$_{sk}$ is presented as perfusion units (PU), cutaneous vascular conductance (CVC; calculated as BF$_{sk}$/MAP) and also normalised to maximal skin blood flow (%CVC$_{max}$). Values are means (SD) compared by paired t-test and fully repeated measures 2 (1h vs 9h) x 2 (normoxia vs hypoxia) ANOVA.

By design, hypoxia decreased SpO$_2$ (NO, 99(1)% vs HY, 87(4)%; \(P=0.00\)), which resulted in increased HR (NO, 58(8) vs HY, 81(11)beats/min; \(P=0.00\)) and MAP (NO, 88(10) vs HY, 92(13)mmHg; \(P=0.038\)). No differences in baseline BF$_{sk}$ were detected between NO and HY (NO, 15(7) vs HY, 13(7)PU; \(P=0.65\)). In contrast IP (NO, 121(33) vs HY, 153(39)PU; \(P=0.00\)), PP (NO, 166(39) vs HY, 204(39)PU; \(P=0.00\)) and BF$_{sk_{max}}$ (NO, 198(49) vs HY, 229(43)PU; \(P=0.05\)) all were elevated during HY (see figure 1). However, when expressed as CVC, IP (NO, 1.5(0.5) vs HY, 1.9(0.4)CVC; \(P=0.01\)), and PP phase (NO, 2.1(0.6) vs HY, 2.4(0.6)CVC; \(P=0.01\)) remained elevated whilst CVC$_{max}$ (NO, 2.5(0.7) vs HY, 2.7(0.6)CVC; \(P=0.31\)) was not. In contrast, when expressed as %CVC$_{max}$, IP remained increased (NO, 61(9)% vs HY, 70(9)%CVC$_{max}$; \(P=0.02\)) but the PP was unaltered (NO, 78(8)% vs HY, 80(9)%CVC$_{max}$; \(P=0.43\)).

These data indicate that acute hypoxemia alters thermal hyperemia. Therefore, thermoregulation and exercise capacity may be affected during periods of exposure to hot and hypoxic environments.

Figure 1. Skin blood flow in response to local heating during normoxia (21% O$_2$) and hypoxia (12% O$_2$)

Minson CT (2010). J Appl Physiol 109, 1239-1246

We would like to thank all participants for taking part in the study.

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A bilateral deficit in explosive, but not maximal force production

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INTRODUCTION: The combined force production of the lower limbs during synchronous bilateral contractions (BL) has been reported to be lower than the summed force production of each limb when performed unilaterally (UL), and is known as the bilateral deficit (BLD) [1,2]. Although, the BLD during maximal voluntary contractions (MVCs) has received considerable attention there has been little investigation of explosive contractions (EXP). Further, the investigation of BLD may have been confounded by contrasting UL performance with either: (i) single limb performance in a BL situation; or (ii) combined BL performance, that may under represent the performance of one limb in a bilateral situation (i.e. the best effort of both legs when measured together may not represent the best effort of either leg in the bilateral situation).

METHOD: Thirteen healthy untrained males (24 ± 4 yrs) performed a series of isometric MVCs (3 × 3-s duration, 30-s rest; 2-min between conditions) and EXP (10 × 1-s duration, 20-s rest; 2-min between conditions) in 3 conditions: UL, with each leg, and BL. Contraction order was randomised (UL dominant or non-dominant, BL, remaining UL limb). Additionally, UL and BL twitch and octet (8 pulses at 300 Hz; evokes the muscles maximal rate of force development [RFD]) contractions were electrically evoked via supramaximal stimulation of the femoral nerve. EMG was assessed from three superficial agonists. Two separate strain gauges were used to measure maximum voluntary force (MVF) during the MVCs and explosive force (EF) at 50, 100 and 150 ms during EXP. RFD and EMG were measured over consecutive time windows (0-50, 50-100 and 100-150 ms) from their respective onsets. Peak force (PF) and peak RFD (pRFD) were reported from the evoked contractions. Performance during UL contractions was compared to: single limb performance measured during the BL contractions (BLUL); and combined BL performance (BLBL).

RESULTS: There was no BLD for MVF (UL, 1438 ± 202; BLUL, 1452 ± 212; BLBL, 1444 ± 217 N, ANOVA, P=0.551). BLUL and BLBL had similar EF at 50 ms to UL, but at 100 ms BLUL (-8.9%, P=0.036) and BLBL (-11.5%, P=0.003) had lower EF than UL. EF at 150 ms was lower for BLBL only (-9.6%, P=0.029). RFD 50-100ms was lower for both BLUL (-13.3%, P=0.01) and BLBL (-15.6%, P=0.001) compared to UL. There were no differences in EMG between UL and either BL measurement for either type of contraction (P≥0.107). There was a BLD in twitch PF for both BLUL (-8.9%, P<0.001) and BLBL (-10.2%, P<0.001), but not twitch pRFD, or octet PF or pRFD.

CONCLUSION: There was a BLD during explosive but not maximal isometric contractions, and this occurred due to lower RFD 50-100 ms. There was no difference in muscle activation, but twitch PF was lower during BL contractions, which is a novel finding and may help explain the BLD in explosive force production.


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Myocardial blood flow, oxygen extraction and consumption, and efficiency in elite endurance athletes and control subjects at rest and during exercise

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In our previous study we reported that pronounced left ventricle (LV) hypertrophy shows neither impaired or supranormal blood flow reserve (Heinonen et al. 2008 J Physiol). In the present study we aimed to elucidate the characteristics of athlete’s heart further by applying [15O]-labelled radiotracers and positron emission tomography and measured myocardial blood flow (MBF), oxygen extraction (MOE) and consumption (MVO2), and efficiency in 13 highly trained male endurance athletes and 13 control subjects at rest and during supine cycling exercise (100 watts) by established methods and principles (Iida et al. 1996 Circulation; Laine et al. 1999 Circulation). Cardiac ultrasound was also performed and radial artery was cannulated for measurements of arterial oxygen content and energy substrates. Many of athletes (30 ± 5 yrs) whose VO2max in bicycle test was 60 ± 3 ml/kg/min, had participated in Olympics and various World cups in running, CC-skiing, cycling and walking, but control subjects (30 ± 5 yrs, VO2max 40 ± 5 ml/kg/min) exercised regularly less than three times per week. LV mass (138 ± 18 g/m2, p<0.001) was higher and LV workload (1.6 ± 0.5 and 4.1 ± 1.0 mmHg/mL/min/g, p<0.01 in both) and MBF (0.9 ± 0.4 and 1.8 ± 0.4 ml/g/min, p=0.51 at rest and <0.01 during exercise) were lower in athletes both at rest and during exercise, respectively, compared to untrained men (93 ± 12 g/m2, 2.5 ± 0.9 and 7.1 ± 2.0 mmHg/mL/min/g, 1.2 ± 0.9 and 2.3 ± 0.4 ml/g/min, respectively). MOE increased in response to exercise in both groups, but was also always higher in athletes (71 ± 22 vs. 63 ± 11 % at rest and 88 ± 11 vs. 73 ± 10 % during exercise, respectively, both p<0.05). As a result, MVO2 per gram of myocardium was similar between athletes and controls both at rest and during exercise (0.12 ± 0.06 and 0.32 ± 0.08 ml/g/min in athletes and 0.15 ± 0.05 and 0.35 ± 0.08 ml/g/min in controls). Myocardial efficiency was similar between the groups at rest (13.4 ± 11.5 % in athletes and 12.3 ± 2.8 % in controls), but was significantly lower in athletes during exercise (9.7 ± 3.6 % vs. 14.3 ± 5.1 %, p<0.05). Arterial glucose was essentially comparable between the groups, but arterial free fatty acids were significantly higher in athletes at rest and especially during exercise (0.42 ± 0.23 vs. 0.25 ± 0.16 mmol/L), and lactate increased only in control subject in response to exercise (from 1.0 ± 0.2 to 2.8 ± 1.3 mmol/L). In conclusion, elite endurance athletes have lower myocardial blood flow, higher oxygen extraction, but unchanged oxygen consumption at rest and during exercise. Moreover, it is likely that higher arterial free fatty acid levels, and thus their utilization, partly explains the noticed lower myocardial efficiency during applied exercise low for elite athletes but demanding for untrained control subjects.

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Intermittent hypoxic training - should sportsmen really perform it?

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Intermittent hypoxic training (IHT) gained popularity as a method of altitude acclimatisation and thus as potentially beneficial for sports performance. IHT is based on the idea that short, intermittent, intense hypoxic stimuli evoke similar or stronger physiological responses than standard altitude acclimatization, which may in turn prove ergogenic. Extensive validation of IHT is, however, still lacking and was therefore the aim of the present study.

Fourteen healthy, well trained, male junior cyclists participated in the study, which was performed in the middle of their competition period. During the course of the study, their training regimen remained unchanged. Seven subjects performed IHT one hour daily, five days per week, for four weeks, and seven acted as controls. During every IHT session, the IHT group inspired a hypoxic gas mixture (FiO₂=11.4%; simulated altitude of ≈4500 m) for seven minutes, which was followed by three minutes of normoxic breathing. The control group did not perform any IHT. The protocol of the study was approved by the Ethics Committee of the Republic of Slovenia.

In the IHT group, oxygen saturation (SaO₂; %) was measured during every bout and every session of IHT. Haematological parameters (Hb, Htc, erythrocytes, reticulocytes, S-Fe, S-ferritin) were measured at the beginning of the study and after the four weeks. Incremental cycle ergometry at sea level and at simulated altitude of 3000 m (FiO₂=13.3%) was performed in a balanced manner by both groups, both, before and after IHT. Oxygen consumption (VO₂; ml/min kg), oxygen saturation (SaO₂; %), heart rate (HR; bt/min), ventilation (L/min), and ratings of perceived exertion (modified Borg’s scale) were measured throughout cycle ergometry. Blood lactate (L:mmol/L) and maximal work load (WLmax; W) were determined at the end of cycling. Multifactorial ANOVA for repeated measures on one factor was used for statistical analysis and the level of p<0.05 was adopted as statistically significant.

Six subjects of the IHT and four of the control group completed the study. Incremental cycle ergometry, both, at sea level and at simulated altitude of 3000 m, provided identical results before and after the IHT, in both, IHT and control group. No changes were observed in HR, SaO₂, WLmax, VO₂max, or ratings of perceived exertion, in neither control nor IHT group. Similarly, haematological parameters remained virtually unchanged in both, control and IHT group, when values obtained prior to and after IHT intervention were compared.

The results of the present study suggest that the use of IHT for four weeks does not result in altitude acclimatisation and induces no physiological effects. The current knowledge of molecular responses associated with altitude acclimatisation speaks in favour of the present results. Proposing IHT as an ergogenic method seems highly speculative and should be thoroughly reconsidered.

<table>
<thead>
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<th>AVG (SD)</th>
<th>Control group</th>
<th>IHT group</th>
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<tbody>
<tr>
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<td>Before</td>
<td>After</td>
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<tr>
<td>Erythrocytes (10¹²/L)</td>
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<td>Hemoglobin (g/L)</td>
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<td>141 (9)</td>
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<td>0.41 (0.02)</td>
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<tr>
<td>Reticulocytes (10⁶/L)</td>
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<td>26 (11)</td>
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Effects of short-term low-load hypoxic resistance exercise training to failure on quadriceps mass and performance

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Low-load resistance exercise training with reduced muscle blood flow (ischemic training) has been shown to induce gains in muscle mass and strength comparable to heavy-load training (Takarada et al., 2000; Abe et al., 2006). As we recently demonstrated, it can also enhance muscle endurance and oxygen delivery (Kacin & Strazar, 2011), which may be useful in preventing muscle atrophy and deconditioning after joint injury or surgery. However, vascular occlusion with cuffs is limited to extremities and may augment oedema formation, compress nerves and induce discomfort. Given that short-term hypoxia is most likely a key trigger for adaptation, we hypothesised that inhalation of normobaric hypoxic gas mixture during low-load resistance exercise can give similar results with fewer hindrances.

Seven healthy males performed knee-extension exercise for 4 weeks (4 sessions a week) at 15% 1RM. Subjects trained one leg (H-leg) while inhaling hypoxic gas mixture (FO2 = 12 %), whereas the other leg (N-leg) was trained in normoxic conditions. Values are means ± S.D., compared by ANOVA. Levels of mixed blood oxygen saturation were 84 ± 2 % and 97 ± 1 % during hypoxic and normoxic exercise, respectively. After training, no significant increase in quadriceps maximal voluntary isometric contraction force at 90° of flexion and cross-sectional area (MRI) on either of the two legs was observed. Number of full-ROM knee extensions at 15% 1RM to failure (endurance test) increased (P<0.01) by 28 ± 5 % in H-leg and 34 ± 5 % in N-leg when performed in normoxia, and 22 ± 6 % in H-leg and 26 ± 5 % in N-leg when performed in hypoxia. Exercise-induced decrease in oxygenated haemoglobin concentration (near-infrared spectroscopy) in v. lateralis muscle during normoxic endurance tests was attenuated (P<0.025) by 5.69 ± 0.51 μM % in H-leg and 5.57 ± 0.44 μM in N-leg, whereas total haemoglobin concentration was increased (P<0.025) by 5.52 ± 0.28 μM % in H-leg and 5.02 ± 0.24 μM in N-leg. A transient increase (P<0.05) in RMS EMG amplitude of rectus f. was noted in both legs during the normoxic endurance test. In conclusion, inhaling hypoxic gas mixture during low-load resistance exercise does not enhance muscle mass, performance, activation or oxygen delivery more than exercise performed in normoxia. Exercise stimuli for positive muscle adaptation during short-term hypoxic exercise training is clearly not comparable to the stimuli induced by equal ischemic exercise training (Kacin & Strazar, 2011). Differences in level of tissue hypoxia and local metabolite accumulation may play an important role in this regard.


We would like to thank Gasper Podobnik and Nina Cvetek for their valuable assistance with data collection and analysis. This study was supported by a Slovenian Research Agency postdoctoral grant (Z3-9625) and Slovenian Sports Foundation grants (RR-07-39 and RR-08-512).

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Bioelectrical activity of the brain, cardiac autonomic profile and element balance in elite soccer players

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The aim of this study was to examine functional state of the central nervous system (CNS), cardiovascular system (CVS) and autonomic nervous system (ANS) related to the macro- and microelements content in elite soccer players in background exposure.

20 professional soccer players were examined (mean age 19 years) for electroencephalogram (EEG), evoked and even-related potentials (EP and ERP); heart rate variability (HRV: time and frequency domain parameters), which reflects the autonomic balance and cardiovascular parameters (stroke volume (SV), cardiac output (CO), total peripheral resistance (TPR), blood pressure (BP).

Concentration of 24 chemical elements determined in hair by X-ray spectrophotometry was within normal ranges in the studied group compared to the control group of non-athlete healthy individuals (n=36) of same age where concentration of essential elements was significantly lower (0.01<p<0.05 Mann-Whitney U-test).

Spearman correlation analyses showed that most of the significant correlations for CNS parameters in athletes were revealed between hair Zn, Mo, As content and spectral power of all the EEG rhythms (0.40<rs<0.58; 0.01<p<0.05), indicating that Zn has influence mostly on basic brain’s electric activity and higher levels of Mo are associated with lower excitability (beta1 and beta2 frequency: 0.43<rs<0.47; p<0.05).

While for the cardiovascular parameters (SV, CO, TPR, systolic BP) recorded at rest and after exercises associations were determined with hair Mo, Fe, Ca, Mn, Zn and Cu levels (0.49<rs<0.69; p<0.05). Whereas HRV parameters revealed correlation only with Ca and Zn (0.41<rs<0.59; p<0.05). Correlation between hair Ca and RRNN, SDNN, HF at rest and after exercises suggested that calcium affects parasympathetic nervous system more strongly, while Zn tend to decrease HRV (SDNN) at higher concentrations of this element (rs=-0.42; p<0.05).

These results support associations of the macro- and microelement balance with EEG components and HRV characteristics. Future studies are needed to clarify the interaction among different elements and their neurophysiological effects in athletes.

Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.
Correlation between fitness and body size in handball players

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This study aims to determine the relationship between body size and fitness characteristics of Portuguese handball players. A total of 210 male handball players (age, 23.73±5.25 years) participated in this study. Two anthropometric measures were taken from each player, i.e., body mass (in kg) and stature (in cm). Fitness profiling considered 18 variables: 30-m sprint time (30-m, s); (2) Squat jump (SJ) and countermovement (CMJ) height, height ratio and Pavg; (3) SJ adapted to arms (SJA) and CMJ adapted to arms (CMJA), height and height ratio; (4) Handgrip indices, namely dominant, non-dominant, mean score of dominant and non-dominant (mean) and difference between dominant and non-dominant; (5) Number of executions (#) in sit-up test (in 60-s); (6) Distance (m) and the position (1, <1000-m; 2, [1000-m;1300-m]; 3, [1300-m;1600-m]; 4, ≥1600-m) in Yo-Yo Intermittent Endurance Test - Level 2 (Yo-Yo IE2). The Pearson product moment correlation coefficient was used to assess the relationship between selected parameters. The results of the present study show that heavier handball players performed worse in 30-m sprint, vertical jump height, sit ups and Yo-Yo IE2 distance. Moreover, players with higher body mass or stature performed better in handgrip strength (dominant, non-dominant and mean) and average power in vertical jump. However, the indexes in jump height ratio (SJ and CMJ) were inferior. This is in favour of strength and power activities but, represents a limiting factor for weight-bearing activities such as running endurance efforts. The present study suggested that stature is the most significant predictor of vertical jump performance. However, results don’t support that body mass is the most significant predictor of 30-m sprint performance. Nevertheless, according to the gained results, it seems that, in handball players, fitness performances were highly dependent of individual anthropometric characteristics.

Pearson Correlations between body size variables and fitness variables

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**, p<0.01 level (2-tailed); *, p<0.05 level (2-tailed).

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A tissue engineered human skeletal muscle construct to study exercise related phenotypic alterations

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Regulation of skeletal muscle mass and phenotype is critical in order to optimise training and performance in numerous athletic disciplines. However, whilst in vivo models used to examine skeletal muscle regulation and adaptation have given us some understanding of this field, in vitro studies allow for deeper exploration of the processes governing skeletal muscle growth and phenotype. Most in vitro studies in this discipline utilise two dimensional cultures, or use cells from non-human sources, bringing into question the validity of the results when related to humans. The purpose of this work was to temporally examine the maturation and bio-mimetic structure of human muscle derived cells when in both two and three dimensional culture. 4 x 10⁵ human muscle derived cells were seeded into six well plates (2D) or onto a fibrin gel matrix (3D) which self assembled over time (1). The cells were maintained in DMEM with 20% FCS until confluent, at which point constructs were switched to DMEM with 2% FCS and 10ng/ml IGF-1 to encourage myotube formation. At 7, 14 and 21 days RNA was extracted using the TRizol® method and mRNA expression of myosin heavy chain (MYH) isoforms was detected using RT-qPCR and analysed using the 2⁻ΔΔCT method. Myotube diameters were measured using image J software and over 30 myotubes were counted from each condition. n=4-6 for each condition. MYH expression was analysed by ANOVA, and morphology by t-test. In 2D, myotubes began to pull off the plate surface after 14 days in culture, and therefore analyses were only conducted at 7 and 14 days in 2D cultures. In 2D culture, immature (MYH 3 and 8) and slow (MYH 7) MYH relative mRNA expression was decreased at 14 days versus 7 days (p<0.05), whereas MYH 2 showed no change (0.87 to 0.98,) and MYH 1 expression showed a non-significant mean increases in expression (0.96 to 1.38, p=0.13) . In 3D, all MYH transcripts were lowly expressed at 7 days, but gradually increased in expression at 14 days before peaking significantly at 21 days (p<0.001). At 21 days in 3D culture, all MYH transcripts were significantly increased versus all other time points across conditions (p<0.001). Myotube width was significantly greater in 2D than in 3D (45.21 ± 23.62 vs 23.41 ± 4.81, p<0.001), however in 3D the myotube width was far less variable (range 10.88-101.89 in 2D vs. 15.06-34.01 in 3D). In addition, 52% of myotubes were branched in 2D compared to a complete lack of branching in 3D culture. This data demonstrates that human muscle derived cells can be cultured for longer in 3D versus 2D and at 21 days show advanced maturation based on MYH transcripts. Morphologically 3D culture is also more biomimetic than 2D, showing similar characteristics to in vivo muscle. Phenotypic changes in human skeletal muscle can now further be investigated using this model which is amenable to electrical stimulation.


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Is exercise tolerance limited by muscle fatigue in humans?

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It has been recently challenged the long-standing assumption that muscle fatigue causes exhaustion during high-intensity aerobic exercise. Indeed, in spite of a drop in maximal voluntary cycling power after exhaustive cycling exercise, evidence shows that human subjects are still able to produce immediately after exhaustion a power output three times higher than that required by the task. Some Authors have argued that the results by Marcora and Staiano failed to describe what actually happens at the point of exhaustion. In fact, the crucial power-velocity relationship was not addressed because of the different cadence between exhaustion and the following maximal voluntary cycling power assessment (around 40 vs 137 rpm). In addition, a possible recovery of power might be occurred during the 3-4 seconds time delay necessary for changing the mode of the ergometer between the two conditions. Aim of this study was to measure maximal voluntary cycling torque (MVCP) before and immediately after a time to exhaustion cycling exercise (TTE). Participants (n=11) were required to cycle at a cadence of 60 rpm during a TTE at 80% of their predetermined peak aerobic power and exhaustion was defined as the inability to maintain 60 rpm for more than 5 seconds. MVCP was measured in isokinetic mode with the cadence fixed at 60 rpm, before and immediately after the TTE avoiding any time delay between the two conditions. Maximal voluntary contraction (MVC), neural and contractile properties of the quadriceps were investigated using femoral nerve electrical stimulation before and after the MVCP pre and post TTE. Electromiography was recorded during the MVCPs and TTE. Values are means ± SD. Repeated measures ANOVA (followed up by Bonferroni test) and paired t tests were used for the analysis. All data are referred to the right leg. Peak torque during MVCP pre and post decreased by 34% but was still two times higher than the torque required by the TTE (p<0.001) (Fig 1). EMG activity of the vastus lateralis muscle during pre and post MVCP resulted unchanged (p=0.127). On the contrary, EMG at the end of TTE was found to be 49% significantly lower than that found in the MVCP post. Two minutes after the end of the MVCP post a decrease in MVC (p<0.01) with unaltered voluntary activation (p=0.150), compared to baseline values, was showed. At the same time all parameters of muscle contractile properties were significantly decreased (all p values < 0.001). According to Marcora and Staiano, these results challenge the assumption that muscle fatigue causes exhaustion during high-intensity aerobic exercise. The higher EMG activity immediately after the TTE suggests that central fatigue is far from being a factor limiting this type of aerobic exercise. These results further suggest that exercise tolerance is ultimately limited by perception of effort.

Effect of time to exhaustion cycling exercise on the torque production during a maximal voluntary cycling power test. MVCP pre, maximal voluntary cycling torque before the time to exhaustion cycling exercise; MVCP post, maximal voluntary cycling torque immediately after the time to exhaustion cycling exercise; TTE, time to exhaustion cycling exercise. * Significant difference between TTE and MVCP pre (post hoc Bonferroni, p<0.001), # Significant difference between TTE and MVCP post (post hoc Bonferroni, p<0.001), § Significant difference between MVCP pre and MVCP post (post hoc Bonferroni, p<0.001).


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ACTN3 and ACE genotype affects muscular performance in response to high-speed power training in older Caucasian women

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The angiotensin-converting enzyme (ACE) and the alpha-actinin-3 (ACTN3) genes have been associated with power phenotypes and both have been suggested to influence skeletal muscle function in response to strength training (Lima et al. 2010). However, conclusions have been inconsistent across investigations. There is a paucity of research data concerning exercise training-induced adaptations in older population (Adams et al. 2000).

The purpose of this study was to investigate the possible associations between ACE I/D and ACTN3 (R/X) polymorphisms and maximum strength, power and muscle function in older Caucasian women (n=139; age= 62.5±8.1; ID:n=52; DD:n=52; II:n=35 and RX:n=54; RR:n=52; XX:n=33) and their adaptation during 12-weeks of high speed power training. Period of intervention consisted of 40% of one repetition maximum (1RM) to 75% and 3 sets 4–12 reps in countermovement jump (CMJ) (Pereira et al., 2012). Strength was measured dynamically in leg extension exercise (1RMLE), power was evaluated by CMJ and functional capacity was recorded by sit to stand test (STS). ACE I/D and ACTN3 R/X polymorphisms were determined by polymerase chain reaction. Significant differences were performed by ANOVA (means±SD). The trainingxgenotype effects were analyzed by repeated-measures ANOVA.

Whole body was independent of ACE and ACTN3 genotypes. At baseline no significant effects of both ACE and ACTN-3 genotype were found for all considered strength parameters. Over the 12-weeks training period, the subjects significantly increased maximum strength (62.9% in 1RMLE), lower limbs muscle power (30% in CMJ) and functional capacity (22.5% in STS test).

Genotype effect for ACE showed no statistically difference only in 1RMLE (P=0.187). But subjects with genotype DD had higher maximal strength than others after the high-speed power training. Although, genotype effect for ACTN3 showed significant effects for all measures: 1RMLE (p=0.011), CMJ (p=0.050) and STS (p=0.033). RR genotype exhibited a positive and a prevalence of strength (1RMLE: 33.1±6.4), power (CMJ: 0.164±0.02) and functional capacity (31.3±4.7) comparing to RX and XX genotypes.

The combined influence of ACE DD+ACTN3 RR vs. ACE II+ACTN3 XX polymorphisms was also studied. In response to high-speed power training, the results showed that the D-allele carriers and R-allele combination seems to induce significant increases but only for 1RMLE.

These data suggest that ACE genotype is not associated with muscle strength adaptation to high-speed power training. On the other hand, strength training response seems to be significantly affected by the presence of the ACTN-3 RR genotype alone or in combination with the ACE DD genotype in older Caucasian women’s. The results provide a novel insight that these genetic variations may interact to determine muscle performance in older women.


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The metabolic profiling of exercise intervention: exercise metabolomics

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In exercise intervention science, the nascent fields of exercise ‘omics’ (metabolomics and proteomics) encompass the identification, characterisation, and quantification of the changes over time for metabolite and protein content of whole cells, tissues, or body fluids. In order to establish the current status, a literature search was completed using a metasearch engine linked to academic database resources using a combination of the keywords; ‘exercise’, ‘sport’, ‘intervention’, ‘metabol*’ which resulted in 235 potential studies. The search was refined to relevance based on the following inclusion criteria: healthy human subjects; exercise/training intervention; analysis of targeted or untargeted metabolites in muscle, blood or urine; published between 1999-2011. Studies were excluded that concerned animal subjects, humans diagnosed with mechanical or medical problems, no exercise/training intervention, diet-based intervention or nutritional studies, reviews, abstracts without full-text available and duplicates. These inclusion/exclusion criteria were used to select relevant studies to extract and compare data on the change in metabolites induced by exercise intervention, and compare the analytical techniques used over the past 12 years. The refined search resulted in a final selection of eighteen studies that were reviewed. Data on study design, populations, exercise intervention, outcome measures and analytical techniques, were compiled and analysed. Studies have been reported for targeted and nontargeted biological profiling of blood and urine, based mostly on gas chromatography-mass spectrometry (GC-MS) and high performance liquid chromatography-mass spectrometry (LC-MS). Standardised protocols for sampling and analysis are at an early stage and few studies report chemometric analysis of the data. GC-MS and nuclear magnetic resonance (NMR) spectrometry data for urine, plasma and saliva from baseline studies in our laboratory are presented.

AL acknowledges a PhD scholarship from the School of Health, Sport and Bioscience, University of East London

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The benefits of long term aerobic activity on dimensions of sense of coherence type resistance in glaucoma patients with correlation to blood and intraocular pressure

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Glaucoma is an asymptomatic, progressive optic neuropathy which loss of retinal ganglion cells and atrophy of optic nerve. Glaucoma is often accompanied by an increased intraocular pressure caused by worse intraocular liquid outflow. The severe psychical stress could increase the intraocular pressure and may be one of the important factors to influence the genesis and progress of glaucoma. Physical activity plays an important role in reducing psychological stress. Therefore, appropriate aerobic exercise, as one of the essential physical activity component, could reduce the intraocular pressure in glaucoma patients and prevents progress of damaging retinal ganglion cells. The aim of this pivotal study was to assess the impact of physical activity on dimensions of sense of coherence type resistance in glaucoma patients with correlation to blood and intraocular pressure. The study group consisted of 19 glaucoma patients–women, age 51.4±3.4 years. Intraocular pressure was measured twice before and after 3-month exercise program in eye clinic. Blood pressure (BP) was measured within week, 5 times a day before and just after each exercise session. The exercise group (n=11; mean age 48.9±4.8 years) was enrolled in the 3-month aerobic exercise program twice a year. The control group (n=8; mean age 53.9±6.2 years), matched in age without any organized physical activity. Pharmacotherapy of all glaucoma patients was non-specific beta-blockers and prostaglandin analogue drops. The exercise program, with a frequency of 2 times per week and duration 55 to 60 minutes per session. The aerobic dance and fit ball aerobic exercises were alternated. The questionnaire was used to evaluate the psychological state (The Subjective Perception Scale SUPOS) with 7 components represented in its overall integrated structure. Values are means ± S.E.M., compared by ANOVA. The intraocular pressure varied before the intervention in the normal range and significant (p≤0.05) reduction was detected after the program in the exercise group, except one patient. After the intervention both the systolic and diastolic BP was no different from initial values with no significant fluctuation during the day. No significant changes of BP were detected in 35% of the exercise group immediately after the session, ranged only between 5-15%. The values of disintegrity block (uncomfortable feeling, depression, feeling of uncertainty and sad were higher (not significantly) in control group. In individual evaluation of values of activity block 50% of the subjects of control group and 91% of exercise group showed the values higher than 35% (as in non-clinical population). The results of our study suggest a positive impact of aerobic program on retention the risk factors of glaucoma patients.

Supported by VEGA 1/0503/11

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Chronic endurance training results in abolishment of the age-associated slower rate of adjustment of oxygen uptake kinetics

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The rate of adjustment of oxidative phosphorylation has been shown to be slower in older compared to young adults and in untrained compared to endurance-trained individuals. However, it remains unclear to what extent chronic endurance training can speed oxygen uptake (VO2) kinetics in groups of young, middle-aged, and older adults. We examined a group of young, middle-age, and older chronically trained and untrained males to compare the rate of adjustment of oxidative phosphorylation in each age-group and condition. Pulmonary VO2 (VO2p) was measured during repeated step transitions from 20 W to moderate-intensity cycling (80-90% of estimated lactate threshold). VO2p was measured breath-by-breath using a volume turbine and a mass spectrometer. VO2p profiles were modeled as a mono-exponential using non-linear regression. The groups consisted of young (Y) trained (n= 4) and untrained (n= 9) (24 ± 5 yrs.; mean ± SD), middle-aged (M) trained (n= 5) and untrained (n= 4) (51 ± 3 yrs.), and older (O) trained (n= 3) and untrained (n= 6) (68 ± 4 yrs.) males. Maximal VO2 (VO2max) was lower in O (2.55 ± 0.48 L/min) compared to Y (4.12 ± 0.51 L/min) and M (4.00 ± 0.50 L/min) (p< 0.05) and it was larger in trained (4.04 ± 0.74 L/min) compared to untrained (3.37 ± 0.84 L/min) individuals. The rate of adjustment of oxidative phosphorylation, as represented by the phase 2 VO2 time-constant (τVO2p), was longer in O (39.1 ± 13.3 s) compared to Y (22.2 ± 8.7 s) and M (24.1 ± 7.3 s) (p< 0.05) and it was shorter in trained (19.4 ± 7.4 s) compared with untrained (32.9 ± 11.8 s) individuals (p< 0.05). There was a significant age-group by condition interaction indicating that O untrained (46.8 ± 8.1 s) but not trained (23.8 ± 4.2 s) had a slower VO2 kinetics compared to the other groups (p< 0.05). These preliminary data support the idea that: 1) Chronic endurance trained results in faster rate of adjustment of oxidative phosphorylation; 2) the age-related slowness of VO2 kinetics is only observed in older but not middle-aged males; 3) chronic endurance training results in complete abolishment of any age-related lengthen of the phase 2 VO2p.

Supported by: NSERC, Standard Life Assurance Company of Canada.

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Acute mechanical overload in vitro induces a hypertrophic transcriptional response

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Skeletal muscle mass contributes to strength and power performance. Understanding the cellular and molecular mechanisms that underpin hypertrophy and the types of contraction modalities that induce such a response, will allow for more specific training programmes for the athletic and clinical populations. In vivo models have provided an insight into the basic mechanisms underpinning the hypertrophic and atrophic processes. In vitro models will allow for the investigation of the intrinsic response of the muscle cell to increased or decreased loading, controlling for the role of systemic influences. An established 3D in vitro model was used, to investigate the effect of two mechanical overload regimes. 4m/ml C2C12 mouse myoblasts were seeded in 3ml of type-1 rat tail collagen and plated into standard dimension chamber slides (n= 4 Control (CT), n= 5 Static Load (SL) and n= 3 Ramp Load (RL)). Each chamber held a custom made A-frame and floatation bar at either end to provide attachment points for the gel, to allow longitudinal tension for the alignment of the myoblasts. The constructs were cultured in growth medium (GM, 20% FBS) for 4 days, before inducing differentiation (DM, 2% FBS and 10ng/ml IGF-I). Following a further 10 day maturation period in DM, the constructs were prepared for experimentation. The constructs were transferred to the tensioning culture monitor (t-CM) for the following regimes of acute mechanical overload; SL = 10% strain for 60 mins, RL = continuous increasing load to achieve 10% strain at 60 mins. CT constructs were tethered to the t-CM without stretch. Constructs were sampled immediately at 60 mins for RNA extraction. Transcript changes in MMP9, IGF-I and genes associated with muscle tissue breakdown or reduced protein synthesis; MuRF-1, MAF-Bx and Myostatin were all investigated using qRT-PCR. Significant (ANOVA) increases in MMP9 were found in both SL (16.4-fold) and RL (22.7-fold) compared to CT (p<0.05). IGF-I significantly increased in the SL condition (71.4-fold, p= 0.001) but not in RL (17.4 fold, p>0.05) compared to CT. A mean reduction in Myostatin expression was observed in both SL and RL conditions compared to CT (p>0.05). However, no differences were found in MuRF-1 and MAF-Bx in either experimental condition, suggesting components of the Ubiquitin Proteasome Pathway have not been manipulated in this system. These findings provide corroborating evidence for the early role of MMP9 and IGF-I in response to mechanical overload. A reduction in Myostatin has been shown to contribute to an increase in net protein accretion. Together, these data have suggested that the mechanical overload has induced a molecular response which has been shown to induce a hypertrophic response. This study has provided evidence for the use of an animal-free bio-mimetic in vitro model to investigate the response of skeletal muscle to mechanical overload.

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Glutathione S-Transferases are a superfamily of ubiquitous multifactorial enzymes, which play a key role in cellular detoxification, protecting macromolecules from attack by reactive electrophiles, including reactive oxygen species (ROS) and chemotherapeutic agents. GSTs are the most abundant intracellular enzymes, and have multiple roles as anti-oxidant enzyme (Ramalhinho et al., 2011). The purpose of this study was to examine the association between the GSTM1 and GSTT1 null polymorphisms and event expertise in elite Portuguese swimmers. After informed consent, a group of elite swimmers (n=33, 20 males and 13 female, 18.84 ± 2.97 years), designated as Olympic candidates, were recruited. The swimmers were stratified into two groups, based on their current distance event of expertise (Costa et al., 2009): short distance swimmers, between 50 and 200 m (mainly anaerobic events) and middle distance swimmers, between 400 and 1,500 m (mixed anaerobic and aerobic events). A control group of healthy individuals (n=52, 38 males and 14 females, 20.5±1.52 years) was also selected from the Portuguese population (college students), with no background in swimming. Genomic DNA was extracted from blood samples, and genotyping analyses were performed by PCR methods. Odds ratios (ORs) and 95% confidence intervals were calculated. The results showed that the presence of GSTT1 protein (in the absence of GSTM1 protein) seems to be important for both short distance (p=0.048) and middle distance swimmers (p=0.048) when compared with the controls (GSTT1 present + GSTM1 null: 22.8% for controls; 27.0% for short distance swimmers, and 18.0% for middle distance swimmers). The genotype distribution by gender showed that the presence of the GSTM1 protein alone appears to be relevant female middle distance performance (GSTM1 present: 15.4% for controls and 12.0% for middle distance female swimmers, p=0.020). The results seem to support an association between the presence of the GSTT1 protein and athletic performance in both genders. However, the GSTM1 null polymorphism seems to confer some advantageous characteristics to female swimmers for middle distance swimming performance.


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Aerobic capacity, speed and abdominal resistance discriminate handball players from different playing levels

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The purpose of this study was to identify the anthropometric and fitness variables that allows distinguishing handball players with differ-
ent performance level. A total of 210 male handball players (age, 23.73±5.25 years) participated in this study divided into five groups: (1) top elite (TE, n=41); (2) moderate elite (ME, n=53); (3) sub elite (SE, n=35); (4) moderate trained (MT, n=32); (5) next21 (n=49). Two anthropometric measures were taken from each player, i.e., body mass (in kg) and stature (in cm). Fitness profiling considered 18 variables: 30-m sprint time (30-m, s); (2) Squat jump (SJ) and countermovement (CMJ) height, height ratio and Pavg; (3) SJ adapted to arms (SJA) and CMJ adapted to arms (CMJA), height and height ratio; (4) Handgrip indices, namely dominant, non-dominant, mean score of dominant and non-dominant (mean) and difference between dominant and non-dominant; (5) Number of executions (#) in sit-up test (in 60-s); (6) Distance (m) and the position (1, <1000-m; 2, [1000-m;1300-m]; 3, [1300-m;1600-m]; 4, ≥1600-m) in Yo-Yo Intermittent Endur-
ance Test - Level 2 (Yo-Yo IE2). It was observed that sprint time in 30-m, performance in CMJ, average power in CMJ, abdominal strength and the position in Yo-Yo IE2 measures best dicriminated between five playing status groups. Results also showed that the position in the Yo-Yo IE2, sit up and speed (i.e., sprint time in 30-m), more clearly distinguished between groups, and classification results showed that 48.3% of original group cases (and 41.5% of cross-validated grouped cases) were correctly classified.Sprint performance can be consid-
ered an important fitness component of handball physical performance and, probably, it is correlated to high intensity activity during actual match-play. Moreover, the ability to repeatedly perform intense intermittent exercise also discriminated playing status groups in handball players, and suggests that: (1) the training of “lower” playing status team’s coaches may have favoured resistance training over the training capacity in the anaerobic metabolism is predominant, or (2) this may be explained not by lack of training capacity, but said with issues related to the recovery of players after training or competitions. Nevertheless, the functional capacity seems to be associated with the competitive level of the subjects.

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Modelling diverse skeletal muscle loading modalities in vitro: metabolic and transcriptional responses

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Research surrounding the mechanisms that govern skeletal muscle adaptation to exercise, have begun to be characterised in vivo. Developing an in vitro model of skeletal muscle, which can predictably respond to stimulation in a similar way to in vivo exercise, will combat experimental issues including; subject recruitment and biopsy sampling. This investigation used 3D in vitro skeletal muscle model, to examine the metabolic and transcriptional responses to two diverse stimulations. 4m/ml C2C12 myoblasts were seeded in 3ml of type-1 rat tail collagen and plated into standard dimension chamber slides (n= 3 each for Control (CT), Continuous Cyclic (CC) and Intermittent Cyclic (IC)). Each chamber held an A-frame and floated bar at each end to provide attachment points for the gel, for the alignment of the myoblasts. The constructs were cultured in growth medium (GM, 20% FBS) for 4 days. Following a further 10 day period in differentiation media (DM, 2% FBS and 10ng/ml IGF-I), the constructs were used for experimentation. The constructs were transferred to the tensioning culture monitor (t-CM) for the following regimes of mechanical overload; CC = 7.5% strain continuous cyclic for 60 mins. IC = 10% strain cyclic stretch for 10 reps followed by a 90 sec delay (1 set), a further 3 sets were performed (total = 1 ‘exercise’), with a 3 mins rest between each exercise of which 4 were completed. CT constructs were tethered to the t-CM without stretch. Conditioned media was sampled every 5 mins for the CC condition and post every ‘exercise’ in the IC condition for [Lactate] analysis. Constructs were sampled immediately at 60 minutes for RNA extraction. Transcript changes in metabolically activated associated genes were investigated using qRT-PCR. CC significantly increased [Lactate] from 1.73 ± 0.31 mmol.L to 5.57 ± 0.85 mmol.L after 5 mins (p<0.01). There were no observed significant differences in media [Lactate] between any time-points from 5-60 mins (p>0.05), indicative of a steady state in Lactate production. IC significantly (ANOVA) increased conditioned media [Lactate] from 1.73 ± 0.31 mmol.L to 7.05 ± 2.61 mmol.L after the first ‘exercise’ (p<0.01). No significant increases in [Lactate] were observed for CT across samples. CC reduced the expression of Cytochrome C by 0.23-fold compared to CT, whilst significantly increasing NRF-2 (p<0.05). IC significantly increased PGC-1α, Cytochrome C, NRF-1 and NRF-2 mRNA expression compared to CT (p<0.05). Increases in PGC-1α, Cytochrome C, NRF-1 and NRF-2 mRNA, suggests an adaptation towards increasing the oxidative potential of the tissue. An increase in NRF-2 mRNA despite a moderate, but non-significant change in NRF-1 mRNA in the CC condition proposes a greater role for NRF-2 in the response to CC stimulation. This system can be manipulated to induce responses similar to those seen with in vivo exercise.

Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.
The effects of warm-up protocols on counter movement jump performance in elite Australian Rules Football

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The purpose of this study was to investigate the acute effect of 3 warm-up protocols on power production during countermovement jump (CMJ) testing in order to compare practical protocols that may be applied immediately before competition and/or training. Fourteen elite Australian Rules Football players performed three warm-up protocols over three sessions in a randomized order. The protocols included a series of dynamic movements followed by an intervention of 1)barbell squats, 2)band resisted squats or 3)static stretching. Three repeated CMJ’s were before and five and ten minutes after each warm-up protocol on an unloaded Smith machine. Power output was measured using a linear position transducer. Mean and peak power was not significantly different from baseline at five or ten minutes following any of the warm-up interventions (p>0.05). These results suggest that, although such warm-up protocols may have some benefits related to improved athletic performance, they do not appear to augment lower limb power output.

KEY WORDS: vertical jump, warm-up

Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.
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Symposium speakers’ manuscripts
Peter Snell winning the 1500 m in the 1964 Tokyo Olympics

The scientific basis of human performance has been of great interest to me since I submitted to the treadmill of Drs Fred Kasch and John Boyer at San Diego State University in 1965 just prior to running the mile in 3 min 55 s. The subsequent publication in *Research Quarterly* (one subject, six authors), in which my maximal oxygen uptake was recorded as 72 ml kg$^{-1}$ min$^{-1}$, clearly indicated that there was more to mile running than possession of a high maximal oxygen uptake. The other physiological characteristics that might account for superior performance are anaerobic capacity and the efficiency of running at race pace. Experience of top runners indicates that as they reach peak form, the sustained fast-pace running demanded by middle-distance events seems almost effortless. Subtle changes in mechanics and metabolic machinery induced by repeated race-pace interval training may contribute to improved overall efficiency and explain this effect. However, the biomechanical and physiological mechanisms explaining the changes remain to be identified.

The San Diego State treadmill experience led to my devouring books on exercise physiology and, ultimately, in 1974 led to my pursuit of education at UC Davis and Washington State University, which I hoped would provide me with a greater understanding of how the body responds to the stimulus of training that varies in intensity, duration, frequency and mode. I became acquainted

with the glycogen depletion studies performed in Sweden by my mentor, Philip Gollnick, who showed how fast-twitch fibres were recruited during moderate-intensity exercise of up to 2 h duration and provided a rationale for this form of training that was the basis of renowned coach Arthur Lydiard’s training programmes.

In my experience, physiologists are not noted for new innovations in training, but they often provide valuable insight into why a particular training technique is successful. It seems, with hindsight, ridiculous that in the early 1950s, many thought that a sub-4-min mile was impossible. Now, thanks to the efforts of scientist contributing to the symposia on the Biomedical Basis of Elite Performance, reported in this themed issue of *Experimental Physiology* and in *The Journal of Physiology*, we can predict with great precision the limits of human performance.

Sir Peter Snell

*Dallas, October 2011*
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Symposium speakers’ manuscripts

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Introduction

Cardiac, respiratory and vascular aspects of performance

This issue of Experimental Physiology contains symposium papers from the conference The Biomedical Basis of Elite Performance held on the 19–21 March 2011 at The Queen Elizabeth II Conference Centre, London which covers the theme of Cardiac, respiratory and vascular aspects of performance.

In the first of these, Green et al. (2012) asks the question, if there is an athletes heart, is there an athlete’s artery? They make a compelling case for training-induced remodelling of the vasculature at the conduit and resistance artery level in athletes. These adaptations are necessary to cope with the substantial cardiac output seen during heavy exercise and explain the well-known observation that, even with their exceptional cardiac output, athletes do not experience abnormally raised arterial blood pressure during maximal exercise. Green et al. (2012) also point to findings from the study of athletes as well as laboratory-based training studies on ‘lesser mortals’ that have led to better understanding of vascular adaptations to exercise. These have important implications for the health of the general populace. They speculate that intravascular laminar shear stress may be the key factor in causing the vascular adaptations they describe. As this is increased by exercise and experienced by the whole conduit and resistance artery network, there is potential for system-wide beneficial adaptation to exercise, not only in the vessels supplying the exercising muscle.

DeVan & Seals (2012) extend the theme of vascular adaptation to exercise with their review of ‘vascular health in the ageing athlete’. As population statistics indicate a rapid worldwide increase in the number of older people, the topic is very timely. They explain that master athletes who perform endurance training-based activities demonstrate a more favourable arterial function–structure phenotype, including lower large elastic artery stiffness, enhanced vascular endothelial function and less arterial wall hypertrophy. Mechanisms behind these adaptations are still uncertain, but once again shear stress is a strong candidate. This combination of vascular traits is associated with a lower risk of clinical cardiovascular disease in endurance-trained master athletes. As such, DeVan & Seals (2012) suggest that this group may represent an exemplary model of healthy or ‘successful’ vascular ageing. In contrast, master athletes engaging in resistance-type exercise do not show this beneficial profile; indeed, they may exhibit less favourable arterial function–structure than their endurance-trained peers and, in some instances, untrained adults. These findings have obvious implications for ‘best advice’ to be given to those striving to reduce risk factors for cardiovascular disease in old age.

Next, Amann (2012) shifts our attention to ‘pulmonary system limitations to endurance exercise performance in humans’. He emphasizes three respiratory system-related mechanisms which may limit performance by impairing oxygen transport to the locomotor musculature. In fact, two of these mechanisms operate by compromising cardiovascular system responses to exercise, which clearly illustrates the need for an integrative approach to the study of human exercise physiology. The first limitation to performance may be caused by a restriction of stroke volume and cardiac output during exercise, due to excessive intrathoracic pressure fluctuations during breathing. A second mechanism is reflex limb muscle vasoconstriction that is triggered by thin fibre muscle afferent activation in response to fatiguing respiratory muscle work. Both mechanisms can therefore compromise locomotor muscle blood flow. The third mechanism relates to the fact that in some endurance-trained athletes, and perhaps more commonly in female athletes, there is a susceptibility to arterial desaturation during exercise and, rather surprisingly, in some individuals this occurs at submaximal exercise intensities. The finding that this arterial...
hypoxaemia occurs in the absence of hypoventilation remains unresolved but will doubtless be the subject of future investigation.

In the article by Theisen (2012), special consideration is given to respiratory and cardiovascular limitations in the paralympic athlete. He notes the rapid improvements in athletic performance by spinal cord-injured athletes over the past 30 years and attributes this in large part to optimization and specialization of training methods, which challenge the athlete’s body to its limits. The consequences of a spinal cord injury are numerous and concern voluntary muscle function, deep and superficial sensitivity, and autonomic function to a degree determined by the level and completeness of the spinal lesion. From a cardiovascular system perspective, blood redistribution from body areas lacking autonomic control is impaired, thus reducing venous return and limiting cardiac stroke volume during exercise. Thermoregulatory function is also affected through a lack of afferent neural feedback and limited efferent vasomotor and sudomotor control below the lesion. Theisen (2012) describes and explains strategies to support venous return and to promote body cooling, which could potentially improve physiological responses and athletic performance, especially in individuals with high lesion levels.

Together, these symposium reports provide an up-to-date and thought-provoking summary of our current knowledge about the physiology of elite performance. It is a well-worn argument but it bears repeating here. The study of extreme adaptation seen in elite performers often leads to greater understanding of fundamental physiological mechanisms. The insight gained from this often integrative approach to research then spreads to the study of normal and pathophysiological states. All told, these symposium reports will surely further increase our admiration of the performances that we will witness in the forthcoming Olympic Games.

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References

Vascular adaptation in athletes: is there an ‘athlete’s artery’?

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Whilst the existence of a specific phenotype characterized as ‘athlete’s heart’ is generally acknowledged, the question of whether athletes exhibit characteristic vascular adaptations has not been specifically addressed. To do so in this symposium, studies which have assessed the size, wall thickness and function of elastic, large muscular and smaller resistance arteries in athletes have been reviewed. Notwithstanding the caveats pertaining to cross-sectional comparisons between athletes and ‘matched’ control subjects, these studies reveal increased conduit artery size, including enlargement of epicardial arteries and those supplying skeletal muscle. Evidence that peak limb blood flow responses are enhanced in athletes further suggests that resistance arteries undergo increases in total cross-sectional area. Such increases can be localized to those arteries supplying active muscle leading to speculation, supported by exercise training studies in humans and animal and cellular data, that arterial enlargement is associated with repetitive episodic increases in arterial shear stress which elicit endothelium-mediated remodelling. Such structural remodelling at conduit and resistance artery level may play a role in accommodating the substantial increase in cardiac output apparent in endurance athletes; arterial pressure is not increased at rest or during exercise in athletes (versus control subjects). Arterial wall remodelling also occurs in athletes but, in contrast to the impact of shear stress on remodelling of arterial lumenal dimensions, the impact of endurance athletic status on wall thickness may be a systemic, rather than localized, phenomenon. Finally, the question of whether the arteries of athletes exhibit enhanced function is moot. Somewhat paradoxically, measures of conduit and resistance artery endothelial function may not be enhanced, compared with healthy control subjects. This may relate to the inherent difficulty of improving arterial function which is already normal, or the time course and transient nature of functional change. It may also relate to the impact of compensatory structural remodelling, as arterial lumen size and wall thickness both affect functional responsiveness. In summary, there is clear evidence for an impact of athletic status on arterial structure and function, at least with respect to the impact of endurance training. Arterial adaptation may, to some extent, emulate that evident in the hearts of endurance athletes, and it is tempting to speculate that similar mechanisms may be at play.

‘Athlete’s heart’ is now a generally accepted term and, indeed, has been used as a benchmark to characterize athletic status (Maron, 1986). This concept evolved from early observations by percussion and subsequent evidence from radiography, two-dimensional echocardiography and recent magnetic resonance and computed tomography studies (George et al. 1991; Naylor et al. 2008). Although there may be some limits (Naylor et al. 2008; Spence et al. 2011) to the Morganroth schema of athletic adaptation in the heart (Morganroth et al. 1975), it is now generally accepted that chamber volume and mass adapt to prolonged and intense...
physical effort in a manner related to the loading of the ventricles.

In contrast, the impact of athletic status on arterial characteristics has not been fully characterized, due in part to the historical difficulty of assessing the size, structure and function of different arteries in vivo. In this symposium report, we examine the question of the existence of a characteristic ‘athlete’s artery’, by reviewing studies of resistance and conduit vessel adaptation in elite-level athletes. In most cases, such studies have recruited a matched control group, and we frankly acknowledge the limitations of cross-sectional comparisons when addressing the question of the impact of exercise training, as previously described (Naylor et al. 2008). Nonetheless, much can be achieved by observing an athletic benchmark (Chakkravarty & Booth, 2004) and in many cases we provide supporting evidence from longitudinal training studies and those in animals and cellular models.

Do athletes possess enlarged conduit and resistance arteries?

Whilst the impact of aerobic exercise training on capillary growth is well established (Andersen & Henriksson, 1977; Brown, 2003), vascular resistance and blood pressure regulation are primarily the province of upstream arterioles and (small) arteries, many of which lie outside the muscle interstitium (Snell et al. 1987; Segal, 1992; Thijssen et al. 2010). Such arterial vessels can adapt to training functionally or by outward structural remodelling (Thijssen et al. 2010).

Resistance vessel remodelling in athletes

Historical perspectives. In his Physiological Review of 1977, J.-P. Clausen pointed out, on the basis of studies using arterial cannulation and dye-dilution techniques, that maximal cardiac output increases as a result of training, whereas maximal mean arterial pressure does not (Clausen, 1977). He stated that ‘...it seems justified to conclude that training reduces total peripheral resistance at maximal exercise’. This makes the point that any hypotensive impact of exercise training must be predicated on functional or structural arterial adaptation, which accommodates enhanced output reserve (Green et al. 2008). Furthermore, studies that attempted to determine whether training-induced increases in oxygen uptake were associated with increased central (cardiac output) or peripheral (vasodilator capacity) adaptations, using one-legged training models (Saltin, 1969; Klausen et al. 1982), suggested that the capacity for vasodilatation in skeletal muscle after training exceeds that of cardiac output to maintain blood pressure, a finding recently endorsed by Calbet and Saltin (Calbet et al. 2004; Saltin & Calbet, 2006).

This literature emphasizes the plasticity of the vasculature in response to exercise training and suggests that arterial adaptation is *sine qua non* for exercise performance in endurance athletes.

Remodelling of lumen dimension. Changes in the collective cross-sectional area of the resistance vasculature have been studied in athletes by measuring peak vasodilator responses elicited by prolonged ischaemia, or ischaemic exercise (Thijssen et al. 2010). The assumption is that, while at rest and in submaximal exercise conditions there are a number of competitive functional influences on vascular tone that conspire to determine flow, peak stimuli reveal the structural ‘capacity’ of a vascular bed (Conway, 1963; Takeshita & Mark, 1980; Naylor et al. 2005). Athletes typically exhibit enhanced peak vasodilator capacity in such studies (Martin et al. 1987; Snell et al. 1987). In 1986, Sinoway and colleagues reported significantly higher peak reactive hyperaemic forearm blood flow responses in the preferred limbs of elite tennis players, relative to their non-preferred arm and those of non-athletic control subjects (Sinoway et al. 1986). This study established that resistance arterial remodelling specific to the active muscle bed occurs in athletes. A subsequent hand-grip training study indicated that localized resistance vessel remodelling occurs, largely independent of skeletal muscle hypertrophy, sympathetic or circulatory influences (Sinoway et al. 1987). Taken together, these findings, later independently confirmed (Green et al. 1994, 1996; Rowley et al. 2011a), indicated that resistance artery remodelling is apparent in athletes and can occur as a result of localized and intrinsic vascular stimuli.

It must also be acknowledged that studies involving single-legged exercise indicate that peak blood flows can be enhanced with hypoxia or haemodilution, suggesting that excess dilator capacity exists in untrained subjects and that sympathetic restraint may play a role in active muscle blood flow control during exercise (Secher & Richardson, 2009). Nonetheless, much higher peak vasodilatation is apparent in trained subjects than in untrained control subjects (Richardson et al. 1995; Green et al. 1994), and the localized exercise training studies presented above indicate that adaptations in vasodilator capacity as well as arterial remodelling contribute to the athletic phenotype.

Conduit artery remodelling in athletes

Coronary arteries. Autopsy studies were the first to suggest structural conduit artery enlargement, in particular of the coronary arteries, in athletes (Currens & White, 1961) and fit individuals (Rose et al. 1967; Mann et al. 1972). However, these studies may be polluted by the lack of active tone and the potential impact of post-mortem tissue change. An *in vivo* angiographic study by Haskell et al. (1993) demonstrated that coronary
artery dilator capacity (in response to nitroglycerine) was enhanced in runners, whereas no differences in coronary dimensions were evident at rest. This finding emphasizes the importance of eliciting dilator responses to uncover differences between athletes and control subjects, confirmed by others (Kozákova et al. 2000; Nguyen et al. 2011). It also suggests that basal arterial tone may be enhanced in athletes, a finding endorsed by the study of Sugawara et al. (2007), who illustrated that leg exercise training enhanced α-adrenoceptor-mediated vasoconstrictor tone and resting plasma noradrenaline concentrations, partly in compensation for enhanced vasodilator capacity. Interestingly, repeat coronary angiographic studies involving exercise training interventions generally confirm that functional coronary adaptations are apparent, but are less consistent with respect to the impact on vasodilator capacity (Windecker et al. 2002) or, by association, arterial remodelling. This may relate to the study time course, with studies involving shorter interventions being less likely to demonstrate structural remodelling (see ‘Athlete paradox: why is arterial function not enhanced?’; Hambrecht et al. 2000, 2003). The studies of Hambrecht and co-workers also suggest that coronary flow reserve (indicative of resistance vessel adaptation) increases in response to short-term training, in the absence of changes in epicardial dilator responses to nitroglycerine or adenosine, suggesting that adaptation of resistance arteries may precede that associated with conduit vessels. Finally, transthoracic echocardiographic studies in which epicardial diameters were imaged (Pelliccia et al. 1990; Hildick-Smith et al. 2000) have also reported similar resting, but enhanced nitroglycerine-induced, coronary vasodilatation in athletes compared with control subjects.

Peripheral conduit arteries. Several studies have used ultrasound techniques to compare large artery size in athletes relative to control subjects. Zeppilli et al. (1995) reported enlarged aortic, carotid, and subclavian arteries in track cyclists and long-distance runners, relative to matched sedentary control subjects, which persisted after correction for body surface area. Aortic arch and subclavian artery size were enhanced in wheelchair athletes, whereas abdominal aorta and mesenteric artery values were lower in these subjects. In 2000, Schmidt-Trucksass and co-workers reported that cyclists, middle-distance runners and triathletes had wider femoral, but not carotid, arteries than control subjects and paraplegics (Schmidt-Trucksass et al. 2000). This was followed by a similar study in which larger femoral arteries were observed in cyclists than in healthy control subjects, paraplegic subjects and below-knee amputees (Huonker et al. 2003). Paraplegic subjects exhibited larger subclavian diameters than control subjects. There were no differences between any of the groups in this study in the size of the aorta, suggesting that muscular conduit arteries are subject to changes in diameter more than larger elastic vessels.

In another recent study, brachial artery diameters were significantly larger in elite canoe paddlers and wheelchair athletes compared with control subjects, and superficial femoral artery diameters were significantly larger in runners/cyclists than in control subjects and paraplegic subjects (Rowley et al. 2011b). The plasticity of arteries in elite athletes was further suggested in a study of elite rowers, in whom significantly enlarged brachial arteries exhibited further expansion following the resumption of regular training (Naylor et al. 2006). Finally, the preferred limb of tennis players exhibited larger subclavian diameter than the contralateral limb (Huonker et al. 2003), a finding reinforced by the observation of larger racquet arm brachial artery diameters in elite squash players (Rowley et al. 2011a).

Remodelling of arterial size: mechanisms

Strong evidence supports the role of repetitive shear stress and the endothelium in chronic changes in arterial size. Arterial ligation in rabbits, which decreases flow, also decreases arterial size, and this effect is endothelium dependent (Langille & O’Donnell, 1986). Manipulation of flow and shear stress leads to similar findings (Kamiya & Togawa, 1980; Tuttle et al. 2001), indicating that wall shear is homeostatically regulated in a nitric oxide-dependent manner (Tronc et al. 1996). In recent studies in humans, unilateral manipulation of blood flow and shear stress using partial cuff occlusion during bilateral hand-grip exercise training bouts resulted in changes in hyperaemic flows and conduit artery dilatation that were shear stress dependent (Tinken et al. 2010). Similar findings were also apparent after exercise-independent increases in flow induced by repetitive forearm heating (Green et al. 2010a; Naylor et al. 2011). These studies implicate changes in shear as a principal physiological stimulus to adaptation and vascular remodelling in response to exercise training in healthy humans.

Summary: remodelling of arterial size in athletes

Collectively, evidence from resistance and conduit artery studies strongly supports the notion that athletes possess larger arteries than control subjects. Whilst some evidence suggests that diameter may not be obviously enlarged in all circumstances at rest, this may be due to compensatory increases in vasoconstrictor tone to maintain blood pressure (Sugawara et al. 2007). Responses to peak vasodilator stimuli consistently reveal enhanced vasodilator reserve in athletes, strongly suggesting that arteries possess structurally enlarged conduit and resistance vessels. It is likely that this vascular enlargement is related to repeated increases in shear stress.
associated with chronic exercise, but other haemodynamic and humoral stimuli may be involved and have not, to date, been thoroughly investigated in humans. Whilst a consensus exists that peripheral conduit and coronary arteries exhibit arterial remodelling, evidence for enlargement of carotid arteries and the aorta in athletes is less consistent. Caveats regarding arterial remodelling include the lack of data available in weight- or power-trained individuals; most data are derived from endurance sports.

Do athletes exhibit wall thickness changes?

Using high-resolution ultrasound techniques it is possible to image the wall of large and muscular conduit arteries in humans and recent automated edge-detection approaches have improved the reliability and validity of this approach (Woodman et al. 2001; Potter et al. 2007, 2008). Wall remodelling may also have implications for atherosclerotic risk, because wall thickness measures in carotid and peripheral arteries predict cardiovascular events (see Thijssen et al. 2012). Changes in arterial wall thickness also impact upon peak vasodilator capacity and functional responses to vasodilator stimuli (Thijssen et al. 2011b).

Information regarding the wall thickness characteristics of athletes is scarce and somewhat conflicting. Dinnenno et al. (2001) reported lower femoral artery wall thickness in endurance-trained middle-aged men versus control subjects, a finding supported by Galetta et al. (2006) in male runners (∼66 years old). In contrast, Abergel et al. (1998) reported increased wall thickness in the carotid artery of young elite cyclists, and Schmidt-Trucksass et al. (2003) observed increased femoral artery wall thickness in young male cyclists, triathletes and weight lifters, relative to control subjects. In a separate study, however, the same authors reported no difference between femoral or carotid artery wall thickness in endurance athletes and control subjects (Schmidt-Trucksass et al. 2000).

We recently attempted to clarify this literature by assessing carotid, brachial and superficial femoral artery diameter and wall thickness in elite athletes engaged in predominantly lower limb (runners/cyclists) or upper limb exercise (canoe paddlers) and matched able-bodied, recreationally active control subjects. We also studied wheelchair control subjects and athletes. Decreased wall thickness was observed in all arteries of able-bodied athletes compared with control subjects, including wheelchair athletes compared with wheelchair control subjects (Rowley et al. 2011b). A further study of elite squash players also confirmed decreased brachial artery wall thickness, which, in contrast to the effects on lumen diameter reported above, was apparent in both limbs (Rowley et al. 2011a). This finding may suggest that the mechanisms responsible for localized adaptations in arterial diameter may differ from those associated with changes in wall thickness in athletes, which appears to be a systemic phenomenon. In support of this notion, our recent experiments implicate shear stress as a mechanism in the response of arterial function and diameter to exercise training (Tinken et al. 2010; Naylor et al. 2011), whereas manipulation of shear stress during exercise training had no impact of arterial wall thickness (Thijssen et al. 2011a).

Cross-sectional studies of athletes which suggest a decrease in wall thickness are, on the whole, supported by data which indicate that physical activity and fitness levels are inversely related to carotid artery wall thickness (see reviews: Green et al. 2011; Thijssen et al. 2012) in humans. Finally, longitudinal training studies suggest that, whilst the effects of exercise training on atherosclerosis of the carotid artery may require intense exercise or interventions performed over prolonged time periods, relatively short-term studies of peripheral arterial wall thickness indicate that an aerobic exercise training programme decreases femoral (Dinenno et al. 2001), popliteal and brachial intima–media thickness (Green et al. 2010b).

Remodelling of arterial wall thickness: mechanisms

Our recent study in elite squash players indicated that, whilst brachial artery diameter was unilaterally affected, arterial wall thickness was similar between the limbs (Rowley et al. 2011a). These data were confirmed in other athletic groups, who performed predominantly upper or lower limb events, but had generalized decreases in conduit artery wall thickness (Rowley et al. 2011b). These findings suggest that, whilst the arterial lumen dimension can be modified by local mechanisms (such as shear stress), wall thickness may be affected by systemic factors. One such factor that is known to affect the artery wall is transmural pressure, which is modulated during exercise as a result of generalized changes in blood pressure (Laughlin et al. 2008; Newcomer et al. 2011). Chronic and sustained increases in blood pressure have pro-atherogenic effects on endothelial cells (Laughlin et al. 2008), including lower bioavailability of nitric oxide and higher levels of VCAM-1, ICAM-1, endothelin-1 and reactive oxygen species. In addition, hypertension is associated with arterial wall thickening in large and small arteries (Folkow et al. 1958; Dinenno et al. 2000). However, exercise generates intermittent increases in blood pressure. Whilst speculative, it may be that the nature of the pressure stimulus, chronic versus intermittent, may modify the adaptive arterial response. This will be an interesting area for further research.

Summary: remodelling of carotid and peripheral conduit artery wall thickness

Taken together, studies performed in athletes and healthy subjects who were exercise trained indicate that arterial
wall thickness decreases as a result of prolonged training interventions, particularly in peripheral arteries supplying active skeletal muscle (Thijssen et al. 2012). As these studies were undertaken in athletes and healthy younger subjects, the decrease in wall thickness should not necessarily be taken to reflect atherosclerotic change, but rather a physiological impact on wall remodelling in healthy arteries, the long-term health implications of which are currently unknown.

Do athletes exhibit enhanced vascular function?

Although several short-term exercise training studies, usually in patients with coronary disease, have reported enhanced vascular (endothelial) function (Hambrecht et al. 2000, 2003; Windecker et al. 2002), to our knowledge there have been no reports of coronary endothelial function in athletic populations. Studies of athletes have predominantly been undertaken in peripheral arterial beds. Resistance vessel function has been assayed via infusion of vasoactive agents into peripheral arteries and measurement of vasodilator responses, whilst conduit artery function has often been studied by measuring the responses of larger arteries to increases in blood flow (shear stress) or pharmacological activation.

Peripheral resistance arteries

The first study of athletes and resistance vessel function used the between-limb model in elite tennis players (Green et al. 1996). This study confirmed previous findings (see section ‘Remodelling of lumen dimension’) that peak dilator responses were enhanced in the racquet arm. However, endothelium-dependent and -independent vasodilator responses to acetylcholine and sodium nitroprusside were not enhanced in the preferred limb, and the impact of inhibition of nitric oxide on basal forearm flows was also unaltered. Unilateral forearm hand-grip training was, likewise, not associated with improvement in resistance vessel responses to intrabrachial vasoactive drug infusion (Green et al. 1994). In a study using similar methodology, cyclists and triathletes demonstrated enhanced forearm vascular resistance in responses to one dose of acetylcholine, but not to L-arginine or sodium nitroprusside (Kingwell et al. 1996), whilst cycle training did not modify responses to acetylcholine (Kingwell et al. 1997). Likewise, forearm responses to acetylcholine and sodium nitroprusside were similar between young sedentary and endurance-trained runners (DeSouza et al. 2000). Interestingly, older athletes exhibited enhanced responses to acetylcholine compared with older sedentary subjects, and exercise training in older sedentary subjects improved acetylcholine-mediated vasodilatation, suggesting that athletic status may retard the effects of ageing on the vasculature (DeVan & Seals, 2012), findings which are generally supported by those of Galetta et al. (2006). These studies suggest, somewhat paradoxically, that peripheral resistance vessel function may not be enhanced in athletes, a conclusion broadly in keeping with findings from exercise training studies (Thijssen et al. 2010).

Peripheral conduit arteries

In studies of elite rowers, paddlers, runners and cyclists, we have not observed enhanced flow-mediated conduit artery vasodilatation in the limbs (Rowley et al. 2011b; Fig. 1). Likewise, Petersen et al. (2006) in a cine-magnetic resonance imaging study, observed that endothelial function in the brachial artery was not enhanced in rowers. In contrast, Walther et al. (2008) reported higher flow-mediated dilator responses in cyclists and swimmers, compared with control subjects. However, smooth muscle sensitivity to nitric oxide was also increased, suggesting that endothelial function may not, in fact, be enhanced in these athletes (Walther et al. 2008). Overall, these studies suggest that vascular function may not be enhanced in athletes, a finding which is consistent with the lack of consensus regarding the impact of exercise training interventions in healthy volunteers (Thijssen et al. 2010; Green et al. 2011).

Athlete paradox: why is arterial function not enhanced?

Whilst it is tempting to speculate that vascular function should be enhanced in arteries of athletes, evidence derived from the resistance and conduit artery studies, described above, using a variety of technical approaches, generally suggests that athletes possess functionally ‘normal’ arteries. There are several possible reasons for this apparent ‘athlete paradox’.

Time course of functional versus structural adaptations.

Based on their extensive animal studies, Laughlin and colleagues suggested that functional arterial adaptations may precede those in arterial size and structure (Laughlin, 1995). Conceptually, this suggests that rapid and transient changes occur in arterial function in response to exercise training, which may then be superseded by structural remodelling, which normalizes shear stress and negates the need for ongoing functional enhancement. Whilst this notion remains hypothetical, evidence for differences in the time course of changes in arterial function and structure exists, including recent human data showing initial improvement, and subsequent return to baseline, of flow-mediated dilatation in humans in response to exercise training (Tinken et al. 2008, 2010). In one of these
studies, changes in arterial function were not observed in the absence of exercise-related manipulation of shear stress (Tinken et al. 2010). These data suggest that the lack of apparent increase in vascular function in athletes may be related to arterial remodelling. In other words, it is possible that functional changes have resolved in athletes who have already undergone a process of structural arterial remodelling.

Impact of structural adaptations on functional responses. Conduit artery dilator responses are indirectly related to arterial diameter (Celermajer et al. 1992; Silber et al. 2005). Although this relationship has been attributed, in part, to the relationship between smaller arteries and shear stress (Silber et al. 2005), we recently demonstrated that lumen dimension correlates highly with flow-mediated dilatation responses, independent of

Figure 1. Representation of data derived from distinct groups of elite athletes
Conduit artery function, in this case femoral artery flow-mediated dilatation (FMD%) is, somewhat paradoxically, not enhanced in elite athletes. This may be due to the inherent problem of ‘supra-normalizing’ function of a priori healthy arteries, or a consequence of structural changes in the artery which negate the ‘need’ for functional upregulation. For example, arteries of athletes are larger in lumen dimension (diameter) and also possess distinctly different wall thickness-to-lumen ratios than arteries of healthy matched control subjects. Both wall thickness-to-lumen ratio and size of the artery impact on arterial function. The arteries of athletes may, therefore, be normal in function, but at different structural remodelling ‘set points’ from the arteries of healthy non-athletic control subjects.
shear (Thijssen et al. 2008, 2009) and that arterial size and shear-independent nitroglycerine responses are also inversely related. Whatever the mechanism responsible for the relationship between arterial size and functional responses, athletes have characteristically enlarged arteries and this may have implications for the interpretation of vasodilator responses. A further consideration is the impact of wall thickness changes. Folkow and co-workers established that arteries with enlarged wall thickness, relative to lumen size, exhibit exaggerated functional responses to a range of stimuli (Folkow et al. 1958). We recently confirmed this phenomenon across conduit arteries of different size in humans (Thijssen et al. 2011b). It is conceivable, therefore, that changes in the wall thickness of arteries in athletes may impact on functional vasodilator responses in athletes.

Summary. Functional responses in arteries are complex and influenced by the myriad humoral, paracrine and neural mechanisms that impact on arterial tone. All of these can be, and probably are, affected by athletic status (Green et al. 2011). For example, changes in autonomic balance may affect vasomotor tone in athletes, although conflicting evidence is apparent in reduced sympathoexitation in the medulla (Mueller, 2010) versus enhanced basal vasoconstrictor tone (Sugawara et al. 2007) and muscle sympathetic nerve activity (Alvarez et al. 2005) as a result of training. In any event, the evidence presented above suggests that arterial function, and in particular but not exclusively endothelial function, is not necessarily enhanced in athletes. This apparent ‘athlete paradox’ may be at least partly explained on the basis of structural adaptations in the arteries that impact upon function.

References

Summary
Despite the many lessons that can be derived from the study of athletes as a model of physiological adaptation, far less attention has been devoted to changes that occur in their arteries than in their hearts. At this time, sufficient evidence exists to conclude that endurance athletes possess enlarged arteries, which may also exhibit decreased wall thickness (Fig. 2). These structural changes may impact on arterial function, because there is limited evidence for enhanced arterial responsiveness to pharmacological or physiological stimulation in athletes. Haemodynamic signals may contribute to arterial remodelling, with shear stress implicated in localized effects of repeated exercise bouts on conduit and resistance artery enlargement. Changes in wall thickness may be systemic rather than localized in nature, and the mechanisms responsible are not well defined. The impact of distinct forms of exercise on arteries, exemplified in comparisons between strength/power and endurance athletes, have not been specifically investigated but may provide important insights. Just as there is ‘athlete’s heart’, we provide evidence for the existence of ‘athlete’s artery’ in humans.


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Acknowledgements

Professor Green’s work is supported by the National Heart Foundation of Australia and the Australian Research Council.
Vascular health in the ageing athlete

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The demographics of ageing are changing dramatically such that there will be many more older adults in the near future. This setting is projected to produce a new ‘boomer-driven’ epidemic of physiological dysfunction, disability and risk of chronic degenerative disorders, including cardiovascular diseases. Standing out against this dreary biomedical forecast are Masters athletes, a group of middle-aged and older adults who engage in regular vigorous physical training and competitive sport. Compared with their sedentary/less active (untrained) peers, Masters athletes who perform endurance training-based activities demonstrate a more favourable arterial function–structure phenotype, including lower large elastic artery stiffness, enhanced vascular endothelial function and less arterial wall hypertrophy. As such, they may represent an exemplary model of healthy or ‘successful’ vascular ageing. In contrast, Masters athletes engaged primarily/exclusively in intensive resistance training exhibit less favourable arterial function–structure than their endurance-trained peers and, in some instances, untrained adults. These different arterial properties are probably explained in large part by the different intravascular mechanical forces generated during endurance versus resistance exercise-related training activities. The more favourable arterial function–structure profile of Masters endurance athletes may contribute to their low risk of clinical cardiovascular diseases.

(Received 30 November 2011; accepted after revision 12 January 2012; first published online 20 January 2012)

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The world is ageing; the number of older adults is on the rise. This phenomenon comes with serious physiological and health implications, including increases in cardiovascular dysfunction and disease (CVD). Indeed, it has been projected that without effective intervention 40% of all adults in the USA will have at least one form of CVD by 2030, with a tripling of attendant medical costs, due largely to the ageing of the population (Heidenreich et al. 2011).

In the midst of this impending epidemic of age-associated dysfunction and disease stands a physiologically exceptional group of middle-aged and older adults referred to as ‘Masters athletes’. These individuals exercise vigorously on most, if not all, days of the week, often engaging in athletic competitions and demonstrating enhanced age-normalized physical function and remarkable sports performance (Tanaka & Seals, 2008). Importantly, at least for those performing aerobic exercise-related training and competitions, Masters athletes have greater cardiovascular capacity (e.g. maximal cardiac output and oxygen consumption; Tanaka & Seals, 2008) and a lower risk of CVD (Laure & Binsinger, 2009) compared with their more sedentary peers.

Many physiological and/or pathophysiological changes are likely to contribute to declines in cardiovascular function and increases in CVD risk with ageing. Among the most important are changes to the arterial system, including stiffening of the large elastic arteries (aorta and carotid arteries), development of endothelial dysfunction and wall thickening (Lakatta & Levy, 2003). Here we summarize and update recent discussions (Seals et al. 2008, 2009) of evidence suggesting that these adverse vascular changes may be less manifest (or even absent) in certain subgroups of Masters athletes and, therefore, might help explain their more favourable cardiovascular capacity and health.

Large elastic artery stiffness

Large elastic artery stiffness, most commonly assessed by aortic pulse wave velocity (aPWV) or the local compliance...
of the carotid artery (via ultrasound and tonometry), has emerged as a major independent risk factor for CVD in older adults and is linked to a greater risk of systolic hypertension, left ventricular hypertrophy and other disorders of ageing, such as cognitive impairment (Lakatta & Levy, 2003; Mitchell et al. 2010). As reflected by increased aPWV and decreased carotid compliance, large elastic artery stiffness increases with age even in non-hypertensive adults free of clinical CVD (Tanaka et al. 1998; Lakatta & Levy, 2003).

Middle-aged and older male and female Masters endurance athletes (triathletes, cyclists, runners and swimmers) demonstrate lower aPWV (Vaitkevicius et al. 1993; Tanaka et al. 1998) and greater carotid artery compliance (Tanaka et al. 2000; Monahan et al. 2001; Moreau et al. 2003, 2006a; Nualnim et al. 2011) compared with their non-exercise-trained or sedentary (herein referred to as ‘untrained’) peers (Fig. 1). The aPWV in these Masters athletes is similar to that in trained and/or untrained young adults (Vaitkevicius et al. 1993; Tanaka et al. 1998), whereas carotid compliance is lower than that observed in young adult control subjects (Tanaka et al. 2000; Moreau et al. 2003). The lower large elastic artery stiffness in Masters endurance athletes compared with middle-aged/older untrained adults is associated with other cardiovascular benefits, including lower 24 h systolic and pulse pressures (Seals et al. 1999) and enhanced baroreflex sensitivity (Monahan et al. 2001; Nualnim et al. 2011). Little is known about the mechanisms by which these Masters athletes maintain lower large elastic artery stiffness with age, but less oxidative stress-related suppression of arterial compliance may play an important role (Moreau et al. 2006a). A lower ‘subclinical’ CVD risk factor burden in the Masters endurance athletes also could contribute, although subjects with major risk factors were excluded in the aforementioned studies. In contrast to their peers performing endurance training/competitions, Masters athletes engaged in sports requiring intensive resistance training have greater large elastic artery stiffness than untrained adults, as indicated by lower carotid artery compliance (Miyachi et al. 2003). Interestingly, Masters rowers, a group of athletes who perform both intensive resistance and endurance training, demonstrate enhanced carotid artery compliance compared with untrained control subjects (Cook et al. 2006), suggesting that even some element of endurance training can offset the apparent negative consequences of intensive resistance training. No differences in peripheral large (femoral) artery compliance have been observed among groups of Masters athletes and untrained healthy adults (Cook et al. 2006; Nualnim et al. 2011), suggesting age- and training-specific influences on large elastic arteries.

Vascular endothelial function

Vascular endothelial function is most commonly assessed in humans by measuring endothelium-dependent dilatation (EDD) using either brachial artery flow-mediated dilatation (FMD) or the forearm blood flow.
responses to brachial artery-infused acetylcholine (Seals et al. 2011). Endothelium-dependent dilatation is reduced with advancing age in untrained adults, even in the absence of CVD risk factors/disease (Seals et al. 2011). Unlike their untrained peers, however, male Masters endurance athletes have largely or completely preserved EDD with ageing (Fig. 2; DeSouza et al. 2000; Taddei et al. 2000; Eskurza et al. 2004, 2005; Franzoni et al. 2005; Black et al. 2009; Pierce et al. 2011a). These athletes also appear to be at least partly protected from impairments in EDD in response to acute ischaemia–reperfusion injury (DeVan et al. 2011).

Reduced vascular oxidative stress is a key mechanism by which EDD is preserved with age in male Masters athletes (Taddei et al. 2000; Eskurza et al. 2004; Franzoni et al. 2005). Indeed, there is now direct evidence of reduced oxidant stress in the vascular endothelial cells of these athletes compared with untrained control subjects, and this is associated with reduced endothelial cell expression of the oxidant enzyme NADPH oxidase and redox-sensitive transcription factor nuclear factor κB, as well as increases in the expression of the antioxidant enzyme manganese (mitochondrial) superoxide dismutase (SOD) and activity of endothelium-bound SOD (Pierce et al. 2011a). Reduced endothelial oxidative stress in these Masters athletes causes less destruction/greater bioavailability of the endothelium-dependent dilating molecule, nitric oxide (NO), resulting in a greater NO-mediated EDD (Taddei et al. 2000). Greater bioavailability of the critical cofactor for NO production, tetrahydrobiopterin (BH₄), also plays an important role in the maintenance of EDD in these athletes (Eskurza et al. 2005). This could be due to less oxidation of BH₄, increased endogenous BH₄ synthesis, or both. Basal NO production also is preserved in male Masters endurance athletes (Seals et al. 2008), perhaps also a result of reduced oxidative stress and enhanced BH₄ bioavailability.

The mechanisms for this endothelium-protective phenotype of male Masters athletes remain to be established, though it is not clearly or consistently related to differences in clinical characteristics (Seals et al. 2008, 2009, 2011). Rather, training-induced increases in intravascular laminar shear (via increases in systemic and active limb blood flow), differences in one or more presently unidentified (protective) circulating humoral factors and/or greater resistance to a given level of potentially endothelium-damaging factors (e.g. plasma low-density lipoprotein cholesterol or glucose) all have been proposed (Seals et al. 2008, 2009, 2011).

In comparison to men, far fewer data are available on vascular endothelial function in female Masters endurance athletes, and all of these data are based on brachial artery FMD. Initial reports on small groups of women suggested greater EDD in female Masters endurance athletes compared with untrained age-matched control subjects (Hagmar et al. 2006; Black et al. 2009). A recent study of a much larger sample found no differences in brachial FMD in endurance-trained and untrained postmenopausal women, while confirming past observations in men (Pierce et al. 2011b). Extensive analysis revealed no obvious physical or clinical characteristics that could explain the
sex-specific differences. However, all of the women were estrogen deficient, and it is possible that a certain critical level of estrogen bioavailability is necessary for exercise-generated physiological signals to modulate vascular endothelial function in this group.

Finally, among Masters endurance athletes, it is possible that vascular endothelial function is influenced by the type of activity performed. A recent investigation found that brachial artery FMD was greater in middle-aged and older Masters runners compared with age- and sex-balanced groups of Masters endurance swimmers and healthy untrained control subjects (Nualnim et al. 2011). To our knowledge, no cross-sectional studies are available on vascular endothelial function in primarily/exclusively resistance-trained Masters athletes.

**Arterial wall thickness**

Carotid and femoral artery intima–media thickness (IMT) are independent predictors of CVD and increase two- to threefold with adult ageing in the absence of major risk factors or clinical diseases (Lakatta & Levy, 2003; Seals et al. 2008). This large artery wall thickening with age is mediated by hypertrophy of both the intimal and the medial layers and is likely to represent one aspect of a vascular remodelling process in response to changes in intravascular mechanical forces with ageing (Seals et al. 2008). Age-associated increases in IMT also may reflect the development of subclinical or clinical-grade atherosclerotic plaques, although the latter is less likely in healthy adults.

The carotid IMT of male and female Masters endurance athletes does not differ from untrained age- and sex-equivalent untrained adults, nor are the age-related differences in carotid IMT different in endurance athletes compared with untrained adults (Moreau et al. 2002, 2003; Tanaka et al. 2002). This also is the case in resistance exercise-trained Masters athletes (Miyachi et al. 2003). The absence of an effect is probably due to the fact that ‘central’ (e.g. carotid artery) blood pressure, a key determinant of IMT among healthy adults, does not differ in Masters athletes and health untrained control subjects.

In contrast, femoral artery IMT is smaller in male and female Masters endurance athletes compared with age- and sex-matched untrained control subjects, and the age-associated difference is smaller in endurance-trained athletes compared with untrained adults (Fig. 3; Dinenno et al. 2001; Moreau et al. 2002, 2006b). The smaller femoral IMT and accompanying increase in lumen diameter in Masters endurance athletes are features of ‘expansive arterial remodelling’, a process presumably aimed at normalizing wall stress in response to exercise-evoked increases in femoral blood flow required to meet the demands of the active muscles in the legs (Dinenno et al. 2001). Rather than being smaller, femoral IMT is greater in resistance-trained male Masters athletes compared with untrained age-matched control subjects (Miyachi et al. 2005). This may be the result of the different

**Figure 3. Femoral artery wall thickening is influenced by age and type of exercise training**

A, femoral artery intima–media thickness (IMT) of young untrained adults, older untrained adults and Masters athletes (adapted from Moreau et al. 2006b). B, percentage difference in femoral artery IMT from study-specific older untrained control subjects in resistance-trained male and endurance-trained Masters athletes (adapted from Miyachi et al. 2005; Moreau et al. 2006b). Values are means ± SEM. *P < 0.001 versus young untrained subjects of same sex; †P < 0.001 versus older untrained subjects of same sex.
intravascular mechanical forces generated in the systemic circulation during resistance compared with endurance training, particularly the marked increases in arterial pressure during weight-lifting manoeuvres.

Summary and conclusions

Large elastic artery stiffness, vascular endothelial function and large artery wall thickness are major indicators of arterial health and risk of age-associated CVD (Lakatta & Levy, 2003). Overall, Masters endurance athletes demonstrate a more favourable arterial phenotype compared with untrained middle-aged and older adults, which may explain, at least in part, their greater cardiovascular functional capacity and lower risk of CVD. As such, the Masters endurance athlete may be viewed as a model of ‘exceptional vascular ageing’. In contrast, Masters athletes for whom training and competitive sport require primarily or exclusively intensive resistance muscle activities exhibit a less favourable arterial function–structure profile than their endurance-trained peers and, in some cases, compared with untrained adults. The differences in arterial properties between Masters athletes engaging in sports requiring endurance versus resistance training are probably explained by differences in the intravascular mechanical forces generated during these activities.

References


Acknowledgements

Our thanks go to all of the students, postdoctoral fellows and staff who contributed to the work in our laboratory. This work was supported by NIH R37 AG013038, T32 AG000279 and UL1 RR025780.
Pulmonary system limitations to endurance exercise performance in humans

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Accumulating evidence over the past 25 years depicts the healthy pulmonary system as a limiting factor of whole-body endurance exercise performance. This brief overview emphasizes three respiratory system-related mechanisms which impair O\textsubscript{2} transport to the locomotor musculature [arterial O\textsubscript{2} content (\(C_{aO_2}\)) \times leg blood flow (\(\dot{Q}_L\)]), i.e. the key determinant of an individual's aerobic capacity and ability to resist fatigue. First, the respiratory system often fails to prevent arterial desaturation substantially below resting values and thus compromises \(C_{aO_2}\). Especially susceptible to this threat to convective O\textsubscript{2} transport are well-trained endurance athletes characterized by high metabolic and ventilatory demands and, probably due to anatomical and morphological gender differences, active women. Second, fatiguing respiratory muscle work (\(W_{\text{resp}}\)) associated with strenuous exercise elicits sympathetically mediated vasoconstriction in limb-muscle vasculature, which compromises \(\dot{Q}_L\). This impact on limb O\textsubscript{2} transport is independent of fitness level and affects all individuals, but only during sustained, high-intensity endurance exercise performed above \(\sim 85\%\) maximal oxygen uptake. Third, excessive fluctuations in intrathoracic pressures accompanying \(W_{\text{resp}}\) can limit cardiac output and therefore \(\dot{Q}_L\). Exposure to altitude exacerbates the respiratory system limitations observed at sea level, further reducing \(C_{aO_2}\) and substantially increasing exercise-induced \(W_{\text{resp}}\). Taken together, the intact pulmonary system of healthy endurance athletes impairs locomotor muscle O\textsubscript{2} transport during strenuous exercise by failing to ensure optimal arterial oxygenation and compromising \(\dot{Q}_L\). This respiratory system-related impact exacerbates the exercise-induced development of fatigue and compromises endurance performance.

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exchange, even during strenuous endurance exercise. The majority of untrained ($V_{O2\,max} \leq 55 \text{ ml kg}^{-1} \text{ min}^{-1}$), but even most well-trained individuals, are characterized by only a small two- to threefold increase in the alveolar to arterial $O_2$ difference ($A-aD_{O2}$; surrogate of gas exchange efficiency) from rest ($\sim 5–8 \text{ mmHg}$) to $V_{O2\,max}$ ($<30 \text{ mmHg}$). This small change indicates a largely uncompromised and adequate rate of $O_2$ diffusion across the alveolar–capillary membrane (Dempsey & Wagner, 1999).

Furthermore, in most humans, alveolar ventilation during exercise can rise unrestricted and out of proportion to $CO_2$ production as arterial partial pressure of $CO_2$ is reduced to $\geq 10 \text{ mmHg}$ below resting levels. In other words, alveolar hyperventilation can increase sufficiently and raise alveolar partial pressure of $O_2$ ($P_{O2\,a}$) high enough to enable a compensation for the widened $A-aD_{O2}$. The net effect is a nearly unchanged arterial partial pressure of $O_2$ ($P_{O2\,a}$) from rest to $V_{O2\,max}$ and only a fairly small reduction in arterial haemoglobin saturation ($S_{O2}$), which is, however, almost exclusively caused by the exercise-induced increases in core temperature and metabolic acidosis. Also, airway resistance and lung compliance during exercise are maintained near resting levels and, in untrained subjects, breathing requires only $\leq 10\%$ of both $V_{O2\,max}$ and maximal cardiac output (Aaron et al. 1992; Harms et al. 1998b), and intrathoracic pressure changes developed by the respiratory muscles approximate only 40–50\% of their maximal dynamic capacity (Johnson et al. 1992).

Overall, the respiratory system in healthy young individuals might generally be considered as sufficiently ‘equipped’ to handle the pulmonary gas exchange requirements associated with even high-intensity endurance exercise.

Weaknesses and limits of the healthy respiratory system

In some, but not all, trained endurance athletes, the metabolic requirement associated with high-intensity exercise demands extreme ventilation and pulmonary gas exchange, which can reach and outstrip the functional capacity of their respiratory system and eventually compromise arterial oxygenation and limb $O_2$ transport (Dempsey et al. 1984; Williams et al. 1986; Powers et al. 1988; Harms et al. 1997). I briefly cover three respiratory system-related mechanisms which present significant limitations to locomotor muscle $O_2$ transport during exercise.

Exercise-induced arterial oxyhaemoglobin desaturation. High-intensity endurance exercise in some fit athletes causes a time-dependent decrease in $S_{aO2}$ of greater than 5\% from resting levels ($\sim 98\%$), although extreme drops into the mid 80\% range have been reported (Dempsey & Wagner, 1999). The oxyhaemoglobin desaturation during exercise is based on both respiratory and non-respiratory influences. Briefly, non-respiratory influences encompass the rightward shift of the oxyhaemoglobin dissociation curve mediated by metabolic acidosis and hyperthermia (Wasserman et al. 1967; Rasmussen et al. 1991).

In a minority of athletes, frequently those characterized by the greatest fitness (Williams et al. 1986), arterial oxyhaemoglobin desaturation also occurs due to a fall in $P_{aO2}$ (Holmgren & Linderotholm, 1958) secondary to an abnormally widened $A-aD_{O2}$ (Hopkins & McKenzie, 1989; Dempsey & Wagner, 1999). At maximal exercise in healthy untrained individuals, $A-aD_{O2}$ is usually up to 20–30 mmHg; however, in some elite athletes, this difference might be as wide as 35–50 mmHg (Dempsey et al. 1984).

Arterial desaturation during exercise can also occur due to an inadequate hyperventilatory response secondary to low chemoresponsiveness (i.e. attenuated response to circulating chemical stimuli such as protons, catecholamines, adenosine or potassium (Lumb & Nunn, 2000) and maybe also $O_2$ and $CO_2$ (Harms & Stager, 1995; Guenette et al. 2004)) and/or mechanical constraints presented by the airways (Dempsey et al. 1984; Johnson et al. 1992; Dempsey & Wagner, 1999). Inadequate ventilatory responses during exercise have been shown to reduce $P_{aO2}$, which negatively affects arterial blood gas status and $S_{aO2}$ (Johnson et al. 1992).

Some recent studies indicate a greater prevalence of arterial oxyhaemoglobin desaturation in active women compared with their male counterparts (Harms et al. 1998a; Hopkins et al. 2000; Hopkins & Harms, 2004). Various pulmonary structural and functional differences have been found between women and age- and height-matched men (Hopkins & Harms, 2004). For example, women are characterized by smaller lung volumes and airways, a lower resting lung diffusion capacity and lower maximal expiratory flow rates compared with men (McClaran et al. 1998; Guenette et al. 2007). Although the exact effects of these anatomical and morphological gender differences remain elusive, they are considered as key contributors to the greater gas exchange disturbances and ventilatory limitations during exercise in women versus men.

Remaining issues, from a personal communication with Professor Jerry Dempsey:

In the minority of trained individuals characterized by a reduction in $S_{aO2}$ secondary to an excessive $A-aD_{O2}$ and the resulting fall in $P_{aO2}$, it remains unresolved why the reductions in $P_{aO2}$ already occur during submaximal exercise and why they only seem to occur in trained rather than untrained individuals, especially runners. The
idea that we originally had (i.e. that the extraordinary demand for pulmonary O₂ transport exceeds the ordinary structural capacity of the lung in these athletes) does not apply in submaximal conditions because the athletes are not anywhere near maximal demands for O₂ transport and the ‘capacity’ of the lungs for gas exchange is not being challenged in the usual sense. The cause(s) of this arterial hypoxaemia during submaximal exercise in the absence of hypoventilation in these types of endurance-trained athletes remains a mystery to me. It is also a mystery to me that arterial hypoxaemia occurs most often during running and only rarely during bicycle exercise.

A further key unresolved issue is the huge variability in exercise-induced A–aDₐO₂ difference and oxyhaemoglobin desaturation amongst athletes. Many athletes are hardly affected even at maximal exercise, whereas others are characterized by a fall in PₐO₂ even during submaximal exercise which worsens at higher workloads.

**Exercise-induced respiratory muscle work (W.resp) and associated metaboreflex-mediated impact on Q_L.** A further threat to locomotor muscle O₂ delivery is W.resp associated with heavy sustained exercise (>85% VO₂ max). The ventilatory response during heavy exercise, which is often accompanied and impaired by expiratory flow limitations and dynamic hyperinflation (Johnson et al. 1992), requires substantial increases in both inspiratory and expiratory muscle work, often leading to respiratory muscle fatigue. Even though diaphragm force, during tidal breathing, falls during the latter stages of sustained heavy exercise, alveolar ventilation is not compromised, presumably due to accessory muscle recruitment. However, fatiguing contractions and associated accumulation of metabolites in the inspiratory and expiratory muscles activate unmyelinated group IV phrenic afferents (Hill, 2000), which reflexly increase sympathetic vasoconstrictor activity (St Croix et al. 2000) and vasoconstriction of the vasculature of the exercising limb (Harms et al. 1997; Fig. 1). The result is a reduction in Q_L and (presumably) an increase in blood flow to the respiratory muscles, indicating a competitive relationship for a limited cardiac output (Manohar, 1986; Musch, 1993). These effects do not occur during exercise at intensities lower than ~80% VO₂ max (Wetter et al. 1999). During intense exercise (>85% VO₂ max) in the highly trained subject, the respiratory muscles require up to 15–16% of VO₂ max versus ≤10% in the untrained. Thus, in contrast to arterial desaturation, W.resp induced by heavy, sustained exercise has no effect on CaO₂, but the reduction in O₂ transport is caused by reduced Q_L.

**Figure 1. Relationship between respiratory muscle work and leg blood flow**

Fatigue-related metabolite accumulation in respiratory muscles activates group III/IV phrenic afferents, which reflexly cause increased sympathetic efferent discharge and limb vasoconstriction. This sequence facilitates locomotor muscle fatigue and limits endurance exercise performance. Adapted from Dempsey et al. (2002), with permission.
Intrathoracic pressure effects on cardiac output. The ventilatory response during high-intensity exercise is associated with a substantial augmentation of negative and positive intrathoracic pressures. In the presence of expiratory flow limitation and hyperinflation in the well-trained young athlete, these inspiratory pressures may approach 95 and 30% of the maximal dynamic pressure available to the inspiratory and expiratory muscles, respectively (Johnson et al. 1992). The heart and great vessels are exposed to these substantial oscillatory pressures.

Recent studies in exercising humans and animals have used mechanical ventilation and threshold loads to reduce negative inspiratory or increase positive expiratory intrathoracic pressures, respectively. The results of these investigations suggest a substantial effect of these pressures on venous return, stroke volume and cardiac output during exercise. For example, the normally occurring negative inspiratory intrathoracic pressures associated with high-intensity exercise have a significant facilitating contribution (up to 10%) to end-diastolic volume and subsequently stroke volume and cardiac output. Importantly, no additional effects on cardiac output are observed when negative inspiratory intrathoracic pressures are increased beyond normal by imposing additional inspiratory negative pressure via resistive loading (Harms et al. 1998b; Miller et al. 2007). In contrast, even small increases in positive intrathoracic pressures on expiration (5–10 cmH₂O) have been shown to decrease ventricular transmural pressure, which reduces the rate of ventricular filling during diastole and thereby impairs stroke volume and cardiac output (Stark-Leyva et al. 2004; Miller et al. 2006). Increases in expiratory positive intrathoracic pressures of similar and even greater magnitudes occur during the transition from moderate to intense exercise in well-trained individuals and/or with the development of expiratory flow limitations (Johnson et al. 1992).

Taken together, negative inspiratory pressures during exercise appear to promote cardiac output via increasing ventricular preload and therefore stroke volume, whereas expiratory positive pressures during exercise limit cardiac output via increasing the ventricular afterload and thereby decreasing stroke volume. The net effect of intrathoracic pressure changes on cardiac output during high-intensity exercise in the well-trained endurance athlete will depend upon the degree to which the functional consequences of negative inspiratory pressures (i.e. facilitating cardiac output) balance the mechanical consequences of positive expiratory pressures (i.e. limiting cardiac output).

In summary, it should be emphasized that a threat to locomotor muscle O₂ transport secondary to arterial desaturation >4–5% from rest is experienced only by a subgroup of well-trained endurance athletes and can develop even at submaximal exercise intensities. However, the threat to O₂ delivery via the respiratory muscle

![Figure 2. Effect of exercise-induced arterial desaturation on 5 km cycling time trial performance](image-url)

During the iso-oxic trial, S\textsubscript{a}O\textsubscript{2} was maintained at resting levels (~98%) via progressive increases in inspiratory O₂ content (F\textsubscript{I}O\textsubscript{2}). Time to completion and mean power output (331 ± 13 versus 314 ± 13 W) were significantly improved during the iso-oxic time trial. Adapted from Amann et al. (2006), with permission.
metaboreflex occurs in all healthy subjects, but only at sustained, high-intensity endurance exercise (>85% \( V_{O_2\ max} \)). Furthermore, the reduction in \( Q_l \) imposed by the respiratory muscle metaboreflex is potentially even further exacerbated via potentially negative effects of intrathoracic pressure excursions on cardiac output.

Respiratory system limitations: consequences for endurance performance and fatigue

Consequences of exercise-induced arterial desaturation for endurance performance. The impact of arterial desaturation on endurance performance has been revealed by adding just sufficient \( O_2 \) to the inspired air to prevent the fall in \( S_{aO_2} \) during exercise. The measurable threshold of \( S_{aO_2} \)-related limitations to peak aerobic power occurs at a desaturation of >4–5% from rest (Squires & Buskirk, 1982; Powers et al. 1989; Harms et al. 2000a). Beyond this threshold, a linear association between the changes in saturation and \( V_{O_2\ max} \) is observed, such that each further 1% reduction in \( S_{aO_2} \) causes a 1–2% reduction in peak aerobic power.

Likewise, exercise-induced arterial desaturation also limits endurance performance achieved during a time trial-like test modality (Koskolou & McKenzie, 1994; Nielsen et al. 2002). For example, Amann et al. (2006) have recently demonstrated a significant limiting effect of arterial desaturation on 5 km cycling time trial performance (Fig. 2). The \( C_{aO_2} \), and thus \( O_2 \) delivery, was increased by ~8% when the exercise-induced fall in \( S_{aO_2} \) (to ~91%) was prevented by increasing the fraction of \( O_2 \) in the inspired air. This resulted in a substantial 2–5% reduction in the time to completion, and up to a 5% increase in mean power output.

Consequences of \( W_{resp} \) for endurance performance. The effects of the \( W_{resp} \) on endurance performance have been revealed by reducing the normally occurring \( W_{resp} \) during constant-load exercise via mechanical ventilatory assist or heliox breathing. At exercise intensities corresponding to ≤80% of \( V_{O_2\ max} \), significant 20–40% reductions in \( W_{resp} \) have no effect on endurance performance (Gallagher & Younes, 1989; Marciniuk et al. 1994; Krishnan et al. 1996). These observations are not surprising given the fact that blood flow redistribution between the respiratory muscles and the locomotor muscles only occurs at exercise intensities >85–90% of \( V_{O_2\ max} \) (Harms et al. 1997) and not at or below 80% of \( V_{O_2\ max} \) (Wetter et al. 1999). However, when constant-load exercise is performed at intensities greater than 85–90% \( V_{O_2\ max} \), respiratory muscle unloading was found to increase endurance time to exhaustion significantly (Wilson & Welch, 1980; Johnson et al. 1996).

For example, during constant-load cycling at 90% \( \dot{V}_{O_2\ max} \), a 60% reduction in \( W_{resp} \) resulted in increased limb vascular conductance and 3–4% increases in leg \( O_2 \) transport and uptake, even in the face of a reduced cardiac output (Harms et al. 1997). Time to exhaustion was increased by ~14% when \( W_{resp} \) was reduced by ~50%. This significant effect on exercise performance has been confirmed indirectly by increasing \( W_{resp} \) by ~28%, resulting in ~15% reduction in time to exhaustion (Harms et al. 2000b).

Consequences of pulmonary system limitations for the development of locomotor muscle fatigue. Even the relatively small reductions in \( O_2 \) transport associated with exercise-induced haemoglobin desaturation >5% from rest, or the high \( W_{resp} \), exacerbate the rate of development of peripheral locomotor muscle fatigue during exercise (Amann & Calbet, 2008). For example, during constant-load exercise (>90% \( V_{O_2\ max} \)), increases in locomotor muscle \( O_2 \) transport secondary to a ~60% reduction in \( W_{resp} \) (via proportional assist ventilation) alleviated end-exercise quadriceps fatigue by 25–30% compared with control exercise (Romer et al. 2006b, Fig. 3). Furthermore, when exercise-induced arterial desaturation was prevented during constant-load leg cycling (>90% \( V_{O_2\ max} \); via adding supplemental \( O_2 \) to the inspired air), end-exercise quadriceps fatigue was nearly 50% less compared with control conditions (Romer et al. 2006a). In contrast, no

Figure 3. Effects of a 60% reduction in inspiratory muscle work (Insp. Unload) on the pre- to postexercise change in the force–frequency curve of the quadriceps muscle

The \( y \)-axis represents the change for the second of the paired quadriceps twitch amplitudes (\( Q_{tw,y} \)). The work rate and exercise time were identical during control exercise and inspiratory unloading (>90% \( V_{O_2\ max} \); 292 W, 13 min). Adapted from Romer et al. (2006b), with permission.

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effect of maintaining resting $S_aO_2$ on peripheral fatigue was observed in those individuals who sustained haemoglobin saturation above 95% during the exercise (Romer et al. 2006a). The effect of $O_2$ delivery on peripheral fatigue development has been shown to be a key determinant of endurance exercise performance (Amann et al. 2006, 2011). We recently proposed that exercise-induced alterations of locomotor muscle fatigue affect, in a dose-dependent manner, the firing rate (and thus the central projection) of group III/IV muscle afferents, which are known to provide inhibitory feedback to the determination of central motor drive during exercise (Amann et al. 2006, 2009; Amann, 2011). In other words, acting via inhibitory feedback to higher motor areas, the highly $O_2$ delivery-sensitive peripheral locomotor muscle fatigue influences central motor drive and therefore exercise performance.

Exercise at altitude

Additional respiratory limits to exercise performance at or near sea level occur during acute or chronic exposure to the hypoxia associated with altitudes above $\sim 1500$ m (Buskirk et al. 1967). Hypoxia aggravates the proposed threats to limb $O_2$ delivery in two ways. First, the alveolar–capillary diffusion limitation becomes more pronounced, due to a decreased $P_aO_2$ at any given alveolar ventilation. Second, acute, but especially chronic, hypoxic exposures potentiate the hyperventilatory response to exercise and markedly increase $W_{\text{resp}}$ (Thoden et al. 1969; Amann et al. 2007). Therefore, hypoxia exacerbates the rate of development of peripheral locomotor muscle fatigue elicited via high-intensity exercise and reduces exercise performance in two ways, namely, via reductions in $S_aO_2$ and increases in $W_{\text{resp}}$ (Amann et al. 2007). For example, we recently studied identical submaximal constant-load cycling exercise (273 W, 8.6 min) performed at sea level and simulated altitude (inspiratory $O_2$ content = 15%). Haemoglobin saturation was substantially lower during the exercise in acute hypoxia ($\sim 95 \text{ versus } \sim 81\%$), whereas $W_{\text{resp}}$ was about 40% higher compared with sea level (Amann et al. 2007). These drastic changes nearly doubled the rate of development of locomotor muscle fatigue during the cycling exercise and compromised the subjects’ endurance performance (Amann et al. 2006).

Conclusion

Accumulating evidence over the past 25 years indicates a substantial role of the healthy respiratory system in limiting high-intensity endurance exercise in humans. This influence is mediated via the effects of the respiratory system on locomotor muscle $O_2$ delivery and associated consequences on the development of fatigue during exercise and an individual’s aerobic capacity. Reductions in $O_2$ delivery are caused by the failure of the pulmonary system to maintain resting arterial oxygenation during exercise and/or a respiratory muscle metaboreflex, which causes a sympathetically mediated reduction in $Q_L$. Furthermore, intrathoracic pressure excursions associated with the high ventilatory work during intense exercise have been suggested to limit cardiac output. Taken together, the pulmonary system is a key, although highly variable, determinant of endurance performance in healthy individuals.

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**Acknowledgements**

I thank Professor Jerry Dempsey for his comments on this manuscript. This work was supported by the US National Heart, Lung, and Blood Institute.
Symposium Report

Cardiovascular determinants of exercise capacity in the Paralympic athlete with spinal cord injury

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This report briefly summarizes the cardiovascular factors that influence exercise physiology and, eventually, sports performance of athletes with a spinal cord injury (SCI). The consequences of an SCI are numerous and concern voluntary muscle function, deep and superficial sensitivity, and autonomic function to a degree determined by the level and completeness of the spinal lesion. Athletes with SCI perform with their upper body, which limits their maximal exercise capacity and puts them at a disadvantage compared with leg exercise in terms of mechanical efficiency and physiological adaptations to exercise. Studies generally find that maximal oxygen consumption and mechanical power output are inversely related to spinal lesion level. Athletes with cervical or dorsal lesions down to Th6 have limited maximal heart rates owing to a lack of sympathetic drive to the heart. Blood redistribution from body areas lacking autonomic control is impaired, thus reducing venous return and limiting cardiac stroke volume during exercise. Thermoregulatory function is affected through a lack of afferent neural feedback and limited efferent vasomotor and sudomotor control below the lesion. Strategies to support venous return and to promote body cooling potentially improve physiological responses and athletic performance, especially in individuals with high lesion levels. The latter are subject to autonomic dysreflexia, a generalized sympathetic vasoconstriction below the lesion resulting from nociceptive stimulations in insensate body regions. Acute episodes induce high blood pressure, may enhance exercise performance and must be treated as a clinical emergency. Deliberate triggering of this reflex is prohibited by the International Paralympic Committee.

(Received 2 November 2011; accepted after revision 4 November 2011; first published online 16 November 2011)

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The upcoming London Paralympic Games will be one of the major sport events in the world in 2012. Over 4000 expected athletes, representing some 150 nations, will take part in the 20 disciplines of the Paralympic Programme. Once more, wheelchair sports will be one of the highlights, having grown extremely popular ever since the first ‘Olympics of the Disabled’ in Rome in 1960, called into life by Sir Ludwig Guttmann (Labanowich, 1989). Indeed, at that time, eligibility for participation was based on the presence of a spinal cord-related disability, i.e. spinal cord injury (SCI) or post-polio syndrome. While today athletes with other disabilities have integrated into the Paralympic Movement, those with an SCI remain represented in all Paralympic disciplines, except those dedicated to specific disability groups.

The sport performances of athletes with an SCI have improved dramatically over the past 30 years. For example, when the marathon race was introduced for wheelchair athletes in the 1984 Paralympic Games, the Canadian Mel Fitzgerald (International Stoke Mandeville Games Federation class V) covered the distance in 1 h 45 min 54 s. While this is certainly an outstanding accomplishment, it does contrast with the time of 1 h 23 min 17 s that brought the Australian Kurt Fearnley (functional class T54) Paralympic gold in the Beijing 2008 Paralympics. Such a spectacular evolution of sport performance is not only related to improvements of the wheelchair characteristics, technical aids and propulsion technique, but also to the optimization and specialization of training methods that will challenge the athlete’s body to its limits.
The purpose of this paper is briefly to review the cardiovascular factors determining exercise capacity and, eventually, sports performance in athletes with SCI. The particular aspects addressed include the general consequences of a spinal cord injury, the specificities of upper body compared with lower body exercise, the relationship between exercise performance and spinal lesion level and, finally, the cardiovascular background in SCI as a major determinant of exercise capacity and performance.

General consequences of spinal cord injury

The consequences of an SCI are largely determined by the lesion level and the extent of damage to the spinal cord in the transverse plane. The latter characteristic can give rise to a large range of syndromes of incomplete somatic, sensory and autonomic functions below the lesion level, which are beyond the scope of this review. The most obvious consequence of an SCI is the paralysis of muscles innervated from at or below the level of the spinal lesion. In the case of a complete SCI, there will be total interruption of the usual neuronal traffic from higher brain centres via the upper motoneuron. Voluntary control of the concerned muscle groups will be lost, while reflex contractions and spasticity via $\alpha$-motoneurons may appear as a result of lost inhibition from upper control centres. Likewise, sensory feedback about muscle length and joint position no longer reaches the brain, as is the case for any other deep or superficial sensory information (pain, temperature, pressure, etc.). The lesion level and its completeness can be determined quite precisely based on the testing of involved muscles and skin sensitivity. A major distinction is made between a syndrome of paraplegia and tetraplegia. In the former case, the SCI is located at the dorsal, lumbar or sacral level, affecting the lower limbs and the trunk to a variable degree. Tetraplegia additionally involves hand and arm function, so that manual wheelchair propulsion may be compromised (see Fig. 1).

An aspect that is generally less well known is the impact of an SCI on the autonomic nervous system, which also depends on the spinal lesion level (see Fig. 1). The cranial parasympathetic nervous system will remain intact, while the sacral parasympathetic functions will always be involved in the case of a complete SCI. The thoracolumbar sympathetic nervous system will be affected to a variable extent, potentially disturbing heart function and blood redistribution capacity during exercise, especially in athletes with high thoracic or cervical SCI. The ensuing consequences for exercise performance are numerous and will be addressed in greater detail below.

Specificities of upper body exercise

Individuals with SCI rely on their upper body musculature for athletic activity. Any sport performance must therefore be viewed in the light of the fundamental differences in the physiological adjustments between upper body and lower body exercise. Comparisons between these two exercise modes have mainly been drawn from studies investigating dynamic arm cranking and leg cycling in the same (mostly not upper body trained) population. A first observation is that maximal external power output (PO) and peak oxygen consumption ($\dot{V}O_2^{peak}$) are lower during arm cranking. Values generally range between 55 and 60% for PO and between 70 and 80% for $\dot{V}O_2^{peak}$ compared with leg cycling (Glaser, 1985; Pendergast, 1989; Marais et al. 2002). These findings pertain to the smaller active muscle mass involved during arm work,
limiting oxidative capacity and leading to an early onset of muscle fatigue. In contrast, oxygen uptake at any given absolute PO is higher for arm work, illustrating the lower mechanical efficiency of this exercise mode (Pendergast, 1989; Marais et al. 2002). Possible reasons are a greater reliance on less efficient, type II muscle fibres and the different cardiovascular and ventilatory responses elicited by arm cranking (Pendergast, 1989). Although cardiac output (Q) has been reported to be more or less similar in both exercise modes for a given level of oxygen uptake, arm cranking tends to induce a higher heart rate (HR), increased total peripheral resistance, and lower responses for cardiac stroke volume (SV). The lower SV responses may be caused by the absence of the skeletal muscle venous pump in the inactive legs during mere upper body exercise. Finally, the ventilatory response during arm cranking can be higher and the arteriovenous oxygen difference may be lower, especially in untrained individuals (Pendergast, 1989).

The maximal external PO that can be performed in a wheelchair is even lower than that during arm cranking (Haisma et al. 2006). Furthermore, at a given submaximal PO level, mechanical efficiency is lower for wheelchair exercise, with values generally below 10% (Glaser, 1985). Therefore, upper body exercise in general, and manual wheelchair propulsion in particular, elicit greater physiological strain than lower body exercise at a similar PO level, limiting exercise capacity and inducing early onset of muscle fatigue during exercise. As a consequence, individuals may not always reach their age-predicted maximal HR during upper body exercise, especially if they are untrained.

Influence of spinal lesion level on exercise performance

Exercise capacity of athletes with an SCI is inversely related to spinal lesion level (Dallmeijer et al. 1996; Haisma et al. 2006; Theisen, 2006). A review analysing the results of 37 studies including athletes and sedentary participants reported average VO_{2\text{peak}} values between 0.76 and 1.03 l min^{-1} for individuals with tetraplegia and between 1.03 and 2.51 l min^{-1} for subjects with paraplegia (Haisma et al. 2006). Greater average values were found for elite (tetraplegic) wheelchair rugby players (1.67 l min^{-1}; Leicht et al. 2011) and for individuals with incomplete cervical lesions after a 24-session structured hand cycle training (1.43 l min^{-1}; Valent et al. 2009). Elite basketball players with SCI reached high-end levels of 2.47 l min^{-1}, while those with other disabilities had significantly higher values of 3.35 l min^{-1} (Leicht et al. 2011). One reason for the inverse relationship between VO_{2\text{peak}} and lesion level is the amount of functional muscle mass (and thus oxidative capacity) that can be recruited to contribute to PO development. Therefore, maximal PO during wheelchair or arm cycle ergometry is also inversely related to the level of SCI (Dallmeijer et al. 1996; Haisma et al. 2006).

The amount of active muscle mass is not the only SCI-related factor determining athletic performance. Other critical aspects concern respiratory function and cardiovascular adjustments to exercise. Deficits in the ventilatory responses concern mainly expiratory muscles, while individuals with high cervical lesions also have inspiratory deficits (see Fig. 1). In paraplegics, average forced expiratory volume in 1 s amounts to 90% of age-, sex- and height-matched able-bodied control subjects, while in tetraplegics, this value reaches only 60% (Haisma et al. 2006). Some findings indicate that training of respiratory muscles of individuals with tetraplegia increases aerobic exercise performance and could improve maximal PO (Uijl et al. 1999), but the literature remains scarce (Van Houtte et al. 2006).

Cardiac function is impaired in athletes with a complete lesion above Th1. Indeed, these individuals lack sympathetic innervation to the heart and rely solely on parasympathetic withdrawal and circulating catecholamines to increase their HR (see Fig. 1). Heart rate responses during maximal exercise or high-intensity submaximal performance will therefore hardly exceed 110–130 beats min^{-1} (Bhambhani et al. 2010). Athletes with spinal lesion levels down to Th6 are also likely to achieve lower than anticipated maximal HR values (see Fig. 1), while those with lesions below that level will have normal responses (Hopman et al. 1993a; Hopman, 1994; Theisen, 2006).

Blood redistribution capacity during exercise will be affected to a variable degree, depending on the level and completeness of the lesion, owing to altered autonomic control. Compared with able-bodied exercising at the same relative submaximal intensity (oxygen uptake), individuals with an SCI will have lower (Jacobs et al. 2002) or similar Q responses (Hopman, 1994); however, these responses are achieved by a higher HR and lower cardiac SV compared with able-bodied individuals. The reason is mainly related to a lack of sympathetic vasoconstriction in the paralysed body parts, as determined from studies focusing on whole-leg volume changes (Hopman et al. 1993b), skin blood flow (Theisen et al. 2000; 2001a) and blood flow to the splanchnic bed (Thijssen et al. 2009). As a result, venous return is hampered and, in accordance with the Frank–Starling mechanism, the rise in SV during exercise is limited compared with able-bodied control subjects. During submaximal exercise, Q can be maintained at similar levels by a compensatory rise in HR, provided that sympathetic innervation to the heart is intact (lesion level below Th6; Hopman et al. 1993a). In maximal exercise conditions, however, the lower SV could limit maximal Q, oxygen delivery and athletic performance (Hopman, 1994).
Venous return and cardiac responses in exercising individuals with SCI can be improved by different techniques, such as wearing an anti-G suit (Hopman et al. 1992) or electrically stimulating the paralysed lower limb muscles (Raymond et al. 2001); however, these techniques have no practical use in a sports context. Wearing an abdominal binder did not influence the physiological responses of highly trained paraplegic athletes (Kerk et al. 1995), but a decrease in HR and an increase in SV was found in less active tetraplegics when combined with compressive stockings (Hopman et al. 1998).

Thermoregulatory function is influenced by the lack of afferent neural input from insensate body parts and impaired vasomotor and sudomotor control below the lesion level (see Fig. 2). Athletes with a complete SCI lack active vasodilatation to increase skin blood flow and sweating in the body parts without autonomic control (Hopman et al. 1993a; Theisen et al. 2000, 2001b), which limits cooling efficiency and increases the risk of hyperthermia. This is especially critical for individuals with tetraplegia or when exercising in thermally strenuous conditions (Price, 2006). Although exercising athletes with SCI generally endure greater heat storage than their able-bodied counterparts, paraplegic individuals seem to be able to tolerate this additional strain without significant adverse effects when performing in a thermoneutral environment (Price, 2006). Strategies such as hand cooling (Goosey-Tolfrey et al. 2008), feet cooling (Hagobian et al. 2004), or the utilization of cooling vests (Webborn et al. 2005) or spray bottles (Pritchett et al. 2010) prior to or during exercise have shown potential or been proved useful to control core temperature rises. The greatest effects have been found in individuals with tetraplegia, while fewer effects are observed in paraplegics. Although these strategies are interesting, not all have demonstrated significant performance improvements, and their applicability in a sport context can be problematic.

Boosting

A particular aspect of the physiology of individuals with SCI is the occurrence of autonomic dysreflexia (Bhambhani et al. 2010). It is especially prevalent in individuals with spinal lesions above Th6 and consists of a general sympathetic-induced vasoconstriction in the body parts lacking innervation by the autonomic nervous system. This so-called ‘mass reflex’ is generally triggered by a nociceptive stimulus below the lesion (e.g. bladder distension). Typical symptoms include high blood pressure, excessive sweating, headache and, less frequently, shivering and blurred vision (Bhambhani et al. 2010). The blood pressure increase induces a reflex bradycardia and vasodilation in innervated body areas, but the effect is insufficient to counterbalance the blood pressure rise to sometimes dangerously high levels.

Autonomic dysreflexia is a clinical emergency and should be treated without delay by finding and eliminating the nociceptive source. A recent study (Bhambhani et al. 2010) suggests that more than 15% of athletes with an SCI above Th6 have voluntarily induced autonomic dysreflexia to improve athletic performance. This process,

Figure 2. Changes in leg skin blood flow (SKBF), leg skin temperature ($\Delta T_{\text{skin}}$) and oesophageal temperature ($\Delta T_{\text{es}}$) in six able-bodied (AB) and 12 spinal cord injured (SCI) subjects (Th5–Th12 lesions) during an incremental arm crank exercise test

* Significant difference between the two groups ($P < 0.05$). (Redrawn with kind permission from Springer Science+Business Media: Eur J Appl Physiol, Cutaneous vasomotor adjustments during arm-cranking in individuals with paraplegia, 83, 2000, 539–44, Theisen D, Vanlandewijk Y, Sturbois X & Francaux M, figure 2.)
termed ‘boosting’, has been found to increase peak exercise performance, as reflected by higher peak PO, oxygen uptake, HR and catecholamine levels (Schmid et al. 2001). Boosting is potentially dangerous and is considered a doping offense by the International Paralympic Committee. The fact that 40% of Paralympians with a high SCI are unaware of this phenomenon calls for educational programmes to enhance the knowledge of athletes and other stakeholders (Bhambhani et al. 2010).

Conclusion

The cardiovascular adaptations to exercise in athletes with an SCI are influenced by a series of critical factors. First of all, maximal exercise capacity is influenced by the fact that these athletes perform with their upper body, an exercise mode characterized by a lower \( \dot{V}O_2 \text{peak} \) and maximal PO compared with leg exercise. Second, the level of the spinal lesion is inversely related to active muscle mass that can be recruited for PO development, which limits maximal oxygen consumption and endurance capacity. Cardioacceleration may be impaired if the spinal lesion is located higher than Th6, owing to a lack of sympathetic drive to the heart. Blood redistribution via sympathetic vasoconstriction is impaired in the paralysed body parts, inducing lower cardiac SV and higher HR at submaximal exercise intensities. Finally, cardiovascular responses may be further burdened by the less efficient thermoregulation, imposing a higher thermal strain during exercise. Specific strategies supporting venous return and favouring body cooling may improve exercise capacity and sports performance of SCI athletes.

References


Thermoregulatory aspects of performance

This issue of *Experimental Physiology* contains symposium papers from the conference *The Biomedical Basis of Elite Performance* held on the 19–21 March 2012 at The Queen Elizabeth II Conference Centre, London which covers the theme of *Thermoregulatory aspects of performance*.

Elite athletes are capable of prodigious feats of exercise, but for every individual there is a limit to the power output that can be achieved at any given moment. It seems futile to look for a single factor that limits exercise performance in all individuals, in all types of exercise and in all environments. Nonetheless, there appear to be some common factors. Endurance exercise performance is impaired progressively as the ambient temperature increases (Galloway & Maughan, 1997) and, at least in a warm environment, is impaired progressively as the ambient humidity rises (Maughan et al. 2012). These observations, which are a matter of common experience among athletes and industrial workers, suggest that some aspect of thermoregulatory function is linked to the fatigue that accompanies prolonged exercise in hot, humid environments. They do not, however, identify how or where thermal stress is sensed, nor do they offer any clues concerning the physiological mechanisms responsible for the early onset of fatigue.

The metabolic rate increases to meet the needs of exercise, and the marathon runner who wishes to break the men’s world best performance of 2 h 03 min 38 s must sustain a speed in excess of 20 km h$^{-1}$. Assuming a body mass of 55 kg, this requires an energy expenditure of more than 1100 kcal h$^{-1}$, with 20% or more of the energy appearing as heat. Most of this heat is generated in a relatively small muscle mass, and González-Alonso (2012) has considered the implications for the cardiovascular system of the need to dissipate this heat. Some is lost by conduction to the skin overlying the active limbs and thence to the environment, but most must be transferred by convection via blood flow from the active musculature to the body core and thence to the skin. A high flow rate to the muscle and to the vascular bed of the skin requires a cardiac output that may exceed the pumping capacity of the heart, especially if the blood volume is reduced by the high rates of sweating that are necessary to maintain evaporative heat loss.

In order to maintain the rate of evaporative heat loss, there is a need to maintain a high skin temperature and this in turn requires a high skin blood flow. Sawka et al. (2012) have argued that the skin temperature, rather than a high core temperature, plays a key role in the aetiology of fatigue. The problem with a high skin temperature is that it narrows the temperature gradient from the body core to the skin, thus increasing the blood flow requirement for convection of heat from the core to the skin. They provide compelling evidence that high skin temperatures will lead to impairment of maximal aerobic power as well as of endurance performance, and argue against the widely held view that a high core (brain) temperature is in itself the primary limitation to exercise.

The idea that the brain – rather than the heart, lungs or muscles – acts to limit performance is not new. Indeed, this concept was the prevailing one at the end of the 19th century (Lagrange, 1889). Only in recent years, however, has it been possible to investigate events taking place within the central nervous system in exercising man. Nybo and colleagues have made an extensive series of measurements of heat exchange across the brain during exercise and have shown that brain temperature is closely related to body temperature (Nybo, 2012). Transcranial magnetic stimulation and other techniques have been used to show that both passive and exercise-induced hyperthermia impair voluntary muscle activation during intense muscle activity. In contrast to the stance of Sawka et al. (2012), Nybo’s conclusion is that brain temperature plays a key role in performance in the heat. This may be at least partly because a high skin temperature will be
inevitable during hard exercise in a hot environment, with all the implications for cardiovascular function that follow.

Although the combination of heat, high humidity and hard exercise pose a major challenge to thermoregulation and exercise capacity, the elite athlete can defy our expectations if those expectations are based on laboratory measures of physiological responses of moderately trained individuals in the laboratory. Prior to the Atlanta Olympics of 1996, Bodil Nielsen calculated the effects of the ambient conditions on the thermoregulatory capacity of a marathon runner and concluded that a fast time would not be possible in the anticipated heat and humidity. Nevertheless, the winning time in the men’s race was 2 h 12 min and the women’s race was won in a time of 2 h 26 min. Even more remarkable, however, was the performance of Sammy Wanjiru at the Beijing Olympic marathon of 2008. In spite of an ambient temperature of up to 30°C and high humidity, he completed the marathon in 2 h 6 min 32 s. The performance is not in doubt, so we have to revisit our thoughts on the thermoregulatory capacity of the elite endurance athlete.

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References


High skin temperature and hypohydration impair aerobic performance

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This paper reviews the roles of hot skin (>35°C) and body water deficits (>2% body mass; hypohydration) in impairing submaximal aerobic performance. Hot skin is associated with high skin blood flow requirements and hypohydration is associated with reduced cardiac filling, both of which act to reduce aerobic reserve. In euhydrated subjects, hot skin alone (with a modest core temperature elevation) impairs submaximal aerobic performance. Conversely, aerobic performance is sustained with core temperatures >40°C if skin temperatures are cool-warm when euhydrated. No study has demonstrated that high core temperature (~40°C) alone, without coexisting hot skin, will impair aerobic performance. In hypohydrated subjects, aerobic performance begins to be impaired when skin temperatures exceed 27°C, and even warmer skin exacerbates the aerobic performance impairment (~1.5% for each 1°C skin temperature). We conclude that hot skin (high skin blood flow requirements from narrow skin temperature to core temperature gradients), not high core temperature, is the 'primary' factor impairing aerobic exercise performance when euhydrated and that hypohydration exacerbates this effect.

(Received 4 August 2011; accepted after revision 28 November 2011; first published online 5 December 2011)

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Heat stress impairs submaximal and maximal aerobic exercise performance (Sawka et al. 2011). For maximal intensity exercise, cardiovascular mechanisms related to oxygen delivery are likely to limit performance in the heat (Rowell et al. 1966; González-Alonso & Calbet, 2003). The mechanisms limiting sustained, submaximal intensity exercise in the heat include cardiovascular, CNS and metabolic (glycogen depletion) changes (Cheung & Sleivert, 2004; Sawka et al. 2011). Metabolic limitations are minor and specific to particular exercise tasks in the heat (Cheung & Sleivert, 2004). Cardiovascular mechanisms were historically assumed to be the primary factor impairing submaximal performance in the heat (Rowell, 1986), but the sustainment of skeletal muscle blood flow at exhaustion shifted the emphasis towards CNS limitations and the role of high core temperatures (Nielsen et al. 1990, 1993).

Bodil Nielsen and colleagues (Nielsen et al. 1990) proposed that a high core temperature (~40°C) ‘having an effect on the CNS in reducing the motor drive for performance’ is the critical factor impairing submaximal intensity aerobic performance in the heat. During the past decade, the ‘critical’ core temperature hypothesis has been widely attributed as the primary mechanism impairing submaximal aerobic performance in the heat. Deteriorated CNS function may contribute to impaired aerobic performance in the heat (Nybo & Nielsen, 2001a), but the importance of a high core temperature has rarely been questioned. Studies supporting the ‘critical’ core temperature hypothesis have simultaneously induced high core temperatures with hot skin (Nielsen et al. 1990, 1993; González-Alonso et al. 1999). To our knowledge, no study has demonstrated that high core temperature alone will impair aerobic performance.

This paper reviews recent evidence that hot skin (>35°C) alone can impair submaximal aerobic performance. Additional evidence will be provided...
Table 1. Estimated whole-body skin blood flow (SkBF) requirements* during prolonged, severe running exercise at different body core (Tc) and skin temperatures (Tsk)

<table>
<thead>
<tr>
<th>Tc (°C)</th>
<th>Tsk (°C)</th>
<th>Gradient (°C)</th>
<th>SkBF (l min⁻¹)</th>
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<tr>
<td>38</td>
<td>30</td>
<td>8</td>
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<td>39</td>
<td>36</td>
<td>3</td>
<td>2.9</td>
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*Equation for skin blood flow: \( Q_{sk} = \frac{1}{C} \times h(T_c - T_{sk}) \), where C is the specific heat of blood (~0.87 kcal °C⁻¹ l⁻¹), h the heat production (in kcal min⁻¹) and \( Q_{sk} \) the skin blood flow (Rowell, 1986). Net heat production (7.7 kcal min⁻¹) estimated using 60 kg body mass and 325 m min⁻¹ running velocity (approximate pace for men’s world class 42 km footrace) after subtracting for work (20% efficiency) and 50% dry and evaporative heat losses.

that if skin temperatures (Tsk) are cool-warm, aerobic performance can be sustained despite high core temperatures (Tc). Hot skin narrows the Tsk to Tc gradient, which increases skin blood flow requirements (Rowell, 1986) and may be the ‘primary’ factor impairing submaximal aerobic exercise performance in the heat. Body water deficits (>2% body mass; hypohydration) will exacerbate the effect by reducing central blood volume. We therefore postulate that during exercise heat stress, hot skin and hypohydration act in concert to reduce aerobic reserves, which increases the relative exercise intensity and perception of effort.

Physiology of skin temperatures and hypohydration

During exercise in the heat, the most significant physiological burden is to support high skin blood flow for heat dissipation (Sawka et al. 2011). Skin temperature is elevated in proportion to ambient temperature and humidity (Gagge & Gonzalez, 1996), while Tc is elevated in proportion to exercise intensity and is largely independent of the environment during compensable heat stress (Sawka et al. 2011). Warm-hot skin is associated with a greater skin blood flow and cutaneous venous compliance, which augments cardiovascular strain (Sawka et al. 2011). For this review, we define hot skin as 35°C and above, warm skin as 30–34.9°C and cool/cold skin as <30°C. We recognize that skin temperature effects are a continuum and that the Tsk to Tc gradient alters these relationships.

Table 1 illustrates the effects of different Tsk and Tc combinations on estimated (Rowell, 1986) whole-body skin blood flow requirements during combined exercise and heat stress. An elevated Tsk increases skin blood flow at any given Tc, while an elevated Tc reduces skin blood flow requirements at any given Tsk. The rows beginning with Tc 38 and 39°C highlight an often unappreciated point; at any given skin temperature, an elevation in core temperature reduces whole-body skin blood flow and can be viewed as a positive response for sustaining aerobic performance in the heat. For example when comparing Tc of 39°C to a Tc of 38°C at equivalent Tsk of 36°C, SkBF is reduced from 4.4 to 2.9 l min⁻¹. Figure 1 demonstrates the impact of warm-hot Tsk, at constant Tc (~37.5°C), on cardiovascular strain during light-intensity (metabolic rate ~450 W) exercise (Cheuvront et al. 2003). The heart rate (HR) elevation during exercise was an exponential function of skin warming beyond Tsk ~35°C. The high skin blood flow requirements act to reduce cardiac filling and elevate HR for a given cardiac output (Trinity et al. 2010; Stohr et al. 2011b). Conversely, rapidly cooling Tsk has a profound effect on reducing HR and sustaining mean arterial pressure during exercise in the heat (Shaffrath & Adams, 1984). In addition, hot skin can be associated with reduced cerebral blood flow and cerebral oxygen delivery during moderate-intensity exercise (Nybo & Nielsen, 2001b; Nybo et al. 2002; Rasmussen et al. 2010).

During combined exercise and heat stress, hypohydration augments hyperthermia and cardiovascular strain in proportion to the magnitude of body water deficit (Sawka et al. 1985). Hypohydration reduces cardiac filling (Stohr et al. 2011b) and stroke volume during combined exercise and heat stress, making it difficult to maintain cardiac output (Montain & Coyle, 1992) and sustain muscle blood flow when heat stress is severe (Gonzalez-Alonso et al. 1998).

Figure 1. Impact of high skin temperature, with a constant core temperature, on elevating heart rate during light-intensity exercise (metabolic rate ~450 W) From Cheuvront et al. (2003).
Aerobic performance

Heat stress. Warm-hot $T_{sk}$ degrades maximal aerobic power ($V_{O2\ max}$) in proportion to the $T_{sk}$ elevation (Arrgimsson et al. 2003). Thus, when performing exercise at a given metabolic rate, a person with warm-hot skin will work at a greater percentage $V_{O2\ max}$ compared with temperate conditions. Figure 2 demonstrates that marathon race performance is progressively slower with increased environmental (wet bulb globe temperature; WBGT) heat stress (Ely et al. 2007). Skin temperature is elevated with WBGT (Gagge & Gonzalez, 1996), but $T_c$ may or may not be elevated, as it depends upon the sustainment of exercise intensity and heat exchange biophysics (Sawka et al. 2011). Therefore, marathon race performance might slow as a function of elevated $T_{sk}$.

Laboratory studies consistently demonstrate that $T_{sk}$ elevations impair submaximal intensity aerobic performance. González-Alonso et al. (1999) employed a time-to-exhaustion (TTE; 60% $V_{O2\ max}$) test, during which subjects wore a water-perfused suit. When $T_{sk}$ was raised incrementally from $\sim$36 to 38°C, TTE was shortened from 56 to 31 min; however, between the two trials, $T_c$ ($\sim$40°C) and HR ($\sim$188 beats min$^{-1}$) were similar at exhaustion. MacDougall et al. (1974) used a similar combination of TTE test (70% $V_{O2\ max}$) with water-perfused suit to show that when $T_{sk}$ was raised incrementally from $\sim$29 to $\sim$32 and then $\sim$35°C, TTE was shortened from 90 to 75 and then 48 min, respectively, despite similar core temperatures at exhaustion ($\sim$39.5°C). Tatterson et al. (2000) used a time-trial (TT) test and reported that performance was impaired by $\sim$6% in a warm environment when $T_{sk}$ was $\sim$33°C, versus 27°C in a temperate environment. Core temperature and HR levels were again similar at exhaustion ($\sim$39.3°C and $\sim$195 beats min$^{-1}$), as in other studies. Most recently, Periard et al. (2011) reported a $\sim$13% decrement in mean power output during a 40 km TT in hot versus temperate environmental conditions that produced $T_{sk}$ of 36 and 28°C, respectively. Although $T_c$ was higher at exhaustion in the heat (39.8 versus 38.9°C), pacing strategy fell off significantly after 20 min of cycling when $T_c$ was similar in both trials ($\sim$38°C), while $T_{sk}$ was already $>5^\circ$C higher in the heat.

Performance studies cited as directly supporting the ‘critical’ core temperature hypothesis have simultaneously elicited high core temperatures with hot skin. In the original study of Nielsen et al. (1993), subjects completed a TTE test (60% $V_{O2\ max}$) for 9–12 consecutive days in a hot environment as part of a heart acclimation experiment. Heat acclimation increased TTE from 48 to 80 min over the test days, with exhaustion consistently coinciding with $T_c$ $\sim$40°C and $T_{sk}$ $\sim$37°C. González-Alonso et al. (1999) manipulated initial body temperatures prior to a TTE test (60% $V_{O2\ max}$) in a hot environment by applying precooling, no precooling and preheating to subjects. The critical core temperature explanation for fatigue resulted from exhaustion coinciding with a consistently high $T_c$ ($\sim$40°C), but $T_{sk}$ ($\sim$37°C) and HR ($\sim$196 beats min$^{-1}$) were equally consistent, with the HR near maximal levels based on age.

There is evidence that the $T_{sk}$ ($>35^\circ$C) alone can degrade aerobic performance. Ely et al. (2010) measured the impact of two environmental conditions (40 and 20°C) on a 15 min TT performance test where $T_c$ elevation was modest and similar in both trials ($\sim$38.2°C), but the compensable environments produced cool-warm (30°C) or hot skin (36°C). Time trial performance was impaired by 17% with hot $T_{sk}$, although a similar HR ($\sim$180 beats min$^{-1}$) was achieved. These findings are consistent with studies employing uncompensable heat stress to produce hot $T_{sk}$ ($>35^\circ$C) while performing a walking TTE test (Sawka et al. 1992; Montain et al. 1994; Latzka et al. 1998). During those studies, physical exhaustion routinely occurred ($\sim$50% of cases) at relatively low $T_c$ ($<38.5^\circ$C), but with high HR relative to the exercise intensity. Therefore, hot skin will impair performance and induce exhaustion well below levels associated with the ‘critical’ $T_c$ hypothesis.

There is evidence that competitive running performance (velocity) can be preserved despite high $T_c$ $\geq$40°C, if $T_{sk}$ is cool-warm (Ely et al. 2009; Lee et al. 2010). Ely et al. (2009) had highly trained runners perform an 8 km running TT on a 400 m track in compensable environmental conditions eliciting cool-warm $T_{sk}$ ($32–34^\circ$C). They measured running...
velocities over 200 m segments and found no difference when \( T_c \) was below (first 6.5 km) or above 40°C (final 1.5 km). Figure 3 presents the individual data for average running velocities when \( T_c \) was below and above 40°C. Lee et al. (2010) also examined running performance in a warm environment during a longer, 21 km race, in which velocity was determined for 3 km intervals. They too found that high core temperature (≥39.5°C) was common and not associated with reduced performance. Although \( T_{sk} \) was not measured, the \( T_{sk} \) prediction equation of Adams (1977) for outdoor running in the sun would suggest a value near 32°C.

**Hypohydration.** Hypohydration impairs maximal aerobic power in hot environments (Craig & Cummings, 1966) and submaximal aerobic performance in temperate and warm-hot environments (Cheuvront et al. 2005; Castellani et al. 2010; Kenefick et al. 2010). The following studies demonstrated that hypohydration impairs submaximal aerobic performance and that the impairment is augmented by high \( T_{sk} \). In those studies, \( T_c \) was <39°C and therefore well below the ‘critical’ \( T_c \).

Cheuvront et al. (2005) tested the effect of hypohydration on aerobic performance using a 30 min exercise preload at ~50% \( \dot{V}_{O2\max} \) followed by a 30 min TT in temperate and cold environments. Hypohydration by 3% body mass impaired performance by 8% in the temperate (\( T_{sk} \sim 29°C \)) but not in the cold environment (\( T_{sk} \sim 20°C \)). Castellani et al. (2010) used a nearly identical test whereby \( T_{sk} \) was ~32°C in both hypohydration and euhydration trials. Hypohydration by 4% body mass impaired performance by 18%. Kenefick et al. (2010) further characterized the interaction between environmental conditions and hypohydration by having subjects exercise for 30 min (50% \( \dot{V}_{O2\max} \)) followed by a 15 min TT in 10, 20, 30 and 40°C environments (inducing stepwise increases in \( T_{sk} \) from 26 to 36°C) when euhydrated and when hypohydrated by 4% body mass. Hypohydration impaired aerobic performance by 12 and 23% when \( T_{sk} \) was 33 and 36°C, respectively.

Figure 4 plots the impact of hypohydration on aerobic performance from the preceding three studies (Castellani et al. 2010; Cheuvront et al. 2005; Kenefick et al. 2010). These studies employed similar procedures over a broad range of \( T_{sk} \) from 20 to 36°C. Segmented regression (Vieth, 1989) was used to approximate the statistical \( T_{sk} \) threshold for performance impairment using individual study data points (\( n = 53 \) paired observations). The threshold which best minimized the residual sums of squares was shown to be 27.3°C, and warmer skin accentuated the performance impairment by ~1.3% for each additional 1°C rise in \( T_{sk} \) similar to that reported by Kenefick et al. (2010).

**High skin temperature/relative intensity hypothesis**

Cheuvront et al. (2010) proposed that impaired submaximal aerobic performance in the heat might be explained by warm-hot \( T_{sk} \) reducing \( \dot{V}_{O2\max} \). A large \( \dot{V}_{O2\max} \) is a prerequisite for success in sports where

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**Figure 3.** Time trial running velocities of 12 highly trained runners in compensable environmental conditions (cool-warm skin temperatures) when their core temperatures were below (mean of first ~32 200 m segments) or exceeded a core temperature of 40°C (mean of last approximately eight 200 m segments). From Ely et al. (2009).

**Figure 4.** Percentage decrement in submaximal aerobic performance from euhydration as a function of skin temperature (\( T_{sk} \)) when hypohydrated by 3–4% of body mass. Data are means (error bars are 95% confidence intervals) compiled from three studies (Cheuvront et al. 2005; Castellani et al. 2010; Kenefick et al. 2010) employing similar experimental procedures and time trial (TT) performance tests. Filled circles represent 15 min TT tests; open circles represent 30 min TT tests. At a \( T_{sk} \) intercept of ~27°C, the percentage decrement in aerobic exercise performance declines linearly by ~1.3% for each 1°C rise in \( T_{sk} \), similar to the single study of Kenefick et al. (2010). The best-fit equation for the second line segment is \( y = -1.26x + 26.37 \).
aerobic metabolism predominates (Bassett, & Howley, 2000), and \( V_{O_2\text{max}} \) is reduced incrementally with warm-hot \( T_{sk} \) (Arngrimsson et al. 2003). When \( V_{O_2\text{max}} \) is reduced, the resultant increased percentage \( V_{O_2\text{max}} \) results in impaired submaximal exercise capacity (Gleser & Vogel, 1973a,b). If relative exercise intensity is increased, constant-rate exercise (TTE) will be more difficult to sustain (earlier fatigue) or require a slowing of self-paced exercise (TTT) to achieve a similar sensation of effort. An increased percentage \( V_{O_2\text{max}} \) is associated with greater cardiopulmonary stress (HR and respiration) and elevated perceived exertion, while warm-hot skin is associated with elevated thermal discomfort (Gagge et al. 1969; Gonzalez & Gagge, 1973). Other physiological cues being sensed might include cardiopulmonary baroreceptor unloading (Stohr et al. 2011a,b), reduced cerebral perfusion or cerebral oxygenation (Rasmussen et al. 2010) and arterial hypocapnia (Sawka et al. 1980). Likewise, if CNS function is deteriorated by heat stress and contributes to impaired performance, the afferent input from skin (Kunsch et al. 1994), muscle (Todd et al. 2005) and perhaps osmoreceptors/baroceceptors when hypohydrated (Montain & Tharion, 2010) might all contribute to altering the signal processing.

We conclude that: (1) hot skin (high skin blood flow requirements from narrow \( T_{sk} \) to \( T_c \) gradients), not high core temperature, is the ‘primary’ factor impairing submaximal aerobic performance when euhydrated; (2) hypohydration impairs submaximal aerobic performance when skin temperature is \( \sim 27^\circ \text{C} \), and even warmer skin exacerbates (–1.5% for each \( 1^\circ \text{C} \) increment of \( T_{sk} \)) these decrements; and (3) high core temperature (\( \sim 40^\circ \text{C} \)) alone does not impair aerobic performance.

References


Brain temperature and exercise performance

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Events arising within the central nervous system seem to be a major factor in the aetiology of hyperthermia-induced fatigue. Thus, various studies with superimposed electrical nerve stimulation or transcranial magnetic stimulation have shown that both passive and exercise-induced hyperthermia will impair voluntary motor activation during sustained maximal contractions. In humans, the brain temperature increases in parallel with that of the body core, making it very difficult to evaluate the independent effect of the cerebral temperature. Experiments with separate manipulation of the brain temperature in exercising goats indicate that excessive brain hyperthermia will directly affect motor performance. However, several homeostatic changes arise in parallel with hyperthermia, including factors that may influence both peripheral and central fatigue, and it is likely that these changes interact with the inhibitory effect of an elevated brain temperature.

(Received 28 September 2011; accepted after revision 23 November 2011; first published online 24 November 2011)

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Hyperthermia, brain temperature and fatigue

During exercise, hyperthermia-induced fatigue becomes important when endogenous heat production surpasses the capacity for heat release to the environment, because it will elevate the skin temperature and augment the core temperature response. During exercise that elicits maximal oxygen uptake, muscle fatigue arising secondary to reduced arterial oxygen delivery may, to a large extent, explain the hyperthermia-induced reduction in performance (see Nybo, 2008 for discussion). During prolonged hyperthermic exercise trials, however, performance is markedly impaired, although the muscle oxygen uptake remains similar to that of control exercise at the same workload (Nybo & Nielsen, 2001a,b). During exercise with progressive hyperthermia, the brain temperature increases in parallel with that of the body core and, as discussed in the present paper, this could be an important factor influencing the fatigue arising during prolonged exercise as well as central fatigue provoked by passive heating.

Until the beginning of this millennium, there were no direct assessments of the cerebral temperature response during exercise or data directly supporting the hypothesis that hyperthermia was associated with central fatigue. However, the absence of peripheral fatigue and failure to observe increased levels of muscle metabolites or ion changes that could explain the marked reductions in performance indicated that a central fatigue mechanism could be involved (Nielsen et al. 1990). Fatigue is influenced by a number of muscular as well as cerebral factors, and the relative importance of central fatigue is still debated; however, several studies have provided experimental evidence for central fatigue as a consequence of exercise-induced hyperthermia (Nybo & Nielsen, 2001a) or following passive hyperthermia (Morrison et al. 2004; Todd et al. 2005; Racinais et al. 2008). Maximal isometric contractions combined with superimposed electrical nerve or muscle stimulation or transcranial magnetic stimulation of the motor cortex are the commonly applied models to investigate the contribution of central versus peripheral fatigue, but some studies have also investigated the issue during isokinetic contractions (Gandevia et al. 1995; Gandevia, 2002). Figure 1 is modified on the basis of results from the first experimental study that used this approach to demonstrate that hyperthermia reduces voluntary activation during a prolonged maximal knee extension (Nybo & Nielsen, 2001a). The reduction of the integrated EMG and the lowered voluntary activation percentage during the latter stage of the isometric contraction clearly show that the ability to maintain maximal activation of the skeletal muscles is reduced during a sustained contraction.

Figure 1. Force production (A), voluntary activation level (B) and rectified integrated surface electromyography (IEMG; C) from the vastus lateralis muscle during 2 min of sustained maximal knee extension during hyperthermia (core temperature of \(\sim 40^\circ\text{C}\)) and control conditions (core temperature of 38\(^\circ\text{C}\)). The subjects were instructed and verbally encouraged to make a maximal effort throughout the contraction, and electrical stimulation (EL) was superimposed every 30 s to assess the level of voluntary activation, which was calculated as voluntary force divided by the force elicited when EL was superimposed. Data are means \(\pm\) SEM for eight subjects (error bars not included in A and C). *All values in this period are significantly lower than control values, \(P < 0.05\). (Modified from Nybo et al. 2001a, with permission.)

and especially during the hyperthermic trial. However, exercise-induced fatigue is seldom related solely to a single factor, and the picture of hyperthermia-induced central fatigue is more complex, because voluntary activation may be maintained during repeated maximal contractions if these are relatively short and interspersed by brief breaks (see Fig. 2).

Two logical questions precede these observations. Firstly, how important is the central fatigue aspect during dynamic exercise, i.e. may dynamic exercise be considered as a continuous effort and compared with the sustained contractions illustrated in Fig. 1 or as a series of contractions and comparable to the data presented in Fig. 2? Secondly, what physiological mechanism(s) may explain the observed performance and fatigue pattern? While superimposed electrical nerve stimulation and transcranial magnetic stimulation are feasible methods for discriminating between central and peripheral fatigue during isometric contractions, it is more difficult to differentiate between factors during dynamic exercise.

Figure 2. Mean maximal force development in seven subjects during 40 repeated MVCs with the knee extensors (total duration 200 s)
The brief maximal contractions (duration of \(\sim 2\) s) were repeated every 5 s, and the two maximal voluntary contraction protocols were performed immediately \(\sim 30\) s after cycling to exhaustion in a 40\(^\circ\text{C}\) hot environment (exercise time of 50 \(\pm\) 3 min with an end core temperature of \(\sim 40^\circ\text{C}\)) or following 1 h of non-exhaustive cycling at a similar exercise intensity in a thermoneutral environment (core temperature of \(\sim 38^\circ\text{C}\)). (From Nybo & Nielsen, 2001a, with permission.)
Therefore, neither question may be answered with certainty, but studies with manipulation of central nervous system neurotransmitter levels, specifically dopaminergic reuptake inhibitors, indicate that the relative influence from central fatigue is significantly enhanced during exercise in the heat compared with normothermic exercise (Roelands & Meeusen, 2010).

**Exhaustion versus fatigue or performance**

During time-to-exhaustion trials in uncompensable hot environments, the core temperature will increase steadily, and it appears that the inability or unwillingness to continue exercise in mammals, including humans, is closely related to the attainment of a high core temperature (Cheung & McLellan, 1998; Fuller et al., 1998; González-Alonso et al., 1999; Walters et al., 2000). There may be differences in the tolerable exercise between species and there is also variation between subjects (González-Alonso et al., 1999). Factors such as motivation, exercise mode, training status, acclimatization and hydration may explain part of these interindividual differences, but for a given individual in a standardized situation, exercise seems to be terminated with remarkable little variation in the end-point core temperature (MacDougall et al., 1974; Nielsen et al., 1993; Fuller et al., 1998; González-Alonso et al., 1999; Walters et al., 2000). Initially, this observation has been misinterpreted as if exhaustion or fatigue was dictated by a ‘critical core temperature’; however, studies with pharmacological alterations of synaptic dopamine levels (Watson et al., 2005; Roelands et al., 2008) or caffeine administration (Cheuvront et al., 2009; Nybo, 2010; Ely et al., 2011; Roelands et al., 2011) have demonstrated that subjects may surpass their ‘normal’ end-point temperature. Furthermore, during competitions, when athletes are maximally motivated, it also appears that they reach slightly higher core temperatures compared with laboratory experiments (González-Alonso et al., 1999; Ely et al., 2009), whereas dehydration will lower the end-point temperature (González-Alonso et al., 1999).

Therefore, hyperthermia-induced central fatigue should not be considered as an all-or-none phenomenon that occurs only when the core and brain temperature reaches a critical point. Rather, there appears to be a progressive inhibition of the brain areas responsible for motor activation when the core temperature increases above normothermic levels (Morrison et al., 2004), and these signals are most probably integrated with sensory feedback from the exercising muscles and cardiovascular changes, as well as other CNS factors, to determine the exercise fatigue response during prolonged activities in the heat (Ely et al., 2010; Maughan et al., 2011).

The suggestion that brain temperature in itself is a very important factor is supported by the experiments from Caputa et al. (1986), who were able to manipulate the brain and body core temperatures separately in exercising goats by changing the temperatures of implanted thermo-elements. Their results demonstrate that elevating the hypothalamic temperature independently of the temperature of the rest of the body core reduced the ability and willingness of the goats to continue exercise. It is therefore hypothesized that inhibitory signals arising in temperature-sensitive areas of hypothalamus as a consequence of an excessive increase in the cerebral temperature will either directly or indirectly hamper motor activity (Nybo & Nielsen, 2001a; Nybo et al., 2002b).

It seems plausible that brain temperature also is a very important factor in humans, and it is clear that the impairment in voluntary muscle activation is related to elevations of the core temperature rather than to changes in skin or local muscle temperature (Thomas et al., 2006). A high skin temperature may indirectly influence the brain by increasing the cardiovascular stress and, in turn, this may impair orthostatic tolerance and reduce the cerebral blood flow (Wilson et al., 2002, 2006). However, the elevated muscle temperature or a high skin temperature in itself seems to have little influence on the reduction in voluntary muscle activation (Thomas et al., 2006).

During exercise with constant power output, hyperthermia-induced fatigue emerges as a steady increase in perceived exertion, and this is accompanied by a gradual slowing of the electroencephalogram (Nybo & Nielsen, 2001b). Hyperthermia-induced fatigue will result in a reduction in power/speed during time trails (Tucker et al., 2004; Watson et al., 2005) and during exercise where subjects are instructed to adjust their power output to maintain a predefined level of exertion (Tucker et al., 2006). Also, average power output during repeated sprinting and intense one-legged knee extensor exercise becomes reduced by hyperthermia (Drust et al., 2005), and it is noteworthy that the ‘performance pattern’ during repeated sprinting and intense dynamic knee-extensor exercise resembles that observed for sustained isometric contractions, and that the impaired performance is accompanied by reduced and not enhanced accumulation of substances involved with peripheral fatigue, such as plasma \(K^+\), \(H^+\) and muscle lactate (Drust et al., 2005).

Furthermore, during isolated knee-extensor exercise, the active muscle mass is relatively small and does not challenge the cardiovascular system maximally. Hyperthermia-induced fatigue during repeated sprints or exercise with a small muscle mass does not appear to be caused by inadequate oxygen delivery or disturbances of muscle homeostasis, but rather by the direct temperature influence on the CNS. Although such evidence for central fatigue is circumstantial (Fritzsche et al., 2000; Kay et al., 2001; Nybo & Nielsen, 2001b; Pitsiladis et al., 2002; Drust et al., 2005), it seems likely that hyperthermia, via some of the same mechanisms that influence voluntary motor...
activation during isometric contractions, also becomes important during ongoing dynamic exercise.

**Why is the brain temperature higher than that of the body core and is it possible to cool the brain selectively?**

At rest and during normothermic exercise, the brain has a metabolic rate of \( \sim 35 \mu l O_2 g^{-1} min^{-1} \) (Lassen, 1985; Nybo et al. 2002a), corresponding to a cerebral heat production of \( \sim 0.6 J g^{-1} min^{-1} \), and heat balance is established with a jugular venous to arterial temperature difference of \( \sim 0.3 ^\circ C \) and a cerebral blood flow of \( \sim 0.50 ml g^{-1} min^{-1} \) (Yablonskiy et al. 2000; Nybo et al. 2002b). During exercise-induced hyperthermia, the cerebral metabolic rate increases, whereas the overall blood flow to the brain decreases (Nybo et al. 2002a) and the temperature difference between the jugular vein and aortic blood temperature is narrowed to 0.2°C. This

![Figure 3](image-url)

*Figure 3. Oesophageal, tympanic, arterial and jugular venous blood temperature responses during cycling with a normal core temperature response (top panel; control trial) and during a similar exercise bout with progressive hyperthermia (bottom panel).*  
Values are means of seven subjects. Standard deviations are omitted for simplicity, but the SDs of all temperatures were in the range of 0.1–0.3°C. (From Nybo et al. 2002b, with permission.)
implies that heat is stored in the brain, and the average brain temperature increases in parallel with the aortic blood temperature, with the brain remaining at least 0.2°C warmer than the body core (see Fig. 3).

In various animal species, selective brain cooling is achieved via precooling of the arterial blood on its passage through the carotid rete, as this specialized complex allows for heat exchange between the venous network and the arterial blood destined for the brain. Humans, however, have no carotid rete, and the ability to cool the arterial blood before it reaches the brain is very limited (Nybo et al. 2002b). The temperature of the connective tissue adjacent to the internal carotid artery may be ~2°C lower than the aortic blood temperature during exercise with hyperthermia (Nybo et al. 2002b), and this thermal gradient would make it physically possible for arterial blood to release a small amount of heat during its passage from the heart to the brain (Hanson, 1974). However, the transit time of blood within the carotid artery is short, and the blood will not equilibrate with the thermal energy content of the surrounding tissues (Crezee & Lagendijk, 1992; Crezee et al. 1994). Even with neck cooling (placing ice packs over the carotid arteries and reducing skin temperature by more than 20°C) there is no change in the aortic to jugular venous temperature difference (L. Nybo & N. H. Secher, unpublished observations). This indicates that the potential for precooling of blood destined for the brain is minimal and far from sufficient to allow for a lowering of the average brain temperature below that of the aortic blood and the body core.

Intranasal cooling can be used to initiate therapeutic hypothermia, and a newly published magnetic resonance imaging study indicates that the cerebral temperature may be markedly reduced by cooling of the nasal cavities via intranasal balloon catheters circulated with cold saline (Covaciu et al. 2011). However, we have recently observed that the decline in brain temperature induced via this method is related to a parallel decline of the core temperature, because the arterial and jugular venous blood temperatures decline in parallel. Therefore, even with this extreme cooling of the nasal cavities there is no sign of selective brain cooling (L. Nybo, M. Wanscher and N. H. Secher, unpublished observations).

Face fanning or other methods that will selective cool the surface of the head (skin and extracranial tissue) has also been proposed to cool the brain directly and selectively (Cabanac & Caputa, 1979). However, while it is true that such cooling will lower the tympanic membrane temperature, it has no direct effect on the cerebral temperature as long as the arterial temperature remains unaltered (see Fig. 3). In contrast, during exercise with severe (uncompensable) environmental heat stress, any cooling (including face fanning or head cooling) will attenuate the overall rise in the core temperature (Racinais et al. 2008; Simmons et al. 2008), and the performance-enhancing or ‘fatigue-relieving’ effects of such cooling seem to relate to the general cooling of the body, rather than any selective brain cooling effect (Nybo et al. 2002b; Simmons et al. 2008).

In conclusion, brain temperature seems to be a major factor affecting motor performance during sustained contractions and most probably also during prolonged dynamic exercise. As selective brain cooling is not feasible during natural circumstances in humans, the brain temperature is predominantly dictated by the general body core and arterial blood temperature response. Future studies able to address the relative importance of various parameters by separately altering single factors, e.g. cerebral blood flow, cerebral oxygen delivery, neurotransmitter levels and peripheral feedback, and inducing independent changes in cerebral and skin temperature would be of great interest to explore the mechanisms underlying hyperthermia-induced fatigue and central fatigue in general.

References


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Symposium Report

Human thermoregulation and the cardiovascular system

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A key but little understood function of the cardiovascular system is to exchange heat between the internal body tissues, organs and the skin to maintain internal temperature within a narrow range in a variety of conditions that produce vast changes in external (exogenous) and/or internal (endogenous) thermal loads. Heat transfer via the flowing blood (i.e. vascular convective heat transfer) is the most important heat-exchange pathway inside the body. This pathway is particularly important when metabolic heat production increases many-fold during exercise. During exercise typical of many recreational and Olympic events, heat is transferred from the heat-producing contracting muscles to the skin surrounding the exercising limbs and to the normally less mobile body trunk and head via the circulating blood. Strikingly, a significant amount of heat produced by the contracting muscles is liberated from the skin of the exercising limbs. The local and central mechanisms regulating tissue temperature in the exercising limbs, body trunk and head are essential to avoid the deleterious consequences on human performance of either hyperthermia or hypothermia. This brief review focuses on recent literature addressing the following topics: (i) the dynamics of heat production in contracting skeletal muscle; (ii) the influence of exercise and environmental heat and cold stress on limb and systemic haemodynamics; and (iii) the impact of changes in muscle blood flow on heat exchange in human limbs. The paper highlights the need to investigate the responses and mechanisms of vascular convective heat exchange in exercising limbs to advance our understanding of local tissue temperature regulation during exercise and environmental stress.

(Received 12 September 2011; accepted after revision 3 January 2012; first published online 6 January 2012)

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Heat production in skeletal muscle

Heat is produced in all body cells from the conversion of metabolic energy into mechanical and thermal energy. This process is very inefficient and thus approximately 30–70% of the energy liberated during muscle contraction appears as thermal energy (Edwards et al. 1975; González-Alonso et al. 2000b; Bangsbo et al. 2001; Krustrup et al. 2001, 2003). Heat production by dynamically contracting human skeletal muscle increases abruptly and markedly at the onset of dynamic exercise, increases further at a lower rate during the early stages of exercise and eventually plateaus if exercise is of a steady-state nature. When exercise is intense, however, heat production does not level off, as illustrated in Fig. 1. A doubling in heat production is seen over 3 min of intense dynamic exercise, with half of the increase occurring during the first 38 s (González-Alonso et al. 2000b). Heat production in dynamically contracting muscle in conditions of unrestricted flow is estimated by measuring the amount of heat accumulated in the contracting muscles, the amount of heat removed by the blood to the body trunk and the amount of heat loss from the exercising limb skin. When looking at each subdivision (in conditions in which heat exchange with the surroundings of the thigh is minimized by a thermostatically isolated system and the circulation to the lower leg is arrested), heat storage in the active quadriceps muscles accounts for the immediate elevation in heat production, whereas heat removal to the body trunk via the blood is dominant at the end of exercise (Fig. 1).

The elevation of total heat production over time in contracting skeletal muscle is tightly coupled with changes in heat liberation during metabolic ATP production early in exercise (González-Alonso et al. 2000; Bangsbo et al. 2001; Krustrup et al. 2001, 2003). As depicted in Fig. 2,
the contribution of anaerobic energy turnover to total energy turnover (i.e. the sum of total heat production and mechanical power output) is greatest at the onset of exercise, becoming smaller as the contribution of aerobic energy turnover increases. In vitro studies have shown that heat production during ATP utilization varies from 35 to 72 kJ (mol ATP)^{-1} depending upon whether creatine phosphate (PCr), glycolysis or oxidative phosphorylation provides the energy for ATP resynthesis (Wilkie, 1968; Curtin & Woledge, 1978). Hence, both in vitro and in vivo studies show that muscle heat production can increase during high-intensity dynamic exercise by a factor of two, with a shift in ATP resynthesis from primarily PCr catabolism to primarily oxidative phosphorylation.

Heat storage in body tissues depends upon the interplay among heat production, heat dissipation and, to a lesser extent, energy exchanged during mechanical work. Excessive heat accumulation or liberation compromises the physiological function of cellular and organ systems, which can lead to impaired human performance (González-Alonso et al. 2008a; Taylor et al. 2008). A well-developed control system is therefore required to regulate heat exchange within the body and between the skin and the environment. The two avenues of heat exchange inside the body are ‘intercellular conductive heat transfer’ and ‘vascular convective heat transfer’. Heat conductance through tissues in the human body is a slow process and, in limbs, is primarily dependent upon the temperature gradient between muscle and skin and the thermal conductivity of muscle. This avenue of heat exchange is particularly important in conditions of exercise in cold environments, which produces a large temperature gradient between deep muscle and neighbouring subcutaneous tissue and skin (Werner et al. 2008). Conversely, it plays no role when there is not a temperature gradient between muscle and surrounding skin, as during exposure to warm environments. In contrast, convective heat transfer (mass flow) from dynamically contracting limb muscles to the core of the body and to the surrounding subcutaneous limb tissues hinges upon tissue blood flow and arteriovenous blood temperature difference (according to the Fick principle; González-Alonso et al. 2000b). Hence, conditions that alter either limb tissue blood flow and/or arteriovenous blood temperature difference, such as exercise and/or environmental stress, are likely to induce profound changes in convective heat exchange within the exercising limbs and between the exercising limbs and the body torso. Conversely, when exercise is performed in ischaemic conditions (e.g. when limb circulation is arrested by inflation of a cuff), convective heat removal to the body...
The body of literature discussing the processes involved in heat liberation from the skin to the environment is very extensive. The reader is referred to two excellent reviews on the biophysics and physiology of heat exchange between the body and the environment (Gagge & González, 1996; Werner et al. 2008). On the contrary, the number of studies directly investigating the processes involved in heat exchange inside the human body, particularly in contracting limb skeletal muscle, is relatively small due partly to the complexity of performing the necessary invasive measurements of tissue blood flow and blood and tissue temperature gradients. The focus of this paper is on the latter aspect of temperature regulation.

Cardiovascular responses to exercise and environmental stress

The circulatory adjustments to exercise and environmental stress are integrative responses to a vast collection of external and internal stimuli. Based on the external stimuli, the haemodynamic responses to environmental stress and exercise are determined by the magnitude of the environmental (heat or cold) load and the duration, intensity and type of exercise. The environmental conditions and the type and intensity of exercise determine the metabolic and thermal demands for local and systemic blood flow, whereas exercise duration defines the regulatory disturbances and constraints in cardiovascular function over time (Rowell et al. 1996; González-Alonso et al. 2008a; Mortensen et al. 2008). Depending upon the amount of muscle mass engaged, exercise can generally be classified as small or large muscle mass exercise. Single-limb exercise, such as forearm and isolated leg exercise, are examples of small muscle mass exercise, whereas whole-body exercise, such as cycling, running and rowing, are considered large muscle mass exercise. The effects of heat and cold stress during exercise on cardiovascular function are most likely to differ during small compared with large muscle mass exercise when exercise intensity is high (Mortensen et al. 2008). From a mechanistic physiology viewpoint, the combination of intense whole-body exercise and heat stress poses the greatest challenge to the regulation of temperature, mean arterial pressure and oxygen delivery to the working muscles, brain and heart, because in these conditions the cardiovascular system is pushed faster to the limit of its regulatory capacity (Rowell et al. 1996; González-Alonso et al. 2008).

Prolonged whole-body exercise in the heat is associated with greater tachycardia, skin and core hyperthermia, but conflicting systemic and exercising limb blood flow responses compared with equivalent exercise in the cold (Claremont et al. 1975; McArdle et al. 1976; Nadel et al. 1979; Sawka et al. 1979; Montain & Coyle, 1992; González-Alonso et al. 1998, 2000a). The lack of control for subjects’ hydration status, randomization of the experimental trials and familiarization of the participants with the experimental conditions might explain, at least in part, the discrepancy in the blood flow responses in the literature. In this context, people lose more body water during exercise in the heat due to higher sweat rates and thus become more dehydrated than during exercise in the cold. The question then arises as to how distinct levels of dehydration impact upon the cardiovascular responses to exercise and environmental stress. Figure 3 depicts the results from a study investigating the influence of environmental temperature and hydration status on the cardiovascular responses to moderately intense leg cycling (González-Alonso et al. 2000a). Subjects were randomly tested at 35 and 8°C ambient temperatures when euhydrated and dehydrated by 1.5, 3.0 and 4.5% of their body weight. When subjects were euhydrated and core temperature and oxygen uptake (V̇O₂) were the same during exercise in both environments, cardiac output was elevated by ~11 l min⁻¹ in the heat, accompanied by a higher heart rate but an unchanged stroke volume. This elevated systemic blood flow might have been a response to the threefold higher skin blood flow during exercise in the heat, as the metabolic and thermal demands for exercising muscle blood flow were apparently the same.

The circulatory responses to a given level of dehydration, however, vary in cold and hot environments. In contrast to the well-characterized cardiovascular strain evoked by dehydration during exercise in the heat (Sawka et al. 1979; Montain & Coyle, 1992; González-Alonso et al. 1998), graded dehydration up to 4% of body weight loss (i.e. 3 kg for a 70 kg person) does not reduce cardiac output, skin blood flow, arterial blood pressure or systemic vascular conductance during exercise in the cold. Consequently, cardiac output, and possibly active muscle blood flow (González-Alonso et al. 1998), is lower during moderate intensity exercise in the heat compared with exercise in the cold when dehydrated by 4%, which is the opposite response to what occurs in the euhydrated state. Hydration status can therefore explain part of the discrepancy in the cardiac output responses to environmental stress and intense whole-body exercise reported in the literature.

The literature directly comparing the effects of environmental heat and cold stress on the haemodynamic responses to small muscle mass exercise is sparse (e.g. Savard et al. 1988), yet evidence in resting limbs is extensive. Studies in resting limbs generally show that heat stress increases blood flow to the arms and legs, whereas cold stress reduces limb perfusion (e.g. Barcroft & Edholm, 1943). A highly controversial issue, however, is whether these differences in limb perfusion reflect changes only in skin blood flow or in both muscle and skin blood.

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flow. With respect to heat stress, early investigations into the partition of limb perfusion between skin and skeletal muscle in the human forearm led to conflicting results, with some studies suggesting an elevation in muscle blood flow (Barcroft & Edholm, 1946; Barcroft et al. 1947), but others not (e.g. Rodie et al. 1956; Detry et al. 1972; Johnson et al. 1976). The negative findings together with the estimate of maximal skin blood flow of 6–8 l min\(^{-1}\), based on indirect measures of cardiac output and visceral blood flow during whole-body heat stress, promoted the idea that increases in skin blood flow with heat stress accounted fully for the rise in systemic hyperaemia and blood flow redistribution (Detry et al. 1972; Rowell 1974; Minson et al. 1998). Recent evidence in the human leg, however, challenges this widely held dogma. Using \(^{133}\)Xe washout or positron emission tomography techniques, Keller et al. (2010) and Heinomen et al. (2011) recently showed that passive leg heating increases calf blood flow by approximately 60–65%. In parallel, we have shown significant increases in leg tissue blood flow, deep femoral venous O\(_2\) content and muscle oxygenation and a parallel significant decline in leg arterial–deep venous O\(_2\) differences during whole-body heat stress, both at rest and during mild knee-extensor exercise, with a small effect or no effect on aerobic metabolism (Pearson et al. 2011). The enhanced muscle blood flow was closely associated with increases in arterial plasma ATP concentration and muscle temperature (Pearson et al. 2011), which is in turn coupled to a temperature-mediated release of ATP from erythrocytes (Kalsi & González-Alonso, 2012). On the other hand, cold stress has repeatedly been shown to reduce limb blood flow in resting humans (e.g. Barcroft & Edholm, 1943; Gregson et al. 2011). For instance, the classic work of Barcroft & Edholm (1943) clearly showed lower forearm blood flows and deep muscle temperatures when the forearm was immersed in a water bath at 13, 20 and 25\(^\circ\)C compared with higher water temperatures. Likewise, Gregson and co-workers recently reported a 35–40% decline in femoral artery blood flow and conductance following 10 min of cold and temperate water immersion (8 versus 22\(^\circ\)C), which evoked drastic decreases in muscle and skin temperatures, but less cutaneous vasoconstriction at 8 than at 22\(^\circ\)C water temperature, possibly reflecting a lower muscle blood flow (Gregson et al. 2011). Taken together, growing evidence from the human leg suggests that heat and cold stress not only alters blood flow to the skin, but also to the skeletal muscle. These circulatory adjustments might have important implications for heat transfer in resting and exercising human limbs.

**Muscle blood flow and limb heat liberation**

The flowing blood transports heat inside the body in relation to blood temperature and flow rate. Heat transfer

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**Figure 3. Cardiovascular responses to exercise and environmental stress at different levels of hydration**

Note that cardiac output was elevated in the heat in normal hydration conditions, but in contrast to exercise in the cold it declined significantly with increasing levels of dehydration owing to the greater decline in stroke volume (González-Alonso et al. 2000a). *Significantly different from the euhydrated control condition. †Significantly different from exercise in the cold at the same hydration level.
in major arteries and veins supplying and draining the limbs is bidirectional. In normal resting conditions, limb muscle and venous blood temperatures are significantly lower than arterial and core temperature (González-Alonso et al. 1999; He et al. 2002; Fig. 4). In fact, blood temperature in the limb muscle microcirculation is normally several degrees lower than core temperature due to rapid thermal equilibration between tissues and vessels (He et al. 2002). At the level of the major supply vessels, the resulting negative arteriovenous temperature gradient indicates that more heat is being transferred from the upper body core to the extremities than vice versa (Fig. 4). This net body core-to-limbs heat transfer helps limbs to maintain tissue temperature when their metabolic heat production is low. For instance, leg $\dot{V}_{\text{O}_2}$ is normally about 23 ml min$^{-1}$ in the resting state, corresponding to a total leg heat production of $\sim 0.5$ kJ min$^{-1}$ based on the heat equivalent of $\dot{V}_{\text{O}_2}$ (Bangsbo et al. 2000). This value is only half of the $\sim 1$ kJ min$^{-1}$ of heat being transferred from the body trunk to each leg when femoral venous temperature is $\sim 0.7^\circ$C lower than femoral arterial blood temperature, resting leg blood flow is $\sim 0.4$ l min$^{-1}$ and the blood specific heat is 3.61 kJ l$^{-1}$°C$^{-1}$ (Fig. 4). These simple estimates demonstrate that more heat is transferred into the resting leg than produced locally in resting conditions. This implies that limb tissue temperature will drop if its circulation is arrested and heat dissipation to the surroundings of the limbs is kept constant. An example of this might occur during knee or elbow surgery.

The impact of alterations in limb perfusion on heat exchange between the body core and limbs can be exemplified from the findings of He et al. (2002) in the rat hindlimb. To the author’s knowledge, comparable limb thermodynamic data are not available for humans.

**Figure 4.** Temperature and heat exchange across the human leg at onset of cycling exercise

Note that muscle ($T_{\text{mus}}$) and femoral venous temperatures ($T_{\text{fv}}$) are lower than core ($T_{\text{Oes}}$) and femoral arterial blood temperatures ($T_{\text{fa}}$) at the onset of submaximal leg cycling exercise (0–3 min for $T_{\text{fa}}$ and 0–8 min for $T_{\text{mus}}$), but increase very rapidly as exercise progresses. A net heat influx in the leg is observed at rest (0 min) and during the initial 5.5 min of exercise. A net heat efflux is seen thereafter. (Modified from González-Alonso et al. 1999.)

**Figure 5.** Influence of limb blood flow on blood temperature and heat exchange across the resting rat hindlimb

Note that increases in blood flow in the resting hindlimb do not change the net limb heat influx, because of compensatory changes in femoral arteriovenous (a-v) temperature differences (drawn from data reported by He et al. 2002). *Significantly different from control.
Femoral artery and vein blood flow was increased by infusion of the nitric oxide donor sodium nitroprusside (vasodilatation), normal (control) or reduced by infusion of noradrenaline (vasoconstriction; Fig. 5). Increases in blood flow were accompanied by parallel reductions in femoral arteriovenous temperature difference, thus net limb heat influx was the same. In contrast, when femoral artery blood flow was reduced, heat influx into the hindlimb was reduced, accompanying an essentially unchanged arteriovenous temperature gradient. Therefore, the findings of He and co-workers (2002) indicate that increasing limb blood flow in resting limbs does not necessarily increase the net amount of heat being transferred from the body trunk to the limbs because of compensatory tissue-to-blood thermal exchange adjustments within the leg tissues. However, reducing blood flow might have an impact on limb heat transfer. In the resting human leg, femoral venous blood temperature has been shown to decrease by up to 0.5°C with progressive increases in blood flow from 0.4 to 8 min\(^{-1}\) evoked by intrafemoral artery infusion of ATP (González-Alonso et al. 2008b). Whether similar thermal adjustments to those described in the rat hindlimb occur in the human leg warrants detailed investigation.

Exercise illustrates a different scenario, in which not only limb tissue perfusion and convective heat exchange, but also heat production increase. During the initial stages of leg exercise, the temperatures of the contracting muscle and the outflowing femoral venous blood increase at a faster rate than the temperatures of the inflowing femoral arterial blood and the upper body core (González-Alonso et al. 1999), yet a negative femoral arteriovenous blood temperature gradient prevails during the early stages of exercise, signifying that more heat is still transferred from the upper body core to the exercising limbs in normal environmental conditions (Fig. 4). After a few minutes of exercise (duration will depend on the initial temperatures and the rate of heat production or exercise intensity), muscle and venous blood temperature becomes higher than arterial blood and upper body core temperature. At this point in time, heat transferred from the exercising limbs to the body torso becomes positive, increasing thereafter to reach a plateau when exercise is of light-to-moderate intensity. To date, data on heat exchange in human limbs in different environmental and exercise conditions are very limited, thus the ideas discussed above require thorough scrutiny.

**Summary and future directions**

Our knowledge and understanding of human thermo-regulation and its interaction with cardiovascular regulation during exercise is largely based upon data from resting limbs. The observation that a significant amount of heat produced by the exercising muscles is liberated directly from the skin of the exercising limbs (González-Alonso et al. 1999) highlights the need to investigate the responses and mechanisms of vascular heat exchange in resting and exercising limbs. Quantification of heat production and convective heat exchange in human limbs is likely to shed new light onto the role of muscle blood flow in the control of tissue temperature during environmental stress and exercise.

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Acknowledgements

The author apologises to all the authors whose work has not been cited due to space restrictions and thanks all the collaborators who made possible the completion of the work reviewed here. Recent author’s work has been supported by the Gatorade Sports Science Institute and PepsiCo.
Genomic predictors of trainability

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The concept of individual differences in the response to exercise training or trainability was defined three decades ago. In a series of experimental studies with pairs of monozygotic twins, evidence was found in support of a strong genotype dependency of the ability to respond to regular exercise. In the HERITAGE Family Study, it was observed that the heritability of the maximal oxygen uptake response to 20 weeks of standardized exercise training reached 47% after adjustment for age, sex, baseline maximal oxygen uptake and baseline body mass and composition. Candidate gene studies have not yielded as many validated gene targets and variants as originally anticipated. Genome-wide explorations have generated more convincing predictors of maximal oxygen uptake trainability. A genomic predictor score based on the number of favourable alleles carried at 21 single nucleotide polymorphisms appears to be able to identify low and high training response classes that differ by at least threefold. Combining transcriptomic and genomic technologies has also yielded highly promising results concerning the ability to predict trainability among sedentary people.

Evidence for a genetic component

Several selection experiments have confirmed the concept that there is a substantial genetic component to the trainability of exercise performance traits. For instance, in one study on selection for high and low responses to treadmill training in rats, the mean running distance increase of the founder population was 222 m (Troxell et al. 2003). Pairs of lowest and of highest responders to training were mated, and their offspring were later exposed to the
same treadmill training programme. Offspring from the low line did not differ in trainability from the founders, while those from the high line improved their running distance by more than 60% over the low line. These results also revealed that the narrow heritability of running performance trainability reached 43%.

In a series of experiments that we conducted with pairs of monozygotic (MZ) twins, all sedentary young adults, it was established that individual differences in trainability were not randomly distributed (Prud’homme et al. 1984; Despres et al. 1984a; Hamel et al. 1986). Thus, there was consistently more variance in training responses between pairs of MZ twins than was observed between brothers or sisters (within pairs). These MZ twin intervention experiments revealed that there was a strong genotype–training interaction effect contributing to the variation in \( V_{O_2 \text{max}} \) trainability. Four twin studies were performed with exercise training programmes that differed in duration, intensity and control over dietary intake (Prud’homme et al. 1984; Boulay et al. 1986; Hamel et al. 1986; Simonneau et al. 1986; Bouchard et al. 1994). Intraclass correlation coefficients computed from the within-pairs variance and the between-pairs variance in \( V_{O_2 \text{max}} \) response to training ranged from 0.44 to 0.77, indicating that the aggregate genotype of a person plays a major role in determining the amplitude of \( V_{O_2 \text{max}} \) trainability.

Similar findings have been reported for other laboratory-based measures of maximal exercise performance in experimental studies conducted with pairs of MZ twins. Thus, significant within-pair resemblance in training response was observed for total power output during a 90 min maximal cycle ergometer test (Boulay et al. 1986; Hamel et al. 1986) and in short-term (10 s power output) and long-term maximal power tests (90 s power output; Simonneau et al. 1986).

The high degree of heterogeneity in responsiveness to a fully standardized exercise programme in HERITAGE was not accounted for by baseline \( \dot{V}O_2 \max \) level, age, sex or ethnic differences. In the case of the 99 families of European descent who were part of HERITAGE, the increase in \( V_{O_2 \max} \) showed 2.5 times more variance between families than within families (Bouchard et al. 1999). Thus, the remarkable heterogeneity observed for the gains in \( V_{O_2 \max} \) among adults is not random and is characterized by a strong familial aggregation. A model-fitting analytical procedure found that the most parsimonious models yielded a maximal heritability estimate of 47% for \( V_{O_2 \max} \) response level (Bouchard et al. 1999).

Among other findings of interest from HERITAGE, maximal heritability estimates for the changes with exercise training ranged from about 25 to 55% for the gains in \( V_{O_2 \max} \) and power output at 60 and 80% of maximum (Perusse et al. 2001). Submaximal exercise heart rate, stroke volume and cardiac output at a power output level of 50 W exhibited significant familial aggregation in response to endurance training, with broad heritability estimates of about 35% (An et al. 2000, 2003; Bouchard & Rankinen, 2001).

**Candidate genes**

Multiple nuclear and mitochondrial DNA markers have been significantly associated with haemodynamic traits and indicators of physical performance (Bray et al. 2009; Rankinen et al. 2011). Unfortunately, the vast majority of the studies substantiating these associations were based on observational data, were targeting poorly justified candidates and were grossly statistically underpowered. Moreover, all the positive findings on autosomal markers have been diminished by damaging negative reports. The situation applies also to genomic markers of trainability.

Few candidate genes have been found to be associated with the trainability of cardiorespiratory fitness traits. The \( ACE \) gene encodes a peptidyl dipeptidase, angiotensin-converting enzyme, a component of the renin–angiotensin system. A few reports have dealt with the \( ACE \) insertion/deletion (I/D) polymorphism and exercise training-induced left ventricular growth as assessed by echocardiography. Montgomery and coworkers reported that the \( ACE \) D allele was associated with greater increases in left ventricular mass and septal and posterior wall thickness of the heart after 10 weeks of physical training in British Army recruits (Montgomery et al. 1997). A few years later, the same group observed that the training-induced increase in left ventricular mass in another cohort of Army recruits was 2.7 times greater in the D/D than the I/I homozygotes (Myerson et al. 2001). The prevalence of echocardiographically determined left ventricular hypertrophy increased only among the DD homozygotes (Montgomery et al. 1997). Similar observations were made in endurance athletes, with DD homozygotes exhibiting larger left ventricular mass and a higher prevalence of left ventricular hypertrophy (Di Mauro et al. 2010).

Expression of the components of the renin–angiotensin system is increased in response to stimuli leading to cardiac hypertrophy. The \( ACE \) D allele is the allele associated with higher \( ACE \) activity and left ventricular growth response (Skipworth et al. 2011). If the DD genotype leads to more heart size growth in response to exercise in healthy individuals, then one would expect that the same genotype would result in higher \( V_{O_2 \max} \) gains as well. However, several studies on this issue have been reported, and the results do not support the a priori hypothesis. For instance, the II genotype subjects increased \( V_{O_2 \max} \) twice as much as the DD subjects in a training study of postmenopausal women (Hagberg et al. 1998). Several exercise training studies have not observed any differences.
in $\dot{V}_O_2$\textsubscript{max} trainability among the three ACE I/D genotypes (Sonna et al. 2001; Roltach et al. 2005; Day et al. 2007). In HERITAGE subjects exposed to 20 weeks of aerobic exercise, the DD individuals increased $\dot{V}_O_2$\textsubscript{max} by 476 ml O$_2$ min$^{-1}$ (SD = 23), while the II subjects gained 417 ml O$_2$ min$^{-1}$ (SD = 26; $P = 0.042$) in the subgroup of adult offspring ($n = 303$) from Whites of European descent (Rankinen et al. 2000). However, there were no differences among ACE genotypes in $\dot{V}_O_2$\textsubscript{max} trainability in the parental group of Whites ($n = 188$) and in the offspring ($n = 196$) and parent subgroups ($n = 75$) of African descent.

Other genes have been considered as candidates for the individual differences in trainability of cardiorespiratory fitness traits. The apolipoprotein (APOE) gene is one of them; however, only two studies have been reported, and their results are discordant (Hagberg et al. 1999; Thompson et al. 2004). Another gene of interest has been the $\alpha$-actinin 3 (ACTN3) gene (MacArthur & North, 2011). A C/T transition in codon 577 of ACTN3 replaces an arginine residue (R577) with a premature stop codon (X577), resulting in $\alpha$-actinin 3 deficiency in the XX individuals. The stop codon variant is quite common in humans, with an estimated 1 billion people worldwide being XX homozygotes (MacArthur & North, 2011). There is a dearth of data on the role of ACTN3 in the training response of cardiorespiratory fitness traits. However, two studies reported to date on its role in the trainability of skeletal muscle performance traits are inconclusive (Clarkson et al. 2005; Delmonico et al. 2007).

Genome-wide exploration

Improvements in microarray-based high-throughput technologies have made it possible to assay hundreds of thousands of single-nucleotide polymorphisms (SNPs) in a single reaction or to quantify the expression level of thousands of transcripts simultaneously. As a result of these advances, it became feasible to undertake genome-wide screens focused on DNA sequence variants (mainly SNPs) or gene transcript abundance. In a nutshell, in appropriate conditions, objective and largely unbiased hypothesis-free tests became possible. To date, only a handful of genome-wide linkage or genome-wide association studies (GWAS) pertaining to trainability have been reported.

Genome-wide linkage analysis was used in HERITAGE to find genes for the response to exercise training. Quantitative trait loci (QTLs) for training-induced changes in submaximal exercise (50 W) stroke volume and heart rate were found on chromosomes 10p11 and 2q33.3–q34, respectively (Rankinen et al. 2002; Spielmann et al. 2007). The QTL on 10p11 for the gains in stroke volume was narrowed down to a 7 Mb region. Among the linkage-positive families, the strongest associations were found with SNPs in the kinesin family member 5B (KIF5B) gene locus (Argyropoulos et al. 2009). Resequencing of KIF5B revealed several sequence variants. The SNP with strongest association modified the KIF5B promoter activity in cell-based systems. Furthermore, inhibition and overexpression studies in C2C12 cells showed that changes in KIF5B expression level altered mitochondrial localization and biogenesis; KIF5B inhibition led to diminished biogenesis and perinuclear accumulation of mitochondria, while overexpression enhanced mitochondrial biogenesis (Artyropoulos et al. 2009).

The QTL for the changes in exercise heart rate at 50 W (HR50) on chromosome 2q33.3–q34 was localized within a 10 Mb region (Rankinen et al. 2010). The strongest evidence of association was detected with two SNPs located in the 5’-region of the cAMP-responsive element binding protein 1 (CREB1) gene locus ($P = 1.6 \times 10^{-5}$). The most significant SNP explained almost 5% of the variance in HR50 response, and the common allele homozygotes and heterozygotes had about 57 and 20%, respectively, greater decreases in HR50 than the minor allele homozygotes. Furthermore, one of these SNPs located about 2.6 kb upstream of the first exon of CREB1 was shown to modify promoter activity in vitro. The A-allele, which was associated with a blunted HR50 response, showed significantly greater promoter activity in a C2C12 cell model than the G-allele.

The first trainability studies incorporating dense genome-wide screening technologies were recently published, and both of them targeted $\dot{V}_O_2$\textsubscript{max} as a response trait (Timmons et al. 2010; Bouchard et al. 2011). In the first report, Timmons and colleagues relied on global skeletal muscle gene expression profiling and DNA markers to identify genes associated with $\dot{V}_O_2$\textsubscript{max} training response (Timmons et al. 2010). RNA expression profiling of pretraining skeletal muscle samples was performed in subjects of two independent exercise training trials. The first study ($n = 24$) identified a panel of 29 transcripts that were strongly associated with the gains in $\dot{V}_O_2$\textsubscript{max}. The predictive value of the 29 transcripts was subsequently confirmed in a second study ($n = 17$). This was followed by genotyping tagging SNPs for the predictor transcripts in the cohort of Whites of HERITAGE. A multivariate regression analysis using the transcriptome-derived SNPs and a set of SNPs defined from positional cloning studies performed in HERITAGE identified a set of 11 SNPs that explained about 23% of the variance in $\dot{V}_O_2$\textsubscript{max} training response. Seven of the SNPs were from the RNA predictor gene set, and four were from the HERITAGE QTL projects.

The second report was based on a GWAS undertaken with more than 320,000 SNPs on the sample of Whites in HERITAGE (Bouchard et al. 2011). A total of 39 individual SNPs were associated with $\dot{V}_O_2$\textsubscript{max} training.
response at $P < 1.5 \times 10^{-4}$. The strongest evidence of association ($P = 1.3 \times 10^{-6}$) was observed with an SNP located in the first intron of the acyl-CoA synthetase long-chain family member 1 (ACSL1) gene. When all 39 SNPs were analysed simultaneously in multivariate regression models, nine SNPs explained at least 2% (range 2.2–7.0%) of the variance ($P < 0.0001$ for all), while seven markers contributed between 1 and 2% each. Collectively, these 16 SNPs accounted for 45% of the variance in $V_{O2\text{max}}$ trainability, a value comparable to the heritability estimate of 47% reported previously in HERITAGE (Bouchard et al. 1999).

A predictor score was constructed using all 21 SNPs that entered in the final regression model. Each SNP was coded based on the number of high $V_{O2\text{max}}$ training response alleles; low-response allele homozygote was assigned 0, heterozygote received 1, and homozygote for the high-response allele was assigned 2. While the theoretical range of the score was from 0 (no beneficial alleles) to 42 (two copies of the beneficial alleles at all 21 loci), the observed scores ranged from 7 to 31. The difference in $V_{O2\text{max}}$ training response between those with the lowest (9 or less, $n = 36$, mean $= +221 \text{ ml O}_2 \text{ min}^{-1}$) and the highest scores (19 or more, $n = 52$, mean $= +604 \text{ ml O}_2 \text{ min}^{-1}$) was 383 ml O$_2$ min$^{-1}$, as depicted in Fig. 1.

![Figure 1](image-url)

**Figure 1. Age-, sex- and baseline maximal oxygen uptake ($V_{O2\text{max}}$)-adjusted $V_{O2\text{max}}$ training responses across nine genome-wide association study predictor single nucleotide polymorphism (SNP) score categories in HERITAGE Whites**

A predictor score was constructed using all 21 SNPs that entered in the final regression model. Each SNP was coded based on the number of high $V_{O2\text{max}}$ training response alleles. The difference in $V_{O2\text{max}}$ training response between those with the lowest (9 or less, $n = 36$, mean $= +221 \text{ ml O}_2 \text{ min}^{-1}$) and the highest scores (19 or more, $n = 52$, mean $= +604 \text{ ml O}_2 \text{ min}^{-1}$) was 383 ml O$_2$ min$^{-1}$. The number of subjects within each SNP score category is indicated inside each histogram bar. ‘le’ stands for ‘less than or equal to’ and ‘ge’ ‘greater than or equal to’. From Bouchard et al. (2011), with permission.

**Epilogue**

Even though these genome-wide-based results have not been comprehensively replicated yet, they suggest that it may be possible to define panels of expressed transcripts and DNA variants that would predict $V_{O2\text{max}}$ responsiveness to regular exercise. However, a lot more work remains to be done. One key question is whether the response pattern in a given individual is specific to the given exercise mode and regimen. Another issue is that of the duration of the exercise intervention or the training programme. Would the fitness outcomes be different if the exercise programme lasted for years instead of months? The impact of these rather practical issues on the genomic predictors of true cardiorespiratory fitness trainability is unknown.

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Blood doping practices in sports have been around for at least half a century and will likely remain for several years to come. The main reason for the various forms of blood doping to be common is that they are easy to perform, and the effects on exercise performance are gigantic. Yet another reason for blood doping to be a popular illicit practice is that detection is difficult. For autologous blood transfusions, for example, no direct test exists, and the direct testing of misuse with recombinant human erythropoietin (rhEpo) has proven very difficult despite a test exists. Future blood doping practice will likely include the stabilization of the transcription factor hypoxia-inducible factor which leads to an increased endogenous erythropoietin synthesis. It seems unrealistic to develop specific test against such drugs (and the copies hereof originating from illegal laboratories). In an attempt to detect and limit blood doping, the World Anti-Doping Agency (WADA) has launched the Athlete Biological Passport where indirect markers for all types of blood doping are evaluated on an individual level. The approach seemed promising, but a recent publication demonstrates the system to be incapable of detecting even a single subject as ‘suspicious’ while treated with rhEpo for 10–12 weeks. Sad to say, the hope that the 2012 London Olympics should be cleaner in regard to blood doping seems faint. We propose that WADA strengthens the quality and capacities of the National Anti-Doping Agencies and that they work more efficiently with the international sports federations in an attempt to limit blood doping.

**Abbreviations**

ABP, Athlete Biological Passport; Epo, erythropoietin; Htc, haematocrit; LHTL, Live High – Train Low; NADA, National Anti-Doping Agency; nHb, total haemoglobin mass; rhEpo, recombinant human erythropoietin; VO2max, maximal oxygen uptake; WADA, World Anti-Doping Agency

**Introduction**

Blood doping practices in sports have been around for several decades and will likely remain for several years to come also. After the Olympic Games in Mexico City in 1968, focus was on the role of the Hb concentration and the total amount of Hb (nHb) for maximal oxygen uptake (VO2max) and endurance performance. In two studies by Ekblom and colleagues, it was convincingly demonstrated that the elevation in Hb concentration led to a higher VO2max and an improved performance (Ekblom et al., 1972; 1976) (Figure 1). The effect was equally apparent in subjects with low and high aerobic fitness, as well as whether the basal [Hb] was low, normal or high. This latter finding was surprising since the individuals with a high starting [Hb] point would enter the steep part of the [Hb]–blood viscosity curve (Pirofsky, 1953), and when reaching a certain threshold, this may limit maximal cardiac output and hence exercise performance. However, the elevation in VO2max as well as in power output was a function of increase in [Hb] regardless of its level. A few years later came the first accounts of blood manipulations in sports. In January 1984, Francesco Moser cycled 51.151 km in 1 h and thereby shattered the
previous record set by Eddy Merckx in 1972 by more than 1.5 km. Later, it was revealed that Moser had been ‘prepared’ by Dr Francesco Conconi by means of blood transfusions for this event. The same year, blood transfusions were used with success by the American track cycling team during the Olympic Games held in Los Angeles to win several medals. Blood transfusions were also practiced by Finish cross-country skiers in the 1970s and 1980s. Following the ‘transfusion era’, erythropoietin (Epo) abuse took over in the next decennium, as highlighted by the countless doping scandals during the Tour de France bicycle race in those years. The practice with red cell infusion has however received a renaissance as a timed supplement to Epo misuse in the last decade (Waddington and Smith, 2008). The reason why blood doping is a ‘popular’ means of cheating is that it is one of few feasible procedures to increase exercise performance substantially. Physiological factors usually associated to exercise performance such as the maximum pumping capacity of the heart, O₂ extraction and utilization of the exercising muscles, exercise economy and sub-maximal lactate levels (Jacobs et al., 2011) are much more difficult to manipulate with, as compared to the arterial blood O₂ transport capacity which is increased easily by means of blood transfusions or injections with recombinant human Epo (rhEpo). This is also the reason why much focus is given to the haemoglobin concentration ([Hb]; g·L⁻¹ or mM), the total amount of whole body haemoglobin (nHb; g) and other blood variables in ongoing anti-doping work. With this review, we hope to increase the general understanding of blood doping practice by athletes. Throughout the manuscript, data will be given as presented in the original work. To convert haematocrit (Htc) to [Hb; mmol·L⁻¹], divide by 4.7 (Figure 2A). Two prior reviews on the topic published some years ago in the British Journal of Pharmacology are recommended (Elliott, 2008; Spedding and Spedding, 2008).

**Performance gains following blood and Epo doping**

In elite athletes (and also in normal humans), aerobic power and time trial performance correlate well with the individuals’ normal haemoglobin mass (Figure 2C and E), but not with Htc (Figure 2D and F), whereas increases in either value will increase performance (Ekblom et al., 1972; 1976) (Figure 1). This demonstrates that having a naturally high Htc does not guarantee a high performance level, but that manipulations with Htc will affect performance. On the contrary, a naturally high nHb is usually also associated with a high VO₂max (Martino et al., 2002), and manipulations here-with will also alter performance. The performance gain associated to blood manipulations is far from negligible. The first observation in this regard was reported by Pace and colleagues (Pace et al., 1945) where Htc was increased from 47 to 59% by means of blood transfusions, and sub-maximal heart rate at a given workload decreased from 127 beats-min⁻¹ to 113 beats-min⁻¹ in hypoxia. A recent review on the topic revealed that VO₂max is increased by 5–10% when [Hb] is raised 10% by blood transfusion, and that VO₂max is diminished by the same magnitude if [Hb] is decreased by 10% with haemodilution (Calbet et al., 2006). As mentioned above, Ekblom and co-workers (Ekblom et al., 1972; 1976) concluded that VO₂max would increase independent of base [Hb]; however, it must be assumed that, at some point, VO₂max will not increase any further despite increases in [Hb]. Likely due to increased blood viscosity with increasing Htc (Pirofsky, 1953), VO₂max and time trial performance do not increase linearly (or infinite) with increasing Htc. The increased VO₂max following Epo injections are maintained for at least 3 weeks (Figure 2G) (Lundby et al., 2008a). This makes out-of-competition testing crucial (Figure 3) as the detection success in the weeks following termination of rhEpo treatment is virtually zero (Lundby et al., 2008a). Of note is also that sub-maximal exercise performance – such as time trial performance – is increased by much more following blood doping as compared to the increase in VO₂max and, in a recent study, time to exhaustion was increased by 50% in an approximately 30 min long bike time trial following 4 and 11 weeks of rhEpo treatment in normal healthy volunteers (Thomsen et al., 2007). Whether this is directly transferable to elite athletes remains unknown (within the scientific community), but seems likely. The increase in sub-maximal exercise performance following Epo injections is likely due to a reduced relative cardiovascular and metabolic stress, and at least part of the explanation seems to be related to a 2 mM
reduction (from approximately 9 to 7 mM) in plasma lactate values during the time trial (Thomsen et al., 2007). Furthermore, rhEpo treatment also increases exercise performance at moderate altitudes (Robach et al., 2008), and, finally, it had for some time been speculated that rhEpo may increase performance by other means than by stimulating erythropoiesis, but this seems not to be the case (Lundby et al., 2008b; Rasmussen et al., 2010; Lundby and Olsen, 2011).

In the absence of mountains and in an attempt to limit blood doping, some countries have built nitrogen houses where it is possible to expose athletes to hypoxia and hence stimulate altitude exposure. Such facilities are used with the intent to increase endogenous Epo production, and thereby stimulate the synthesis of red blood cell mass and thus exercise performance. The concept of Live High (i.e. living at altitude) – Train Low (LHTL) (i.e. training at sea level) was introduced by Levine and Stray-Gundersen in the early 1990s, and demonstrated feasible a few years later (Levine and Stray-Gundersen, 1997). It should be noted, however, that the performance gains following LHTL are limited to around 1% (Bonetti and Hopkins, 2009), which may of course be relevant for elite performance, but which is still miles away from the performance gains observed following, for example, rhEpo injections (Thomsen et al., 2007). For-
thermore, the to date only placebo-controlled and double-blinded LHTL study using nitrogen houses demonstrated no performance gains following 4 weeks of LHTL (16 h per day at 3000 m) (Siebenmann et al., 2011). Thus, altitude training cannot be ‘offered’ as an alternative to blood doping to athletes. Of greater concern, however, is that altitude exposure

Figure 3

Based on testimonies from caught cyclists, confiscated diaries, etc., it is easy to unravel the blood doping strategy in present-day cycle sports. Here is the example of a cyclist aiming to perform well in Paris-Nice (seven stage race in March), a spring classic (one day race) in late April, and then in the Tour de France (three week stage race) in July, and also the world championships held in the autumn. Epo could then be abused in December, January and February in order to increase red cell mass. Once a sufficient red cell mass has been synthesized, one to three bags of blood are withdrawn and stored for later use. One to 2 days before Paris – Nice two blood bags will be infused and there will be likely no trace of Epo in blood or urine at this time point (Lundby et al., 2008a). Once the race is completed, the two blood bags will be withdrawn, stored and re-infused 1 to 2 days before the spring classic and again withdrawn and stored upon completion of the race (likely on the very same day). Epo injections are likely to occur in May where no major competitions are planned. Following 2–3 weeks of treatment, the gained blood is withdrawn and stored for later use. For the Tour de France in July, two blood bags are infused 1 to 2 days before the start, and then one blood bag is re-transfused on the two resting days. Since there is no direct doping test against autologous blood doping, this makes the doper very difficult to detect during competition, whereas a chance of getting caught exists during the Epo injection period by direct test, whereas the chance of getting caught by the blood passport seems minimal – again highlighting the importance of frequent out-of-competition testing. In this regard, it is surprising that a cyclist finishing within the very top in the 2010 world championships, and hence also a top contender for the 2011 championships, has not been tested since October 2010 (as of July 1st 2011, personal communication). Black circles indicate rhEPO injections, open red circles each indicate the withdrawal and storage of a one unit blood bag (i.e. two dots = two bags), and closed red circles each indicate the re-transfusion of one unit of blood. Red vertical lines indicate competitions. It should be kept in mind that this review only deals with blood doping and that substances such as growth hormone, IGF-1, anabolic steroids and doping ‘maskers’ are very likely also misused by this type of athlete.

seems misused by some athletes as a masking procedure since sports federations such as the International Cycling Union (ICU) exclude the analytical results of blood samples obtained for the Athlete Biological Passport (ABP) (see later) in conjunction to altitude exposure, and, hence, it cannot be ruled that some athletes may go to altitude with the specific
aim to dope knowing that potential blood samples will not be used.

New substances mimicking the actions of Epo
A recent review (Jelkmann and Lundby, 2011) describes the potential for new substances developed to induce endogenous Epo synthesis and agents mimicking the actions of Epo. Very few of these, however, have been tested in humans, and, here, we have chosen only to include substances proven effective in humans.

The research group around Kai-Uwe Eckardt from Germany published a ground-breaking study. Using the prolyl hydroxylase inhibitor PHD-I FG-2216 provided by FibroGen in San Francisco, CA, USA, they proved that oral administration of this phase 1 drug increases endogenous Epo synthesis in healthy volunteers and haemodialysis (Bernhardt et al., 2010), and it is likely only a question of short time before other companies will launch similar products. Although presently unknown, it must be assumed that long-term administration of such drugs will also increase nHb and [Hb], and accordingly also exercise performance. At present, the physiological response to long-term suppression of hypoxia-inducible factor (HIF) breakdown in humans remains unknown, but it should be recognized that HIF controls the expression of a variety of prominent genes within angiogenesis and metabolism besides being the master switch for erythropoiesis. Provided that side effects are not occurring, the immense importance of this study for patients worldwide is obvious. From an anti-doping point of view, however, it is a setback. It will become virtually impossible to develop specific tests for every drug intended to block the breakdown of HIF or to stimulate the Epo receptor. Realizing that the ABP approach is unable to detect doping (Ashenden et al., 2011) with rhEpo injections (see later), it seems very unlikely that this would be the case for these new drugs.

Detection methods for blood transfusions
Although autologous blood transfusions (i.e. withdrawal and re-transfusions with one’s own blood) are a prohibited method of the World Anti Doping Agency (WADA), there is no direct detection method for the procedure. Several non-direct methods have been proposed including the analysis of plastic residues (diocetyl phthalate) (Solymos et al., 2010) present in blood following storage in certain transfusion bags (but not all), gene expression changes (Pottgiesser et al., 2009), microRNAs and proteomics (ongoing projects) and the concept of the ABP first introduced some 10 years ago (Cazzola, 2000; Malcovati et al., 2003), perhaps with the addition of nHb to the ABP (Mørkeberg et al., 2011). Of these, only the ABP seems realistically applicable at the present time and will be discussed following the section on Detection of rhEpo injections since the ABP is also designed to detect rhEpo misuse.

Detection of rhEpo injections
The main difficulty in detecting doping with rhEpo is based on the fact that rhEpo is structurally very similar to endogenous Epo. However, endogenous Epo and rhEpo glycosylation can be distinguished by their charge (Wide and Bengtsson, 1990; Wide et al., 1995), isoelectric point, which is the method used in WADA-accredited laboratories (Lasne et al., 2002), molecular mass (Kohler et al., 2008; Reichel, 2011) or by interaction with specific lectins (Franco Fraguas et al., 2008; Lönnpér et al., 2012). The methods differ in how well they distinguish a certain type of glycosylation (the Epo isoform resolution), and how much Epo is required for analysis (the Epo detection sensitivity). For an in-depth description of the various test and procedures, the reader is encouraged to read a recent review (Reichel, 2011). Despite the efforts by WADA laboratories for a clean sport, rhEpo detection is very difficult to detect, especially when the injection frequency is kept low, albeit sufficient to increase exercise performance (Lundby et al., 2008a). This study also demonstrated inconsistencies in analytical results between two WADA-accredited laboratories. Hence, other methods are currently being investigated.

Ongoing work in regard to rhEpo detection
A proteomic approach seems feasible (Christensen et al., 2011; Fania et al., 2011) but still has a long way to go, as, for example, the influence of different training modalities, altitude exposure, etc. on the proteomic response needs to be clarified, as does the window of detection. Also, the analytical costs of this approach are not negligible. As for autologous blood doping, the CO re-breathing method was suggested to be included in anti-doping work to determine potential rhEpo-induced increases in haemoglobin mass, but was deemed non-suitable (Lundby and Robach, 2010) due to biological and analytical variations (Lundby and Robach, 2010; Eastwood et al., 2011) allowing room for changes in nHb which would still increase exercise performance tremendously, but also due to practical limitations of the method and concerns towards the investigated athletes.

One of the most promising approaches to detect rhEpo doping in current anti-doping work seems to be the ongoing work with membrane-assisted isoform immunosassays (MAIA) developed by Maria Lonnberg and Jan Carlson from Uppsala in Sweden (Lönnpér et al., 2012). The method can be used to distinguish recombinant protein and peptide hormones from their endogenous counterparts by differences in protein carbohydrate structure while requiring only a small amount of each isoform. Besides possessing superior sensitivity, MAIA is also faster and much cheaper to perform than the present isoelectric focusing (IEF) test used by WADA, and we expect this innovative technology to present a great alternative to the cumbersome IEF test.

Another potential addition to current anti-blood doping work could be hepcidin, a hormone produced in the liver which plays a key role in regulating iron balance. Recent data indicate hepatic hepcidin suppression in healthy volunteers treated with low-dose rhEpo (Robach et al., 2009), and following a single rhEpo injection (5000 IU s.c.), it was observed that serum hepcidin levels were profoundly suppressed in six subjects starting 24 h after the injection, and remained suppressed for 2 weeks (Ashby et al., 2010). However, potential implementation of hepcidin assay for anti-doping purpose is limited by several problems: (i) This hormone is modulated by several other factors. On one hand, inflammation or iron loading increases hepcidin expression (Nicolás et al., 2002). These two conditions can occur in elite sports, which in turn may mask rhEpo-induced hepcidin suppression. On the other hand, hypoxia or iron deficiency is known to promote hep-
cidin suppression (Piperno et al., 2011), highlighting the risk of detecting false positive, particularly for iron-deficient athletes or athletes undergoing altitude training regimen. Evidence from animal studies suggest that massive erythropoietic stimulus would dominate over inflammation on hepcidin expression (Lasocki et al., 2008), but whether this holds true in humans treated with low dose rhEpo remains unknown; (ii) Hepcidin assay is currently performed through various techniques which often requires expensive equipment and dedicated skilled personnel (Kroot et al., 2009). Furthermore, a reference range for normal hepcidin values in the normal population is not available yet.

In summary, using hepcidin as a tool for anti-doping seems promising but still needs further work addressing: (i) the role of the main confounding factors on rhEpo-induced hepcidin suppression; and (ii) most importantly, the development of a rapid, low-cost and easy-to-use instrumental approach.

The ABP
The original idea for a biological passport for athletes was first published by Italian M. Cazzola (Cazzola, 2000), and much work in this regard was initially conducted especially by Australian researchers (Parisotto et al., 2000a,b; 2001). Although blood monitoring programs in single sports federations such as the ICU and the International Ski Federation are generally referred to as ‘Blood Passports’, these are not to be mistaken with WADA’s more strictly regulated ‘Athlete Biological Passport’. Today’s ABP statistical software system was largely developed by Pierre-Edouard Sottas (Sottas et al., 2011) and is based on personalized monitoring of biomarkers indicative of doping, and is hence not a direct test program for a given substance. The approach can be used for a variety of doping practices, but, here, we will focus on blood doping – the haematological module of the program. The overall idea is that if an athlete is doping by means of blood transfusions, rhEpo injections or any other method with the purpose to increase red blood cell mass, this will lead to changes in a variety of haematological parameters such as reticulocyte count and [Hb]. Besides, the analysed haematological values, also heterogeneous factors, such as age, sex and genotype, are included in the ABP as are information on altitude exposure and sample handling. The ABP is a multi-step system where the first part is performed using a software system. The next step is completed by experts who evaluate the blood profiles flagged as abnormal by the ABP software, and only if these experts come to the conclusion that it is very likely that blood manipulations have occurred while excluding all other potential causes a disciplinary procedure is opened. In an anti-doping setting and using the ABP approach, Morkeberg and co-workers (Morkeberg et al., 2011) achieved rather disappointing sensitivity rates of less than 20% in a controlled autologous blood transfusion study, whereas Pottgiesser et al. (2011) reported abnormal samples in eight of 11 transfused subjects when the probability level was set to 99% and five abnormal samples if probability level was set to 99.9% using a blinded doping control investigator. For such a scenario to be effective in real life, the authors concluded that a true expert group is required with specific knowledge in regard to competitions, strategies, training settings, etc. within the specific discipline. As such, the results are promising, but one concern in regard to the later study is that one subject triggered a false-positive result based on high initial [Hb]. Since all values flagged by the ABP software as being suspicious are submitted to the panel of experts, this would likely not have resulted in an anti-doping rule violation procedure, but emphasizes the importance of the quality of these experts. It would be preferable for them to have hands-on experience with blood manipulations gained in conjugation to their scientific or clinical work, and we encourage invited experts only to join doping-related panels if strictly within their field of expertise. The prosecuted athlete may of course question a given conclusion by enrolling his/her own expert panel.

Although the ABP sounds promising, limits and pitfalls have been put forward. Most recently, one of the main critics of the system, Guiseppe Banfi from Milano (Banfi, 2011), raised serious concerns in regard to, among others, the data source used to design the ABP, and furthermore stated that the variance used for cyclists is not correct and that the quality control of the used instruments is not completely assured. Based on the earlier discussion, the ABP seems the best currently available tool for detecting autologous blood manipulations, but it also needs to be acknowledged that the system in its present form is far from bulletproof, and that it is only an indirect evidence for blood manipulations, and, furthermore, that it is impossible to be 100% certain that all prosecuted athletes have in fact practiced doping. Therefore, we propose that ABP abnormalities can elicit a lighter ‘no start’ sanction as compared to the up to 2 years ban issued today (which for some athletes corresponds to ‘death sentence’). It should also be acknowledged that in a scenario as exemplified in Figure 2, where the transfusion and subsequent withdrawal could both be performed on the very same day as the competition (Spring Classic or World Championships), this would make it virtually impossible for the ABP to detect manipulations.

In addition to the ABP, Morkeberg and co-workers investigated the possibility to include nHb to the algorithm, and demonstrated this to increase the sensitivity (Morkeberg et al., 2011). Since, however, it cannot be ruled out that the amount of carbon monoxide (CO) given to the athlete in order to determine nHb will negatively affect exercise performance, or even potentially be of harm to the athlete’s health, it seems very unlikely that the CO re-breathing method will be introduced into anti-doping work, and we certainly do not support such an implementation.

The ABP was designed also to detect rhEpo misuse. Since the reticulocyte count is however decreased after an initial increment to base values with continuous rhEpo stimulation (Lundby et al., 2007), the sensitivity of the ABP with long-term rhEpo should be questioned. Bornå et al. (2010) were the first to test the feasibility of a passport approach in order to detect rhEpo doping and reported a 58% detection rate in subjects injected with moderate rhEpo concentrations. Subsequently, Ashenden and co-workers (Ashenden et al., 2011) from Science and Industry Against Blood doping performed a study where ‘microdoses’ of rhEpo were injected in healthy volunteers for up to 12 weeks. The micro-dosing was sufficient to increase nHb by 113 g (approximately equivalent to the haemoglobin contained in two 450 mL bags of liquid or frozen stored blood) which will increase exercise performance.
tremendously (Thomsen et al., 2007; Lundby et al., 2008b). Disappointingly, the ABP software did not flag a single subject as being suspicious of doping, and the authors concluded that it is possible for athletes to misuse rhEpo without eliciting abnormal changes in the blood variables currently monitored by the ABP. A further concern raised by the study was that one sample before any rhEpo injections had occurred was designated ‘abnormal’. As mentioned above, in real life, this value would be evaluated by an expert panel before any proceedings would be initiated, and we can only hope that the panel would make the right decision.

**Htc and [Hb] in healthy humans**

In order to discuss blood manipulations, one has to appreciate the reference values. At sea level, healthy males in the United States (n = 7426) have an Htc between 40 and 49% (5th and 95th percentile, respectively), whereas the normal limits for females (n = 7704) are 35 and 44%, with a slight tendency to increase following menopause. The 50th percentile for males and females in the ‘exercise competitive age’ is 44.5 and 39%, respectively. For young children, the normal limits are 33 and 40% (Fulwood et al., 1982). Based on the Second National Health and Nutrition Examination Survey (n = 2515), major [Hb] differences exist between black and white males (144.8 vs. 153.2 g L⁻¹), females (128.4 vs. 133.9 g L⁻¹) and children (120.3 vs. 126.8 g L⁻¹) (Perry et al., 1992). Smoking more than 10 cigarettes per day significantly increases [Hb], and when comparing never-smokers to smokers, an increase in [Hb] from 133 to 137 and from 152 to 156 g L⁻¹ is seen in females (n = 2454) and males (n = 2250), respectively (Nordenberg et al., 1990). Based on this and similar data, and in an attempt to limit doping practice, the upper Htc limit (cut-off value) allowed for males and females by most sports federations was set to 50 and 47%, respectively. The reference values used for these limits as noted earlier, however, are based on normal healthy individuals. With exercise training, haematological adaptations occur, leading to an initial expansion of plasma volume. However, after approximately 30 days of endurance training, the increase in red cell and plasma volume are approximately equal, and hence also [Hb] normalizes (Sawka et al., 2000). The haematological profile of an elite endurance athlete is less well described. To compare the distribution of blood haemoglobin levels in healthy blood donors and elite athletes specifically for anti-doping purposes, a retrospective (2001–2005) cohort study was performed in 85 846 Danish (sea level) blood donors (males = 36 962; females = 48 884, age 18–65 years) and compared to 1406 national team rowers (males = 1116; females = 290, age 16–32 years). In this data set, 3.9% of the male blood donors had a blood [Hb] of above 10.5 mM (corresponding to a Htc of 51%, i.e., above the cut-off value), and 1.6% of the females had a [Hb] above 9.7 mM (equivalent to a Htc of 47%, the female cut-off value). Surprisingly, this data set also demonstrated that the % distribution for the cut-off values are higher (P < 0.0001) in elite athletes, i.e., 10.4 and 8.3% for the male and females, respectively, and thereby demonstrating that high [Hb] levels in blood are seen regularly in normal people and especially in competitive athletes (Johansson et al., 2009). It needs to be stated, however, that this not can be ruled out that at least some of the athletes included in this study may have made use of doping practice, and that this could have affected the values.

In another study, moderately trained subjects (n = 44) were compared to trained runners (n = 19), highly trained cyclists (n = 17) and world class cross-country skiers (n = 21), and in that study, only two of the moderately trained subjects surpassed the upper limit for [Hb], whereas none of the better trained subjects surpassed the upper accepted level. Data from this study also suggest that Htc in Olympic calibre athletes and non-athletes are rather similar, but that the nHb and plasma volumes are increased in highly trained athletes (Jelkmann and Lundby, 2011). Nonetheless, it needs to be acknowledged that some athletes may have blood values above the cut-off values, and this gives national sports federations the very important task to pinpoint such athletes from an early time point in their careers, and then to ensure the needed documentation in order to avoid a ‘no start’ penalty or potential mis-accreditation of the athlete by sports colleagues or the press.

It is also important to realize that blood values undergo biological variation. Based on results from 12 studies of 638 normal healthy adults, the coefficient of within-subject biological variation of Htc is 3%. The normal within-subject biological variation (3%) and analytical variation (3%) may explain a relative change of approximately 12%, e.g., a change from 42 to 47% between two successive Htc values, measured with a time interval between 1 day and 1–2 months (Thirup, 2003). Partly due to haemodilution in warm weather, Htc often has a seasonal variation in normal healthy adults, and, based on results from 18 studies of 24 793 participants, the population mean is approximately 3% lower in summer than in winter. Population mean values that are 7% lower in summer than in winter have been found in some studies, although no seasonal effect may also be seen, especially in temperate climates (Thirup, 2003). Besides ambient temperature, also altitude exposure influences blood values. As compared to sea level, no changes in Htc or red cell mass (nHb) are usually observed with exposure to 1.600 m altitude (Weil et al., 1968), but starting from around 2350 m altitude (Schuler et al., 2007), an increase is noted which becomes gradually more increased with further altitude gain (Weil et al., 1968; Calbet et al., 2003; Lundby et al., 2006). Other external factors affecting plasma volume, and hence Htc and [Hb], include posture, hydration status and acute exercise (Harrison, 1985). In contrast to this, Schumacher and colleagues (Schumacher et al., 2010) concluded from a specifically anti-doping designed study performed on endurance-trained subjects (mostly cyclists) and appropriate controls that fluid intake and ambient temperature over the course of a ‘typical training day’ had no significant effect on [Hb]. The conducted training resulted in an average [Hb] increase of 0.46 g L⁻¹ which however disappeared 2 h into recovery, and, thus, minor changes in [Hb] can be expected following training/racing, but unless a given athlete is extremely dehydrated (and hence not able to train/race in a meaningful manner) (Harrison, 1985), this likely resembles no limitation to anti-doping work. An example of a similar study approach in national team cross-country skiers is shown in Figure 2B where [Hb] is seen not to be significantly influenced by various interventions associated to normal life of such athletes. In more extreme exercise events (Pugh, 1969), however, a decrease in Htc may be observed secondary to an increase in plasma volume while red cell mass remains stable.
Is it worth it/future direction?

Citius, altius, fortius – the Olympic motto – is supposed to be reached solely by natural talent, proper training and diet. How true is that today? Probably not true at all. Today, it may be impossible to reach the top in a number of sports without using one or more suitable doping listed drug(s). In a recent Nature article, Don Catlin is cited to claim, ‘Everyone in cycling was doping’ (Callaway, 2011). Although Catlin may have gone too far, reasonable questions to be asked by the public are: ‘How can it be so bad with all the anti-doping activities and controls taking place?’, and if the situation is as bad as stated by Don Catlin, ‘is it really worth all the efforts and expenses?’

The answer to the first question has in part been given earlier. Valid and sensitive tests are lacking. The timing of when the tests are performed is not appropriate. In regard to blood manipulations, all of the above seem true. For the most commonly abused drug, anabolic steroids, the tests are there, but the timing is critical. The window for positive detection is quite long (weeks–months), but if anabolic steroids are used, they are taken in periods of hard muscle strength training, many months away from competition.

Indeed, many athletes in strength-demanding sports may only have misused these steroids when preparing for a career in the junior/young senior ranks, years ahead of a career with many successes as a senior. Just as Catlin states that all cyclists were doped, one could anticipate that all athletes in ‘strength’ events have at some point in their carrier been on a drug, which, linked with efficient training, enlarged their muscle mass and strength.

Thus, a quite dark account is painted of the doping situation in elite sports. Why do international sports federations, the International Olympic Committee (IOC) and governments continue to spend millions and millions of US dollars on anti-doping articles and controls? Do they have a choice? Probably not! Governments, regions and communities in most countries of the world support sports with tax money. There are many good reasons for this support. However, one may wonder whether, in a political perspective, the public accepts the support of leisure time activities and environments, which are not ‘drug free’, pretended or in reality.

In WADA’s fight against doping, there are three criteria for a substance to be on the doping list:

- Enhances performance.
- Have harmful effects to the body.
- Contradicts the spirit of sports.

The first and second reasons are quite logical, but the third is more difficult to define; however, it illustrates the wish to highlight sports as healthy, ‘clean’ and a good environment for youth development. International sports leaders are most likely well aware of the fact that another statement by Catlin also is correct: ‘No matter how sophisticated (test and controls) for every move to the right the other guys are moving to the left and it balances out again’. Nevertheless, they have to set money aside for anti-doping work. In other words, regardless of the effort and money spent, doping in elite sports (and among many leisure sports-active individuals) is still present and quite common.

It is understandable that IOC and WADA market another picture. Ahead of the Beijing Olympics, the message was that the competitions were the cleanest ever, and, indeed, the numbers of positive tests were few. However, that is, of course, no measure of the real misuse that has taken place for years and months during preparations for the games.

In the Nature article (Callaway, 2011), some WADA laboratory-related persons bring forward the hopes they and WADA have in regard to the ABP. The idea of accepting indirect evidence of doping has been discussed in various circles including WADA for more than a decade and was first formally discussed at a WADA meeting ahead of the Sydney Olympics.

Since then, WADA has promised to develop this approach, but it was not formally decided until late in 2009. The experience so far is primarily related to the control of blood manipulation. Still, the difficulties are more pronounced than the success of its implementation. In sports as cross-country skiing and speed skating, with only a limited number of top class athletes, the passport could in theory be quite effective, both in preventing doping procedures and also in order to detect misuse. The reason being that the athletes can be well characterized when entering the system and also followed not only during the competitive season but also in between. However, in sports like athletics or soccer, it is difficult to implement the ABP, whatever efforts are made. The critical issue is to establish the individual’s ‘normal’ blood values as well as what is the normal or acceptable variation around the normal value. Another prerequisite for the blood pass to function is regular testing, all the year round. This cannot be handled by WADA and an international sports federation. Well-developed National Anti-Doping Agencies (NADAs) with extensive resources are needed, and WADA and not the least IOC should focus more on aiding the NADAs to become well-functioning. IOC also has the power to block for countries to be part of the international sporting community, if a NADA in a given country does not meet the standards. As long as this is not the case, our hopes are low for the ABP to help to limit doping in sports.

Conflicts of interest

None.

References


Blood doping – effects and detection


Muscle protein synthesis in response to nutrition and exercise

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Abstract Muscle protein synthesis (MPS) is the driving force behind adaptive responses to exercise and represents a widely adopted proxy for gauging chronic efficacy of acute interventions, (i.e. exercise/nutrition). Recent findings in this arena have been progressive. Nutrient-driven increases in MPS are of finite duration (∼1.5 h), switching off thereafter despite sustained amino acid availability and intramuscular anabolic signalling. Intriguingly, this ‘muscle-full set-point’ is delayed by resistance exercise (RE) (i.e. the feeding × exercise combination is ‘more anabolic’ than nutrition alone) even ≥24 h beyond a single exercise bout, casting doubt on the importance of nutrient timing vs. sufficiency per se. Studies manipulating exercise intensity/workload have shown that increases in MPS are negligible with RE at 20–40% but maximal at 70–90% of one-repetition maximum when workload is matched (according to load × repetition number). However, low-intensity exercise performed to failure equalises this response. Analysing distinct subcellular fractions (e.g. myofibrillar, sarcoplasmic, mitochondrial) may provide a readout of chronic exercise efficacy in addition to effect size in MPS per se, i.e. while ‘mixed’ MPS increases similarly with endurance and RE, increases in myofibrillar MPS are specific to RE, prophetic of adaptation (i.e. hypertrophy). Finally, the molecular regulation of MPS by exercise and its regulation via ‘anabolic’ hormones (e.g. IGF-1) has been questioned, leading to discovery of alternative mechanosensing–signalling to MPS.

(Received 21 November 2011; accepted after revision 25 January 2012; first published online 30 January 2012)

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Phil Atherton (left) completed his PhD charting signal transduction pathways regulating skeletal muscle metabolism and plasticity. After this, he completed a 3 year post-doc, providing molecular biology input to a large scale exercise-training programme into the effects of ageing on physiological and metabolic adaptations to exercise. In 2008 he took up a Research Councils UK Fellowship (HEFCE funded beyond 2012) with the view to developing an independent career and is currently pursuing work to define the molecular regulation of protein turnover by nutrition and exercise, in health and disease. A particular interest of Phil’s, is back-translating ‘hits and leads’ from humans into more tractable in vitro models, with the objective of achieving both observational and mechanistic understanding. Ken Smith (right) completed his PhD at the University of Dundee under the tutelage of Prof. Mike Rennie where he developed his career long interest in the development and application of stable isotopic methodologies to understand the regulation of human fuel metabolism, in particular amino acid and protein turnover in skeletal muscle, in health and disease; with particular focus on the role of both nutrition and exercise in maintaining muscle mass and function. Currently he is a principal research fellow in the Division of Metabolic Physiology at the University of Nottingham where he oversees the Mass Spectrometry Core facility, a core component of the recently awarded MRC/ARUK ‘centre for musculoskeletal ageing’.

This review is from the symposium Exercise metabolism at The Biomedical Basis of Elite Performance, a joint meeting of The Physiological Society and the British Pharmacological Society, together with The Journal of Physiology, Experimental Physiology, British Journal of Pharmacology and The Scandinavian Journal of Medicine and Science in Sports, at the Queen Elizabeth Hall, London on 20 March 2012.

Background

Skeletal muscles are highly plastic tissues that adapt to cope with the increased locomotory and metabolic demands of exercise. However, successful adaptation to exercise in terms of altered muscle physiology and improved performance varies exquisitely according to the activities imposed (e.g. force, duration, etc.) and by an individual’s genetic makeup, which designates his or her ‘responder status’ (Timmons, 2011). It follows that selectivity over the quantity (i.e. individual proteins or ‘bulk’ subfractions such as myofibrillar, mitochondrial and sarcoplasmic) of muscle proteins synthesised underlies the exquisite adaptive specificity to distinct exercise training regimens, and perhaps even the marked heterogeneity of responsiveness to training (Timmons, 2011).

In healthy, recreationally active individuals, skeletal muscle proteins display turnover rates of \( \sim 1.2\% \text{ day}^{-1} \) and exist in dynamic equilibrium: muscle protein breakdown (MPB) exceeds muscle protein synthesis (MPS) in the fasted state, and MPS exceeds MPB in the fed state. In response to exercise, MPS is transiently increased whereas MPB also increases, or remains the same (the latter of which is on the proviso of sufficient exogenous nutrient supply; Kumar et al. 2009a). It follows that on a cumulative basis, increases in MPS after each exercise bout ‘drives’ adaptation to exercise training.

Stable isotopes: capturing protein turnover in vivo

Dynamic measures of muscle protein turnover can be determined in muscle tissue using stable isotope methodologies (Rennie et al. 1982; Wolfe, 1982). Stable isotopes are non-radioactive naturally occurring ‘heavy atoms’ (NB safe for use in man), which are essentially identical to their endogenous counterparts but can be distinguished by their mass difference (using mass spectrometric techniques). This allows us to measure incorporation of these isotopic ‘motifs’ into biological samples, i.e. isotopically labelled amino acids to measure MPS in protein obtained from biopsy tissue (Rennie et al. 1982; Trappe et al. 2002; Katsanos et al. 2005; Koopman et al. 2008). However, since these methods require constant tracer infusions, they are only suitable for measuring ‘acute’ (~hours) MPS in a controlled laboratory setting. Therefore it is of great interest that new tracer methods have been recently developed where measures of MPS are possible in free-living subjects over weeks to months. This method involves ingestion of deuterated water (D\(_2\)O) to assess cumulative incorporation of deuterium into muscle proteins via deuterium exchange through alanine (Robinson et al. 2011).

The choice of labelled amino acid will determine the method of measurement. Using deuterium (in place of hydrogen) labelling allows measurement of synthesis using gas chromatography–mass spectrometry (GC-MS), whereas the use of \( ^{13}\text{C} \) or \( ^{15}\text{N} \) is traditionally measured using isotope ratio mass spectrometry (IRMS) of fixed gases, i.e. CO\(_2\) or N\(_2\), which requires combustion or release of CO\(_2\), e.g. by reaction with anhydrous hydrogen or in the case of \( ^{15}\text{N} \), combustion to NO\(_2\), followed by reduction to produce N\(_2\). The free amino acids (AAs) from hydrolysis of proteins are separated by chromatography (gas or liquid) and combusted prior to mass spectrometric analysis, e.g. gas chromatography–combustion (GC-C)–IRMS or liquid chromatography–combustion (LC-C)–IRMS (for an introductory review of tracer approaches see Rennie, 1999).

Recent advances in the stability and sensitivity of mass spectrometers, coupled with the availability of multiply ‘heavy atom’ labelled amino acids, e.g. \([1,2-^{13}\text{C}_2]\)leucine (Atherton et al. 2010), \([\text{D}_3]\)- or \([^{13}\text{C}_6]\)phenylalanine (Koopman et al. 2008; Burd et al. 2011), has permitted greater resolution of the acute responses of MPS even over 30–45 min periods (Atherton et al. 2010) and thus measurement of the temporal nature of the MPS response. Technical development and application of methods to measure muscle protein breakdown (MPB) has, however, lagged behind that of MPS and as a result much less is known about the responses of MPB to exercise and nutrition. However, stable isotopes do allow for estimates of MPB by dilution of the tracer across a limb (using an arterio-venous balance model) when assessed in conjunction with limb blood flow, i.e. a greater difference in labelling of an essential amino acid (EAA) between arterial-venous samples indicates a higher rate of release of AAs via MPB (Wilkes et al. 2009).

Regulation of MPS by nutrition

The two principal determinants of adult skeletal muscle proteostasis are physical activity (discussed subsequently) and nutrient availability. The anabolic effects of nutrition are principally driven by the transfer and incorporation of amino acids captured from dietary protein sources, into skeletal muscle proteins. The purpose of this is to compensate for muscle protein that is lost in fasted (postabsorptive) periods due to, for example, amino acid oxidation and/or carbon donation for liver gluconeogenesis (Wackerhage & Rennie, 2006). Critically (assuming good health and mobility), it is this dynamic ‘fasted-loss/fed-gain’ cycle in proteostasis that ensures muscle mass remains constant. But what are the ‘anabolic components’ of nutrition? After early work defining that the anabolic effects of mixed-meal feeding were entirely attributable to essential amino acids (EAA) (Smith et al. 1992), we and others have gone on to
show dose-dependent and saturable effects at 10 g EAAs (Cuthbertson et al. 2005) equivalent to ~20 g protein (Moore et al. 2009). Perhaps unsurprisingly, this anabolic response is transient in nature, which makes sense, as forsaking adaptive increases in MPB one could achieve hypertrophy simply by eating extra protein! The time course of the feeding response with a saturable amount of protein is as follows. After a lag of around 30 min there is a large increase (~3-fold) with MPS peaking around 1.5 h before returning to baseline by 2 h (Atherton et al. 2010) despite continued increased availability of circulating amino acids and sustained ‘anabolic signalling’ (Bohe et al. 2001; Atherton et al. 2010). It is at this point the muscle becomes refractory to stimulation despite sustained elevations of AAs (see Fig. 1). We have termed this phenomenon ‘muscle-full’ (Bohe et al. 2001; Atherton et al. 2010) based on the developmental concept introduced by Joe Millward wherein muscle protein accretion is physically limited by the inelastic collagen connective tissue of the endomysium surrounding each fibre (the ‘bag-full’ hypothesis)(Millward et al. 1994).

What of a role for insulin in regulating anabolic responses to nutrition (via nutrient-induced secretion)? While it is noteworthy that provision of protein alone (i.e. without carbohydrate) causes a rise in insulin similar to that seen following a mixed meal (Atherton et al. 2010), insulin apparently does not contribute to the anabolic effects of EAAs on MPS. To exemplify this, EAA infuses robustly stimulate MPS even when insulin is ‘clamped’ at postabsorptive concentrations (5 μIU ml⁻¹ with the β-cell inhibitor octreotide; Greenhaff et al. 2008). However, this does not mean there is no postprandial anabolic role for insulin. Indeed, in addition to the 3-fold rise in MPS, there is also a significant anti-proteolytic (~40–50%) effect of feeding on skeletal muscle which is apparently entirely attributable to insulin. To illustrate this, a rise in insulin to just 15 μ IU ml⁻¹ (3× postabsorptive concentrations) is sufficient to mimic the 50% inhibition of MPB (NB the maximal effect size) caused by a mixed meal (Wilkes et al. 2009). Moreover, this anti-catabolic effect cannot be recapitulated via large-dose AA infusions (18 g h⁻¹ over 3 h) when insulin is clamped at postabsorptive concentrations (5 μU ml⁻¹) (Greenhaff et al. 2008). Thus, to summarise, EAA regulates anabolic responses via large increases in MPS, while insulin release regulates anti-catabolic (depressions in MPB) responses. It follows that as the change in MPS is far greater than that in MPB, MPS is the major driving force behind nutrient induced anabolism.

**Regulation of MPS by acute exercise**

The magnitude of acute response of muscle to resistance exercise in terms of MPS is dependent upon both workload and intensity. For example, at intensities ≤40% of one-repetition maximum (1-RM), there are no detectable increases in MPS, whereas at intensities greater than 60% 1-RM, exercise increases MPS 2- to 3-fold (Kumar et al. 2009b). However, this does not mean that lower intensity exercise cannot yield anabolic effects. Indeed, increases in MPS at 30% 1-RM of comparable magnitude to a group performing 90% 1-RM are possible but only when exercise is performed to failure and not when work is matched between 30 and 90% 1-RM (Burd et al. 2010). In essence this means that increasing the volume of work at a lower intensity can overcome and even surpass the blunted MPS response with work-matched low-intensity exercise, probably as a consequence of increased type II fibre recruitment due to the fatigue-generating nature of the contractions (Burd et al. 2010). As such, low-load, fatiguing contractions may represent a feasible approach to stimulate muscle hypertrophy and a means of escape from lifting heavy weights.

In terms of contraction mode, although eccentric-type exercise training (i.e. lengthening contractions, not backward running) has been shown to result in greater muscle hypertrophy (Roig et al. 2009), measurement of MPS after both concentric and eccentric contractions has demonstrated only relatively small temporal differences (Cuthbertson et al. 2006). Moreover, when total work is matched between eccentric and concentric contractions there is no difference in training-induced muscle hypertrophy (Moore et al. 2011). As such, increased external loading encountered during eccentric contractions may explain the greater efficacy of eccentric training, rather than contraction mode per se.

It perhaps comes as no surprise that, as with the ‘muscle-full’ response to feeding, the anabolic response to exercise must also be of limited duration. In terms of the time course of MPS response, immediately after exercise there is a latent period (prior to rises in MPS) of a duration which seems to relate to the magnitude of the muscle hypertrophy (Moore et al. 2011). As such, increased external loading encountered during eccentric contractions may explain the greater efficacy of eccentric training, rather than contraction mode per se.

![Figure 1. The ‘muscle-full’ effect. Relationship between MPS, AA and intramuscular signalling](image-url)
of energy/mechanical stress associated with the exercise. This premise was exemplified in a rodent study showing that MPS is suppressed during intense contraction in a duty cycle (i.e. work)-dependent manner (Atherton & Rennie, 2006; Rose et al. 2009). Furthermore, although there exist no equivalent studies in humans (i.e. MPS during exercise), there have been measures made in the acute period of recovery of exercise bouts which may allude to similar mechanisms. For example, while MPS remained unchanged up to 3 h after extremely fatiguing and damaging eccentric contractions (step-up/step-down carrying weight) (Cuthbertson et al. 2006), the latency for lower intensity exercise (6 × 8 repetitions at 75% 1-RM) is <1 h (Kumar et al. 2009b).

After this latent period, MPS rises sharply by 45 and 150 min and may be sustained for up to 4 h (Kumar et al. 2009b) in the fasted state (limited by substrate availability), and in the presence of increased AA availability, up to and beyond 24 h (Cuthbertson et al. 2006). Interestingly, the time course of changes in MPS to the exercise bout is mimicked by that of the epimysial collagen and tendon collagen, thus demonstrating a high degree of coordination between tissues of the musculoskeletal system in response to exercise (Miller et al. 2005).

Exercise × nutrient interactions regulating MPS

A key aspect surrounding acute responses to exercise and subsequent adaptation is nutrient × exercise interactions. This is exemplified by the fact that acute increases in MPS after exercise in the absence of EAA nutrition provide a more prolonged rise in MPB such that the net effect is negative muscle protein balance (Biolo et al. 1995). If such EAA deficiency persisted throughout training, this would lead to maladaptation; you can’t build or remodel muscle without amino acids! It follows that increasing dietary AA availability after exercise enhances both the magnitude and duration of the increase in MPS (Pennings et al. 2011). Therefore, in essence, exercise is able to pre-condition muscle to delay the muscle full ‘set-point’ (illustrated in Fig. 1). Interestingly, addition of carbohydrate to protein affords no greater anabolic effects on protein turnover (neither increases in MPS nor depressions in MPB) after exercise, highlighting the central role of EAs as the principal (and perhaps only!) macronutrients required to optimise anabolic responses in protein turnover to exercise (Staples et al. 2011).

There has been considerable work undertaken to determine the optimal timing of nutritional intake in order to maximise post-exercise MPS and ensuing adaptations to training (Cribb & Hayes, 2006; Hoffman et al. 2009). In general, we believe that it is largely irrelevant whether the feed is given pre-, during or post-exercise. This is because the delaying of the muscle-full response appears to last at least 24 h (Burd et al. 2011) after a single bout of exercise, which may help explain chronic adaptations such as hypertrophy/remodelling of muscle over time, independent of proximity-dependent feeding patterns (see Fig. 2). Therefore, we contend that nutrient sufficiency per se, rather than timing of intake, is the more important aspect to successful hypertrophic adaptation (that is not to say some acute performance/recovery benefits may be afforded by consumption of nutrition in close proximity to exercise) (Ferguson-Stegall et al. 2011). Moreover, there are still limits to how hard the system can be pushed and increasing protein loading to an identical bout of exercise still demonstrates a saturable response at around 20 g (equivalent to the 10 g EAA maximum dose observed with EAs in the absence of exercise), above which amino acid oxidation is increased and excess protein is thus catabolised (Moore et al. 2009). Therefore increasing the EAA load will not fully overcome the muscle-full effect afforded by exercise; rather, it prolongs the anabolic window. As such moderate feeding strategies may be better (~20 g PRO aliquots) but, perhaps, more often (the frequency of which remains to be determined, i.e. how long the muscle remains refractory to the anabolic effects of AAs).

Regulation of MPS by exercise training

The effect of exercise training on MPS is less well studied. Although a number of studies cite increases in ‘basal or postabsorptive’ MPS as a result of training per se, they may simply be confirming the prolonged acute effects, especially where measurements were made less than 24 h following the last bout of exercise (Hasten et al. 2000). Nonetheless, there are data which suggest that exercise training shortens the duration of the anabolic response, which could be due to greater acute adaptive efficiency (Hartman et al. 2006; Tang et al. 2008), or perhaps the laws of diminishing returns in terms of adaptive responses.

Responses in MPS to different exercise modes

As a field we are often guilty of focusing on resistance exercise and nutrition and ways to make muscles bigger. Nonetheless, most studies support the notion that MPS responses are similar irrespective of the mode of exercise, i.e. resistance vs. non-resistance (though the duration of sensitisation may differ). For instance, endurance-type exercise such as running or cycling is also associated with increased synthesis of mixed muscle proteins acutely (~50–60%) (Harber et al. 2010). However, these acute responses are not associated with significant changes in muscle mass, i.e. hypertrophy
observed with resistance exercise. So what do these changes mean? Clearly extrapolating the amplitude of increase in mixed muscle MPS cannot inform on adaptation – so what can we do? As was stated in the initial section of this review, for adaptation to display exercise-mode specificity, there must be distinct responses of different protein fractions (and indeed individual proteins) within muscle. Indeed, this proposition was elegantly displayed in a study where the same individuals performed a 10 weeks resistance (weight-lifting) programme in one leg and a 10 weeks endurance (cycling) programme in the other. After training, post-exercise myofibrillar not mitochondrial protein synthesis increased with resistance exercise (Wilkinson et al. 2008). Conversely, after training mitochondrial protein synthesis increased only in the endurance-trained leg whereas myofibrillar did not. These data seem to suggest a ‘matching’ between MPS responses and phenotypic changes, i.e. muscle hypertrophy in resistance training versus mitochondrial biogenesis in endurance training. Nonetheless, although it would be tempting to conclude that acute responses within specific muscle pools may provide insight into chronic adaptations ensuing, responses in the untrained individual may be less specific (Wilkinson et al. 2008) and be more related to the unfamiliarity of exercise per se (Coffey et al. 2006). Therefore, extrapolation of acute MPS in subfractions to potential adaptive responses after a single bout of unfamiliar exercise should be cautiously interpreted.

**Sensing and signalling regulating MPS**

Despite being a hot-bed of research, the ‘black box’ question relating to the mechanisms regulating MPS and adaptation to exercise still remains poorly defined. Exercise triggers complex mechanotransduction and physico-chemical (i.e. endocrine, auto/paracrine) sensory mechanisms (Glass, 2010; West et al. 2010). Subsequent activation of receptor and non-receptor mediated intramuscular signalling modulates cellular apparatus regulating both short-term post-translational (phosphorylation) control of protein turnover and gene expression (mRNA/miRNA) and long term changes in cellular metabolic capacity.

But what do we know of this black box? First, it is well established that the mammalian target of rapamycin (mTOR) is a key signalling pathway regulating exercise/nutrient-induced alterations in MPS (Drummond et al. 2009; Dickinson et al. 2011). Indeed, mTOR activation ultimately induces phosphorylation of multiple translational initiation factor substrates (4E-binding protein (4EBP1), ribosomal protein S6 kinase (p70S6K1), eukaryotic initiation factors 4 G/A/B (eIF4G/A/B) and formation of the eIF3F scaffold) to promote assembly of the 48S pre-initiation complex. In a parallel pathway, activation of the key guanine exchange factor, eukaryotic initiation factor 2B (eIF2B) eIF2 shuttles the initiator tRNA (Met-tRNAi) to the ribosome during formation of the 48S pre-initiation complex, thereby promoting ‘global’ protein synthesis and co-ordinately enhancing translational efficiency (for detailed reviews of mTOR and associated signalling see Proud, 2009; Goodman et al. 2011).

In terms of ‘what is upstream of mTOR?’, it has for a long time been known that nutrients (EAAs) signal through mTOR independent of proximal insulin signalling (for detailed reviews beyond the scope of this one see Proud, 2009, 2011). However, exercise-induced inputs upstream of mTOR have been more controversial. Much of the early animal (Stitt et al. 2004) and cell (Rommel et al. 2001) work pointed to a canonical signalling pathway whereby increases in insulin-like growth factor (IGF-1, or splice variants like mechano-growth factor (MGF)) production stimulates proximal insulin signalling pathways (IGFr–AKT–mTOR), and thereafter key substrates of mTOR regulating translational initiation.
However, there are a number of lines of evidence from both in vivo and in vitro systems arguing against such a canonical IGF–AKT–mTOR pathway in the regulation of exercise-induced MPS. In an elegantly designed study, resistance exercise was performed in human arm muscles under conditions of either high endogenous hormone (HH; concurrent bilateral leg exercise) or low endogenous hormone (LH; no concurrent leg exercise) concentrations (West et al. 2009). Yet, despite considerable differences in growth hormone, testosterone and IGF-1 concentrations between the LH and HH groups, there were no differences in mTOR signalling, MPS, or in chronic adaptations to training in terms of mass or strength gains (West et al. 2010). These data suggest that systemic induction of IGF-1 is not a pivotal part of the adaptive process. Nonetheless, it could be argued that IGF-1 regulates AKT–mTOR signalling via more ‘local’ auto/paracrine signalling mechanisms. Yet this is also difficult to reconcile as ablation of the IGFr does not compromise chronic adaptations, i.e. hypertrophic responses to loading in pre-clinical models (Spangenburg et al. 2008; Hamilton et al. 2010).

So what else may be upstream of mTOR in response to exercise? Mechanotransduction is the process of converting mechanical (i.e. exercise) stimuli into cellular responses and represents a viable means by which cells can distinguish mechanical inputs and, thus, perhaps confer adaptive specificity (for detailed review see Hornberger, 2011). Importantly, recent work has highlighted that phospholipase D (PLD) and its membrane-derived lipid second messenger phosphatidic acid (PA) are upstream of contraction-induced activation of mTOR, since pharmacological inhibition of PLD effectively ablated activation of mTOR in response to contractions (O’Neil et al. 2009). Perhaps this represents at least one of the intrinsic mechanisms by which muscle can adapt independently of systemic or even locally derived membrane receptor-based signals.

In terms of generation of an endurance phenotype, perhaps the major signalling axis implicated in mitochondrial biogenesis is the 5’-AMP-activated protein kinase (AMPK)–peroxisome proliferator-activated receptor γ co-activator (PGC-1) pathway, probably activated by heightened AMP:ATP ratios due to high energy demands (and/or stress) associated with endurance (Atherton et al. 2005) or even unfamiliar activities (Coffey et al. 2006). Overexpression of PGC-1 promotes mitochondrial biogenesis (Viscomi et al. 2011), and activation of AMPK can both put the brakes on MPS and induce MPB via proteasomal and autophagy related mechanisms (Bolster et al. 2002).

This latter notion that the control of MPS and MPB is co-ordinately regulated via flux through the AMPK–AKT–mTOR ‘pathways’ is intriguing and it is speculated that the balance of these signals (governed by energetic and mechanical impositions) may to some degree determine adaptive specificity and perhaps capacity.

Conclusions and future work

As workers in the field, we tend to ‘pigeonhole’ exercise training regimens into ‘endurance’ activities composing prolonged low-intensity efforts (e.g. prolonged running and cycling), or ‘resistance’ activities (Kumar et al. 2009a) comprising high-intensity efforts (e.g. lifting weights). However, this classification belies the fact that there are exercise regimens that utilise both modalities. For example, high intensity training (HIT) involves very brief bouts of high-intensity, Wingate style contractions but primarily elicits an endurance-type adaptation as its main feature (Burgomaster et al. 2008). Moreover, for Joe Public at the gym and critically for elite athletes, the goal is often to perform cross-style training (also called concurrent training) in order to prepare for events requiring mixtures of strength, endurance and power, the contribution of each required varying according to the demands of the specific event(s). However, whether there exists a conflict between different training modes on a molecular, MPS or adaptive basis still remains largely to be defined.

Despite considerable advances in our biochemical understanding of ‘implicated signalling pathways’ we are a considerable way off understanding their involvement in adaptive specificity in man. For example, how do apparently similar changes in cellular signals regulate specific muscle fractions (mitochondrial, myofibrillar, etc.) in a manner according to the nature of the exercise? Indeed even comparison of exercise regimens providing adaptations at opposite ends of the spectrum (classic endurance vs. resistance) has failed to reach consensus on distinct regulatory signalling events. This is perhaps because responses are profoundly driven by training status (Coffey et al. 2006; Wilkinson et al. 2008; Vising et al. 2011), genetic heterogeneity (Timmons, 2011) and even technical limitations of poor temporal resolution from ‘snap-shot’ measures of phosphorylation. On the other hand we may have to face the prospect that seeking ‘master regulators’ such as AMPK, AKT and mTOR in humans is naive and that spreading our nets wider, i.e. to encompass genomic mRNA/miRNA measures, is necessary to truly understand the role of protein turnover in determining heterogeneity in adaptive specificity and capacity.

References


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**Acknowledgements**

P.J.A. is a designated Research Councils UK Fellow, supported by the Royal Society and Ajinomoto Inc. We also acknowledge the outstanding and lifelong contribution of Professor Michael J. Rennie PhD, FRSE (Emeritus Professor, University of Nottingham) to this field. We graciously apologise to colleagues whose work we could not include in this review due to space restrictions.
Regulation and limitations to fatty acid oxidation during exercise

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Abstract  Fatty acids (FAs) as fuel for energy utilization during exercise originate from different sources: FAs transported in the circulation either bound to albumin or as triacylglycerol (TG) carried by very low density lipoproteins and FAs from lipolysis of muscle TG stores. Despite a high rate of energy expenditure during high intensity exercise the total FA oxidation is suppressed to below that observed during moderate intensity exercise. Although this has been known for many years, the mechanisms behind this phenomenon are still not fully elucidated. A failure of adipose tissue to deliver sufficient FAs to exercising muscle has been proposed, but evidence is emerging that factors within the muscle might be of more importance. The high rate of glycolysis during high intensity exercise might be the ‘driving force’ via the increased production of acetyl-CoA, which in turn is trapped by carnitine. This will lead to decreased availability of free carnitine for long chain FA transport into mitochondria. This review summarizes our present view on how FA metabolism is regulated during exercise with a special focus on the limitations in FA oxidation in the transition from moderate to high intensity exercise in humans.

(Received 21 November 2011; accepted after revision 16 January 2012; first published online 23 January 2012)

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Abbreviations  FA, fatty acid  HSL, hormone sensitive lipase  IMTG, intramyocellular triacylglycerol  LPL, lipoprotein lipase  RER, respiratory exchange ratio  TG, triacylglycerol  VLDL, very low density lipoprotein.

Introduction

The work by Krogh and Lindhard and Christensen and Hansen in the 1920s and 1930s demonstrated, from measurements of the respiratory exchange ratio (RER), that fatty acid (FA) oxidation increased 5- to 10-fold above resting levels during mild to moderate exercise and peaked at exercise intensities around 65% of maximal oxygen uptake ($V_{\text{O}_2,\text{peak}}$) (Krogh & Lindhard, 1920; Christensen & Hansen, 1939). When exercise intensity increased further, FA oxidation progressively decreased. Today a remaining unsolved question is: What are the limitations and regulation of skeletal muscle FA oxidation at high exercise intensities?

This review is from the symposium Exercise metabolism at The Biomedical Basis of Elite Performance, a joint meeting of The Physiological Society and the British Pharmacological Society, together with The Journal of Physiology, Experimental Physiology, British Journal of Pharmacology and The Scandinavian Journal of Medicine and Science in Sports, at the Queen Elizabeth Hall, London on 20 March 2012.
Delivery of FA

Plasma FA to the working muscle is primarily supplied from lipolysis of triacylglycerol (TG) stored in adipose tissue. During low to moderate intensity exercise lipoprotein lipase (LPL) mediated hydrolysis of plasma TG also delivers a minor amount of FA to the total plasma FA concentration (Kiens et al. 1993; Morio et al. 2004; Sondergaard et al. 2011). The contribution of FA derived from plasma TG hydrolysis at higher exercise intensities remains, however, to be elucidated.

When whole body exercise was studied by the use of isotopically labelled long chain FAs in endurance trained men, the results confirmed the classical findings from the 1920s and 1930s that total FA oxidation was higher during moderate exercise at 65% of $V_{O_2}$-peak compared to exercise performed at 25% or 85% of $V_{O_2}$-peak (Romijn et al. 1993). Despite the relatively high rate of energy expenditure during exercise at high intensities, rate of disappearance ($R_d$) of plasma FAs and FA oxidation was decreased to values below those observed during moderate intensity exercise.

The inability of FA oxidation to support the energy demand during high intensity exercise could be reflected in either a failure of adipose tissue lipolysis, and thus insufficient delivery of FA to the exercising muscle, or a limitation in skeletal muscle to oxidize FAs. A failure in adipose tissue to supply the exercising muscle with sufficient FAs could be due to either a lack of stimulus to adipose tissue lipolysis or an inadequate perfusion of the adipose tissue. It has been shown that plasma catecholamine concentration, one of the major regulators of lipolysis in adipose tissue in humans, increases almost exponentially with exercise intensity (Galbo et al. 1975; Romijn et al. 1993). In the study by Romijn et al., glycerol rate of appearance ($R_g$), which was used to determine adipose tissue lipolysis (Romijn et al. 1993), was not reduced during whole body exercise at 85% of $V_{O_2}$-peak compared to exercise at 65% of $V_{O_2}$-peak (Romijn et al. 1993) implying that adipose tissue lipolysis was not reduced at the high exercise intensity. An important point here is that $R_g$ of glycerol reflects both adipose and muscle tissue lipolysis as well as LPL mediated hydrolysis of very low density lipoprotein (VLDL) bound TG. However, even though the net glycerol balance across skeletal muscle points towards glycerol release being substantial at rest (Stallknecht et al. 2004; Wallis et al. 2007), glycerol is both released and taken up by the leg resulting in low net release during exercise (van Hall et al. 2002; Stallknecht et al. 2004; Wallis et al. 2007). In a study performed by Stallknecht et al. (2004), the glycerol concentration in the interstitial space was measured using the microdialysis technique. Here it was demonstrated that the skeletal muscle interstitial glycerol concentration increased during low intensity exercise (25% of $V_{O_2}$-peak), indicative of a net release of glycerol from muscle. However, a net release of glycerol did not occur at moderate and high intensity one legged knee extensor exercise (Stallknecht et al. 2004). In contrast, subcutaneous adipose tissue interstitial glycerol concentration, which was ~10-fold higher than in skeletal muscle, increased with increasing intensities up to 85% of maximal leg work capacity (Stallknecht et al. 2004) supporting that the contribution from skeletal muscle to the arterial glycerol concentration during moderate and high intensity exercise is relatively small compared to that released from adipose tissue. At higher exercise intensities the high plasma catecholamine concentration can lead to inhibition of adipose tissue lipolysis by $\alpha$-adrenergic mechanisms (Frayn, 2010). Moreover, the high sympatho-adrenal response during whole body exercise can induce a reduction in adipose tissue blood flow (Bulow & Madsen, 1981). This might explain why a decrease in long chain FA $R_d$ from adipose tissue was observed during high intensity exercise compared to both low and moderate exercise (Romijn et al. 1993; van Loon et al. 2001). This coincided with a reduction in plasma FA concentration and oxidation. On the other hand, when plasma FA concentrations were increased to 2 mmol l$^{-1}$ by infusion of a lipid emulsion and heparin (increasing the activity of LPL in plasma and thus VLDL-TG hydrolysis) during high intensity exercise, FA oxidation increased only 27% compared to exercise at the same intensity without infusion of intralipid. Importantly, FA oxidation was only partially restored when compared to levels observed at 65% of $V_{O_2}$-peak even though the plasma concentration of FAs was above 2 mmol l$^{-1}$ (Romijn et al. 1995). These findings were further extended by van Loon et al. (2001), who reported a decrease in both plasma FA oxidation and total FA oxidation during high intensity exercise (72% $V_{O_2}$-peak) compared to moderate intensities at 44% and 55% of $V_{O_2}$-peak, despite no change in plasma FA availability. In addition, when whole body exercise was performed in healthy male volunteers the plasma concentration of FA decreased by 23% during high intensity workload (90% $V_{O_2}$-peak) compared to an exercise workload of 65% $V_{O_2}$-peak (Kiens et al. 1999). Concomitantly with the decrease in plasma FA concentration at the high exercise intensity, an accumulation of intramyocellular FA was observed (Kiens et al. 1999). Together these findings strongly indicate that limitations in FA oxidation at high exercise intensities are not due to failure of adipose tissue to deliver FAs and that the decrease in FA oxidation during high exercise intensity is due to limitations within the muscle cell. The decline in plasma FA concentrations at the very high exercise intensities may to be coupled with an inability of muscle to use the FA.
**Transsarcolemmal FA transport**

Within the past years several membrane bound lipid binding proteins have been identified in human skeletal muscle and increasing evidence is emerging that these proteins either individually or in complexes act as regulators of FA transmembrane transport (Fig. 1). However, the mechanism by which this occurs is unknown. The 43 kDa membrane bound fatty acid binding protein (FABPpm) and the 88 kDa fatty acid translocase CD36 (FAT/CD36) proteins are currently the best described lipid binding proteins in human skeletal muscle (for detailed review see Glatz et al. 2010). Recent studies have suggested a role for FAT/CD36 in the acute increase in FA uptake in skeletal muscle seen in the transition from rest to exercise (Bonen et al. 2000; Jeppesen et al. 2011). This idea of FAT/CD36 as a dynamic regulator of FA uptake originates from Bonen et al. (2000), who showed that [³H]palmitate transport into giant sarcolemmal vesicles (GSVs) was higher in GSVs from contracted rat muscle compared to resting muscle. Furthermore, this change was correlated with a contraction induced increase in membrane FAT/CD36 protein content (Bonen et al. 2000). In further support, it was shown that the contraction induced increase in FA oxidation was greater in isolated soleus muscle from transgenic mice over-expressing FAT/CD36 protein compared to their WT controls (Ibrahimi et al. 1999), even though resting FA oxidation was similar. This could indicate that a greater relocation of FAT/CD36 protein from an intracellular compartment to the plasma membrane during muscle contraction had occurred in the transgenic mice. In turn, this might have facilitated the higher flux in FA metabolism compared to WT mice.

The question is whether the transsarcolemmal transport is limiting for FA oxidation at higher exercise intensities. As mentioned above, an accumulation of intramyocellular FAs was observed in human vastus lateralis muscle when exercise intensity was increased from 65% \( \dot{V}_\text{O}_2\text{peak} \) to 90% \( \dot{V}_\text{O}_2\text{peak} \) despite a decrease in plasma FA concentration (Kiens et al. 1999), suggesting that the transport across the sarcolemma was not limiting FA oxidation at high exercise intensities. Data from studies in the perfused rat hindlimb model (Raney & Turcotte, 2006) have revealed a relation between FA uptake and oxidation, but only at low to moderate contraction intensities. When increasing to higher intensities, FA uptake was still elevated compared to basal levels, despite FA oxidation being decreased to resting values (Raney & Turcotte, 2006), supporting the
notion that the transport of FAs across the membrane is not a limiting factor for FA oxidation when switching to higher exercise intensities.

**Intramyocellular TG**

Intramyocellular triacylglycerol (IMTG) stored within striated muscle cells represents a large energy source, contributing to FA oxidation. To what extent IMTG is utilized during exercise varies depending on intensity, duration and mode of exercise, dietary status, pre-exercise IMTG levels, training status of the subjects and sex (for review see Kiens, 2006). When applying the $^{1}$H-MRS technique to male volunteers running at 60–70% of $V_{\text{O}_2\text{peak}}$, a decreased IMTG content in both the soleus and tibialis anterior muscles was observed, whereas running at 80–90% of $V_{\text{O}_2\text{peak}}$ did not cause changes in IMTG content in either muscle (Brechtl et al. 2001). Similarly, IMTG breakdown did not occur at high intensity exercise in the knee-extensor model (Stallknecht et al. 2004; Helge et al. 2007) when different methods for IMTG analysis were applied. However, these observations are all of net breakdown of IMTG. Recent findings from resting conditions in female and male subjects, using pulse–chase methods by intravenous infusions of two distinct isotopically labeled FAs combined with mass spectrometry measurements of intramuscular lipids, revealed that upon uptake by the muscle, plasma FA was not directly converted to long chain acylcarnitine (LCAC) and oxidized, but traversed the IMTG pool prior to oxidation (Kanaley et al. 2009). Whether FA taken up by muscle during exercise also undergoes esterification and then subsequent hydrolysis prior to mitochondrial entry is unknown.

Lipolysis of IMTG is regulated by adipose triglyceride lipase (ATGL), hormone sensitive lipase (HSL) and mono-glyceride lipase (MGL) (Fig. 1). Only a few studies have looked at intensity-dependent lipase activity in skeletal muscle. Watt et al. (2003) showed that HSL activity, when measured in male subjects at three different exercise intensities (30%, 60% and 90% of $V_{\text{O}_2\text{peak}}$), were increased in all trials and did not differ between exercise intensities. Furthermore, HSL activation was shown to increase in untrained subjects from rest to exercise at 70% of $V_{\text{O}_2\text{peak}}$ and remained unchanged when increasing exercise intensity to $\sim$90% of $V_{\text{O}_2\text{peak}}$ (Kjaer et al. 2000). These observations are supported by our own findings that HSL activity was activated by exercise even at low exercise intensities (30% of $V_{\text{O}_2\text{peak}}$) with no further increase in activity at 60 and 87% of maximal oxygen uptake (Kiens B. and Alsted TJ., unpublished data). Despite HSL being activated by exercise, no significant hydrolysis of IMTG was detected (Kiens B. and Alsted TJ., unpublished data). This study is not the first to demonstrate dissociation between HSL activation and IMTG hydrolysis. Watt et al. (2004) showed that reduced plasma FA availability during exercise, induced by nicotinic acid ingestion, increased IMTG hydrolysis despite no HSL activation. An explanation for a dissociation between lipase activity and IMTG breakdown could be that allosteric regulators of HSL (the influence of which is not measured in the *in vitro* assay) override the covalent regulation of HSL by phosphorylation of different serine residues (for review see Watt & Steinberg, 2008). Fatty acyl-CoA, an allosteric inhibitor of HSL, may inhibit the *in vitro* HSL activity especially during exercise at high intensity when intracellular accumulation of FAs has been shown to occur (Kiens et al. 1999). In the study by Watt et al. (2004), the decline in plasma FA concentration by nicotinic acid may have decreased the intramuscular fatty acyl-CoA concentration thereby relieving the allosteric inhibition of HSL and allowing for increased *in vivo* HSL activity. It is, however, remarkable that nicotinic acid in the study by Watt et al. (2004) on the one hand reduced lipolysis in adipose tissue and on the other increased lipolysis in skeletal muscle. These observations may give further support to the view that lipolysis is regulated differently in the two tissues (Watt & Steinberg, 2008). Although from these observations it seem unlikely that IMTG breakdown during high intensity exercise poses limitations for FA oxidation, this warrants further studies.

**Mitochondrial metabolism**

Long chain FAs taken up into cells are activated in the cytosol by reaction with CoA to yield long chain fatty acyl-CoA, an ATP consuming process catalysed by acyl-CoA synthetase (ACS) (Fig. 1). The active site of ACS has been located to the cytosolic surface of the peroxisomal endoplasmatic reticulum and outer mitochondrial membranes (Coleman et al. 2000). It was recently demonstrated in 3T3-L1 adipocytes that long chain ACS is an integral membrane protein also located in the plasma membrane (Gargiulo et al. 1999) and it was suggested that incoming long chain FAs are immediately esterified at the plasma membrane. This efficient esterification will maintain a low intracellular long chain FA concentration and contribute to uptake of long chain FAs.

Regulation of long chain FA entry into mitochondria is a highly regulated process, as acyl-CoA derivatives cannot cross the mitochondrial inner membrane directly. This is in contrast to short and medium chain FAs, which passively diffuse across the mitochondrial membranes. Long chain FAs first have to be converted to their acylcarnitine form, a reaction catalysed by carnitine palmitoyltransferase 1 (CPT-1) located at the outer mitochondrial membrane (Fig 2). Mitochondrial CPT-1 exist in two isoforms: the liver-type (L-CPT1) and muscle-type (M-CPT1). In skeletal muscle the M-CPT1...
is isoform is predominant (McGarry & Brown, 1997). The importance of CPT-1 in long chain FA oxidation was demonstrated when CPT-1 function was blocked by etomoxir resulting in a marked decrease in FA oxidation both in vivo and ex vivo (Hubinger et al. 1992; Dzamko et al. 2008). In addition, when the human muscle isoform of CPT-1 protein was electroporated into skeletal muscle of rats, an increase in maximal CPT-1 activity of \( \approx 30\% \) was paralleled by an increase of \( \approx 24\% \) in paminoyl-CoA oxidation in isolated muscle mitochondria (Bruce et al. 2007). Earlier findings demonstrated that CPT-1 was potently regulated by malonyl-CoA (Bird & Saggerson, 1984) and a close relationship between malonyl-CoA concentration in muscle and decreased FA oxidation was observed in both humans and rats under resting conditions (Bavenholm et al. 2000; Chien et al. 2000). The formation of malonyl-CoA from acetyl-CoA in skeletal muscle is catalysed by acetyl-CoA carboxylase (ACC). One type of regulation of ACC involves phosphorylation and inactivation by 5'-AMP-activated protein kinase (AMPK). During exercise AMPK and ACC phosphorylation are increased, which results in AMPK activation and in turn ACC inactivation (Richter & Ruderman, 2009). This will hypothetically lead to decreased muscle malonyl-CoA content during exercise and, in turn, increased CPT-1 activation, resulting in increased long chain FA oxidation. However, FA oxidation measured at rest and during isolated muscle contractions (Dzamko et al. 2008), and during whole body exercise (Dzamko et al. 2008; Miura et al. 2009) was similar in mice with a genetically reduced AMPKα2 activity as in wild-type (WT) mice. These findings were supported by O’Neill et al. (2011), who demonstrated that FA oxidation during exercise, evaluated by RER, was higher in mice with abolished AMPK activity (muscle specific \( \beta_1 \) and \( \beta_2 \) double knockout (KO)) compared to WT mice, indicating that AMPK is not a major regulator of FA oxidation during exercise in skeletal muscle. Less genetic evidence is available on the role of ACC2, the main isoform of ACC in muscle, in regulation of FA oxidation. But ACC2 deletion in mice did not affect malonyl-CoA content in muscle or RER under resting condition (Choi et al. 2007; Olson et al. 2010), indicating that either overcompensation by ACC1 had occurred or other mechanisms were responsible for regulating FA oxidation in these mice. The relationship between malonyl-CoA and FA oxidation observed at rest is less

![Figure 2. Schematic overview of a proposed interaction between fatty acid metabolism and glycolysis in skeletal muscle during high intensity exercise](image)

At high exercise intensity the high glycolytic rate will cause a production of acetyl CoA which exceeds the rate of the Krebs cycle. Free carnitine acts as an acceptor of the acetyl groups forming acetyl carnitine, mediated by the enzyme carnitine acetyltransferase. This leaves less free carnitine, substrate for CPT-1, whereby forming of acylcarnitine will be reduced and less FA-acyl will be available for \( \beta \)-oxidation resulting in reduced FA oxidation. OMM, outer mitochondrial membrane; IMM, inner mitochondrial membrane; CPT-1, carnitine palmitoyltransferase; FA, fatty acids: CPT II, carnitine palmitoyltransferase II; PDC, pyruvate dehydrogenase complex; CAT, carnitine acyltransferase.

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clear during exercise in humans. In a series of experiments a discrepancy between malonyl-CoA concentrations and FA uptake and FA oxidation in human subjects during exercise has been demonstrated (Odland et al. 1996, 1998; Dean et al. 2000; Roepstorff et al. 2005). When whole body exercise intensity was increased from 65% to 90% of $V_{\text{O}_2,\text{peak}}$ in male subjects, muscle malonyl-CoA content did not change, despite FA oxidation, determined by RER, being markedly decreased (Odland et al. 1998). This notion was supported by Dean et al. (2000), who showed that increasing knee extensor exercise intensity from 60% to 85% of leg work capacity and further until exhaustion was accompanied by a reduction in muscle malonyl-CoA content, despite RER values concomitantly increasing from 0.84 to 0.99. More recent findings, where pre-exercise muscle glycogen levels were manipulated to induced either high or low FA oxidation during exercise at 65% of $\dot{\text{V}}_{\text{O}_2}$ (Roepstorff et al. 2005), showed marked differences in FA oxidation during exercise without differences in muscle malonyl-CoA content. Taken together, this suggests that malonyl-CoA content is not the major regulator of FA oxidation in working muscle. It should be noted that it is unknown whether local changes in malonyl-CoA concentration in compartments close to mitochondria within the muscle, rather than whole muscle content, have effects on FA oxidation.

Besides the effect of carnitine in mediating FA entry into mitochondria, studies in the 1950s on blowfly muscle revealed that carnitine also serves another important metabolic role. The flight muscle from flies is one of the richest sources of carnitine and at the same time these insects do not oxidize FAs when in flight (Childress et al. 1967). When the blowfly flight muscle was studied under flight the concentration of acetyl carnitine increased 4-fold on initiation of flight, which paralleled the increase in pyruvate concentration (Childress et al. 1967). From these studies it was proposed that carnitine could act as an acceptor of acetyl groups from acetyl-CoA, by forming acetylcarnitine, a reaction catalysed by the mitochondrial enzyme carnitine acetyltransferase (CAT), when acetyl-CoA was generated faster than utilized by the Krebs cycle. In this way, CoASH can be regenerated permitting glycolysis to proceed to acetyl-CoA. These early findings were later supported by findings in both animal and human skeletal muscle. Indeed, it has been shown in several studies in humans that with increasing exercise intensities, muscle acetyl carnitine content was increased (Sahlin, 1990; Constantin-Teodosiu et al. 1991; Odland et al. 1998; van Loon et al. 2001) concomitantly with a decrease in the free carnitine content (Sahlin, 1990; Constantin-Teodosiu et al. 1991; van Loon et al. 2001). In the review from Stephens et al. (2007), compiled results from four different studies showed that a short bout of exercise (4 min) at different exercise intensities was followed by a gradual decrease in the vastus lateralis muscle free carnitine content from ~75% of the total muscle carnitine pool at rest to ~20% at 75–100% $V_{\text{O}_2,\text{peak}}$. In addition, data revealed that acetylcarnitine content accounted for the decrease in free carnitine with high intensity exercise. On the other hand, at low exercise intensities neither free carnitine nor acetyl-carnitine content was changed compared to resting values (Stephens et al. 2007). These findings further support the notion that carnitine acts as the acceptor for the acetyl groups, by forming acetylcarnitine, when the rate of acetyl-CoA formation from glycolysis at high intensities is in excess of its utilization by the Krebs cycle. On the other hand, since CPT-1 activity is dependent on the presence of carnitine (McGarry et al. 1983; Harris et al. 1987), a low muscle content of free carnitine is supposed to reduce the activity of CPT-1. Consequently this will lead to a diminished supply of long chain FA CoA to β-oxidation, limiting long chain FA oxidation during high intensity exercise. The importance for carnitine in long chain FA oxidation in skeletal muscle is evident from the findings of an 85% reduced carnitine content and a 75% reduced FA oxidation in skeletal muscle of patients with lipid storage myophathy compared with healthy controls, despite similar levels of CPT-1 and palmitoyl thio kinase in patients and control subjects (Engel & Angelini, 1973). Thus, an increased availability of pyruvate, acetyl-CoA formation, and ‘binding’ of the free carnitine during high intensity exercise also provide a potential mechanism whereby FA oxidation is down-regulated (Fig. 2). On the other hand, $K_m$ of CPT-1 for carnitine in isolated mitochondria from human skeletal muscle is ~0.5 mM (McGarry et al. 1983). Thus, with the usual fluctuations in carnitine content in skeletal muscle of healthy humans between 1 and 4 mM it is not expected to influence CPT-1 activity as CPT-1 would be saturated with carnitine at all exercise intensities. However, partitioning of carnitine between the cytosol and the mitochondrial matrix makes it difficult to estimate the absolute carnitine concentration near CPT-1 and furthermore extrapolation of in vitro enzyme kinetics to in vivo conditions is fraught with assumptions making it difficult to judge the relevance of such measures.

Recently we have shown (Roepstorff et al. 2005) that when pre-exercise muscle glycogen stores were high, FA oxidation was reduced by 2.5-fold during 60 min of moderate intensity exercise (65% $V_{\text{O}_2,\text{peak}}$) compared to when pre-exercise glycogen levels were low. This was paralleled by low free carnitine levels in muscle during the high glycogen trial whereas the free carnitine content was high during the low glycogen trial (Roepstorff et al. 2005). These findings give support to the notion that a reduction in cellular free carnitine will limit the ability of CPT-1 to transport long chain FAs into the mitochondria, and thus also the rate of long chain FA oxidation at moderate exercise intensities.
In a recent study by Wall et al. (2011), 14 healthy male volunteers were given carnitine supplementation (together with carbohydrates) for 24 weeks resulting in an increase in muscle total carnitine by 21%. This increase in total carnitine content was linked to a 55% reduction in muscle glycogen utilisation during exercise at 50% \( V_{\text{O}_2,\text{peak}} \) compared with controls not supplemented with carnitine. In addition, the study revealed an 80% greater muscle free carnitine content and a 31% lower activity in the pyruvate dehydrogenase complex (PDC) during exercise after carnitine supplementation compared to control. This suggests an increased FA oxidation during exercise at 50% \( V_{\text{O}_2,\text{peak}} \), but this was unfortunately not measured in the study. When exercise was subsequently increased to 80% \( V_{\text{O}_2,\text{peak}} \), no differences were obtained between the groups in glycogen utilisation, but muscle lactate content was \( \sim \)44% lower in the carnitine supplemented trial than in the control trial (Wall et al. 2011). These findings indicate that at high intensities the formation of acetyl-CoA, probably mostly generated from a high glycolytic flux, is captured by carnitine and thereby prevents a product inhibition of PDC activation, by an increased acetyl-CoA/CoASH ratio (Cooper et al. 1975). Support for this are their findings (Wall et al. 2011) of a greater activity in PDC (38%) and a greater acetyl carnitine content (16%) in skeletal muscle during exercise at 80% \( V_{\text{O}_2,\text{max}} \) in the carnitine supplemented trial than in control. As RER or other measurements of FA oxidation were not performed in the study by Wall et al., it is not possible from these findings to evaluate the influence of carnitine supplementation on FA oxidation either at the moderate or at the high exercise intensities. Thus, it cannot be ruled out that high availability of carnitine might increase FA oxidation during high intensity exercise as well.

A clue to understanding the regulation of FA oxidation during high intensity exercise may be obtained from comparison of metabolism during whole body exercise to exercise with a limited muscle mass like the knee-extensors. Whereas it is well established that FA oxidation during exercise decreases at exercise intensities above \( \sim \)65% of \( V_{\text{O}_2,\text{peak}} \), as discussed above, different results are obtained with one-legged knee-extensor exercise (Dean et al. 2000; Helge et al. 2007). Thus, when exercise was allocated to the knee-extensors, plasma FA oxidation, measured by constant infusion of \([U-^{13}\text{C}]\) palmitate, increased with increasing exercise intensities from 25% up to 85% of maximal leg work capacity (Helge et al. 2007). Furthermore, total FA oxidation increased from rest to exercise and remained unchanged during increasing exercise intensities (Helge et al. 2007). In addition, Dean et al. (2000) showed (by measuring RER) that FA oxidation was unchanged from 65 to 85% of knee-extensor maximum work capacity but decreased by 34% compared to at 85% when exercise intensity was increased to 100%. Thus when performing exercise with a limited muscle mass it appears that muscle is able to oxidize FA at much higher relative exercise intensities than during bicycle ergometer exercise when more and large muscle groups are involved. How is this explained?

While this cannot be answered conclusively, we offer the following hypothesis. During exercise with a limited muscle mass at 80% of peak leg work capacity there is hardly any increase in plasma catacholamine concentrations compared to rest (Richter et al. 1988), whereas substantial increases are observed when heavy exercise is performed with more muscle mass (Galbo et al. 1975). The low hormonal response during knee-extensor exercise may limit glycogen breakdown (Richter et al. 1982), and thus glycolytic flux, compared to heavy whole body exercise and therefore limit the production of acetylarnitine. In consequence free carnitine availability and therefore CPT-1 activity may be preserved better than during whole body exercise. In addition, when performing one-legged exercise, muscle blood flow is excessive compared to flow during whole body exercise (Saltin 1985) and this ‘superperfusion’ is likely to create conditions in the muscle that favour oxidative ATP production, and thus limit increases in ADP and AMP. This lesser disturbance in energy status of the cell will in turn cause less stimulation of glycolysis. As mentioned above, this again preserves free carnitine in the muscle and therefore creates favourable conditions for FA oxidation. This could explain why FA oxidation is maintained at higher exercise intensities during knee-extensor exercise compared with whole body exercise. In fact the metabolic conditions in the muscle during one-legged exercise may resemble conditions after endurance training where better metabolic control is achieved and decreased glycolytic flux leads to increased FA oxidation at the same absolute work load (Holloszy & Coyle, 1984).

**Conclusion**

FA oxidation during exercise is subject to multiple possible regulatory steps, ranging from adipose tissue lipolysis to mitochondrial metabolism in skeletal muscle. However, when focusing on limitations of FA oxidation in the transition from moderate to higher intensity exercise, one possibility could be product inhibition from the \( \beta \)-oxidation pathway, but evidence for this is not substantial. It seems that the most attractive regulatory candidate for FA oxidation is the muscle metabolite carnitine, which is essential in CPT-1 regulation and, in turn, FA oxidation. At high intensity exercise the rapid glycolysis provides the mitochondria with excess acetyl-CoA, which is buffered by free carnitine to form acetylarnitine. Accordingly a fall in muscle concentration of free carnitine may reduce CPT-1 activity, and thus the
ability to transport FA into the mitochondria and therefore also the rate of FA oxidation. In this way, rapid glycolgen breakdown and glycolysis are suggested to have a major impact on inhibiting FA oxidation. The absence of any other rigorously identified mechanisms for decreasing FA oxidation during high intensity exercise makes us believe that carnitine is the major direct regulator of FA oxidation in the transition from moderate to higher intensity exercise.

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Acknowledgements

We acknowledge the skilled technical assistance of Irene B. Nielsen and Betina Bolmgren. The financial support from The Integrated Project Grant LSHM-CT-2004-005272 funded by the European Commission, the Danish Agency of Science, Technology and Innovation and the Ministry of Food, Agriculture and Fisheries, and an integrated Project Funded by the European Union (no. LSHM-CT-2004-005272) are acknowledged. As well are the financial support from The Novo Nordisk Foundation and, The Lundbeck Research Foundation and the Danish Medical Research Council. This work was carried out as a part of the research program of the UNIK: Food, Fitness & Pharma for Health and Disease (see www.foodfitnesspharma.ku.dk). The UNIK project is supported by the Danish Ministry of Science, Technology and Innovation.
Regulation of glucose and glycogen metabolism during and after exercise

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Abstract
Utilization of carbohydrate in the form of intramuscular glycogen stores and glucose delivered from plasma becomes an increasingly important energy substrate to the working muscle with increasing exercise intensity. This review gives an update on the molecular signals by which glucose transport is increased in the contracting muscle followed by a discussion of glycogen mobilization and synthesis by the action of glycogen phosphorylase and glycogen synthase, respectively. Finally, this review deals with the signalling relaying the well-described increased sensitivity of glucose transport to insulin in the post-exercise period which can result in an overshoot of intramuscular glycogen resynthesis post exercise (glycogen supercompensation).

(Received 21 November 2011; accepted after revision 21 December 2011; first published online 23 December 2011)

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Introduction
Carbohydrate in the form of glucose and intramuscular glycogen becomes an increasingly important energy substrate with rising exercise intensity (Holloszy & Kohrt, 1996). Carbohydrate oxidation accounts for 10–15% of total energy production during low intensity aerobic exercise (~30% $V_{O_{max}}$), increasing progressively to roughly 70–80% of total energy during exercise of about 85% $V_{O_{max}}$ to about 100% of energy consumption at exercise intensities of 100% of $V_{O_{max}}$ and above (Romijn et al. 1993; Holloszy & Kohrt, 1996). There are two sources of glucose molecules available to the working muscle; plasma glucose and muscle glycogen. While very little net glycogen breakdown is observed at low-intensity exercise, glycogen-breakdown becomes the predominant glucose source at higher intensities (Hargreaves & Richter, 1988). In terms of athletic performance, low muscle glycogen deposits seem detrimental to both high and moderate intensity exercise performance (Hargreaves & Richter, 1988). This has resulted in the widespread practice of high-carbohydrate diet regimens to increase pre-exercise glycogen levels (carbohydrate loading) (Hargreaves & Richter, 1988). In this review we discuss the current thinking on the molecular signals that acutely control glucose uptake and glycogen use by the working muscle. Then we discuss the mechanisms by which skeletal muscle may accomplish an increase in glycogen stores above pre-exercise levels, focusing on the mechanisms enhancing insulin-stimulated glucose uptake post-exercise.
Glucose metabolism during exercise-regulation of glucose transport

Glucose delivery to the working muscle is increased by a marked increase in capillary perfusion during exercise as originally described by August Krogh in frog muscle and recently confirmed by real time contrast enhanced ultrasound in humans (Sjoberg et al. 2011). Another way to increase delivery is to increase plasma glucose concentrations by ingestion of carbohydrate rich meals or drinks. The magnitude of increase depends on the type and quantity of carbohydrates and the reader is referred to other reviews for discussion of how to optimize carbohydrate availability during exercise (Hawley et al. 2011). At the fibre level, it is still debated whether the rate-limiting step in vivo is GLUT4-dependent transport across the plasma membrane or intracellular phosphorylation by hexokinase II. However, increased recruitment of GLUT4 from intracellular vesicular structures to the cell surface during acute muscle contraction/exercise is a well-described acute adaptation in both rodents and humans (for refs see Jessen & Goodyear, 2005; Rose & Richter, 2005) and a necessary contributor to increased skeletal muscle glucose uptake in exercising muscle since in mouse muscles where GLUT4 has been genetically ablated, contraction-induced glucose uptake is abrogated (Zisman et al. 2000). In addition, a contribution from an increase in GLUT4 intrinsic activity, which is clearly dissociable from GLUT4 translocation in some studies, cannot be discounted (Klip, 2009) although effects of exercise on GLUT4 intrinsic activity have not been rigorously demonstrated.

Overall, the GLUT4-translocation response to contraction has been proposed to involve feed-forward activation by sarcoplasmic reticulum (SR) Ca\(^{2+}\) release with subsequent fine-tuning by changes secondary to contraction (e.g. mechanical stretch, metabolism, redox-state). The feed forward proposition is supported by ex vivo rat muscle studies where caffeine-stimulated Ca\(^{2+}\) release from the SR was sufficient to elicit an increase in glucose transport in the absence of measurable increases in force development, nucleotide-status or activation of the AMP/ATP and ADP/ATP-sensitive AMP-activated protein kinase (AMPK) (Wright et al. 2004). However, whereas the original studies did not find changes in energy status or AMPK activation by sub-contraction threshold Ca\(^{2+}\) release, more recent studies have reported nucleotide-changes and AMPK activation using similar Ca\(^{2+}\) concentrations (Jensen et al. 2007; Raney & Turcotte, 2008; Egawa et al. 2009), questioning the usefulness of the caffeine-approach to isolate the Ca\(^{2+}\) response independently of energy turnover and other contraction-activated events. Furthermore, an old observation is that the glucose uptake response correlates excellently with the intensity of muscular work during both human exercise and in more reductionistic rodent muscle contraction models (Rose & Richter, 2005). Of particular interest, Ihlemann and coworkers, by adjusting the length of ex vivo stimulated rat muscles and as a consequence force production and metabolic stress, demonstrated that the glucose transport response correlates with the degree of tension development rather than stimulation frequency (Ihlemann et al. 2000). These studies were recently followed up by another approach where pharmacological inhibition of fast-twitch myosin II-dependent crossbridge cycling partially reduced electrically stimulated glucose transport in rat epitrochlearis muscle (Blair et al. 2009). Using a lower intensity tetanic stimulation protocol to minimize energy turnover by e.g. SERCA-dependent Ca\(^{2+}\) reuptake, we have data showing that the increase in glucose transport by electrical stimulation of mouse muscles ex vivo is fully prevented by myosin II inhibition despite normal Ca\(^{2+}\) activated phosphorylation events (T. E. Jensen, E. A. Richter, unpublished data). This suggests that, while some Ca\(^{2+}\) activated proteins provide necessary signals for contraction-stimulated glucose transport (Rose & Richter, 2005), Ca\(^{2+}\) per se is probably not sufficient to increase muscle glucose transport.

Based on experiments using the AMP-mimetic aminooimidazole carboxamide ribonucleotide (AICAR), activation of AMPK appears sufficient to cause a partial increase in glucose transport in rodent fast-twitch muscle. In contrast, this response is lower in the mixed type I and II fibre mouse soleus and often absent in the type I fibre-dominated rat soleus despite activation of AMPK (Jorgensen et al. 2004; Wright et al. 2005). This does not seem to relate to differential expression of potential downstream mediators of GLUT4 translocation such as TBC1D1 and TBC1D4/AS160 in the rat (Castorena et al. 2011) but may relate to differential expression of AMPK \(\beta\) and \(\gamma\) subunits in different rodent muscles (Treebak et al. 2009). In humans, despite a lack of measurable changes in total AMPK phosphorylation early on during intense exercise, the \(\alpha2\beta2\gamma3\) containing subset of AMPK complexes are rapidly activated with exercise consistent with a role in promoting glucose transport (Birk & Wojtaszewski, 2006). A necessary role of AMPK for contraction-stimulated glucose transport is more controversial, with some studies reporting decreased glucose transport in AMPK deficient mouse models and others not, probably due to redundancy of signalling, differential contraction protocols, and transgenic manipulation strategies (Rose & Richter, 2005). Recently, conditional muscle-specific knockout of both \(\beta\)-AMPK regulatory subunits abolished AMPK activity and potently inhibited exercise-stimulated glucose uptake in vivo and contraction-stimulated glucose transport ex vivo (O’Neill et al. 2011). In parallel to AMPK, proposed to act through TBC1D1/4 (Cartee & Wojtaszewski, 2007;
Cartee & Funai (2009) and eNOS (Lee-Young et al. 2009), a number of other pathways have been proposed to signal to increase glucose transport and may include LKB1 signalling through the AMPK-related kinase SNARK (Koh et al. 2010), and stretch-activated p38 MAPK (Chambers et al. 2009). In relation to the latter, it is, however, worth mentioning that at least one of the p38 MAPK inhibitors used by Chambers and co-workers, SB203580, has been shown interact with and inhibit GLUT4 directly (Antonescu et al. 2005; Ribe et al. 2005). Teasing out how AMPK and other signals blend to elicit a given level of increase in glucose transport in different muscle fibre types during exercise remains a challenging subject for future study.

**Regulation of glycogen breakdown**

Glycogenolysis is regulated by glycogen phosphorylase (GP), acting on the terminal α-1,4-glycosidic linked glucose residues, and debranching enzyme, targeting the α-1,6-branchpoints in the glycogen molecule (Roach, 2002). Most studies have focused on the regulation of GP, the activity of which is increased by allosteric binding of AMP or IMP and competed by ATP or glucose-6-phosphate (G-6-P). In addition, since GP requires inorganic phosphate to produce glucose-1-phosphate from glycogen, inorganic phosphate from ATP and creatine phosphate (CrP)-turnover has been speculated to limit GP activity at the substrate level (for refs see Hargreaves & Richter, 1988). Finally, high initial muscle glycogen concentration clearly augments net glycogen breakdown during contractions likely due to activation of GP by glycogen (Hespel & Richter, 1992). Apart from its allosteric and presumably substrate-level regulation, phosphorylation of GP on Ser14 by phosphorylase kinase (PK) increases the activity of GP measured in vitro. Classically, PK is thought to integrate local and systemic signals to promote glycogen breakdown by being activated initially by Ca2+ binding to the PK δ-subunit (identical to calmodulin), and then plasma adrenaline acting though a β2-adrenergic receptor–adenylate cyclase–PKA cascade (Hargreaves & Richter, 1988). In the absence of adrenaline stimulation, GP activity measured in vitro (reflecting its phosphorylation state) increases rapidly at the onset of contraction and then reverts towards resting activity within a few minutes despite continued contraction and therefore presence of Ca2+ transients (Richter et al. 1982). This is probably a result of dephosphorylation at Ser14 following the initial activation by Ca2+ although, to our knowledge, the mechanism behind this has not been studied in detail. With regards to the adrenergic stimulation of glycogenolysis, it is also worth noting that adrenaline-stimulated glycogen breakdown in incubated rat muscles is potently inhibited by the sodium–potassium pump inhibitor ouabain, suggesting a link to adrenaline-stimulated sodium-potassium pump activity (James et al. 1999). Whether this connection relates to local changes in e.g. nucleotides or K+ is not clear. In humans, the evidence for adrenergic stimulation of glycogenolysis is not clear-cut, with some studies reporting increased glycogen use and GP activation with adrenaline infusion, while others do not (see e.g. Kjaer et al. 2000; Watt et al. 2001 and refs therein). As discussed by Watt and coworkers (2001), this may relate in part to the intensity of exercise, with allosteric regulation of GP playing a larger regulatory role with increasing intensity.

Within a given fibre, glycogen particles have been proposed to be present in at least three distinct subcellular locations, with ~80% between the myofibrils in close vicinity to the SR and mitochondria and two smaller compartments located within the myofibrils and underneath the sarcolemma contributing ~10% each (Nielsen et al. 2011; Prats et al. 2011). The detailed roles of these different glycogen compartments to muscle contraction–metabolism remain to be uncovered but the various glycogen pools are differentially depleted and supercompensated by different kinds of exercise and training (for refs and discussion, see Prats et al. 2009; Nielsen et al. 2011). In relation to fatigue, the emptying of intramyofibrillar glycogen correlates somewhat with lower SR Ca2+ release by 4-chloro-m-cresol in vitro ($r^2 = 0.23$) (for ref see Nielsen et al. 2011), suggesting a potential contribution to the unexplained relation between fatigue and low glycogen. It would be interesting to examine if e.g. most of the ~20% depletion of muscle glycogen with 30 s of all-out bicycle sprint exercise (Birk & Wojtaszewski, 2006) preferentially stems from intramyofibrillar glycogen. The regulation of glucose transport and glycogen turnover in working muscle is summarized in Fig. 1.

**Regulation of glycogen synthesis**

Glycogen synthase (GS) catalyses the rate-limiting incorporation of UDP-glucose via α-1,4-glycosidic linkages into the growing glycogen polymer, with branching enzyme catalysing formation of α-1,6-branchpoints (Roach, 2002). Counterintuitively, this UTP-requiring anabolic glycogen synthase is not only stimulated by insulin but also by exercise although unchanged or inhibited GS activity at high intensity has been described (for refs see Nielsen & Wojtaszewski, 2004). In stark contrast to GP regulation, the regulation of GS by post-translational modifications is quite complex, with at least nine phosphorylation sites targeted by multiple kinases (Nielsen & Wojtaszewski, 2004; Jensen & Lai, 2009). The dephosphorylated state of GS, in particular
at sites 2, 2a, 3a and 3b, increases GS activity in vitro. This dephosphorylation is catalysed by protein phosphatase 1 (PP1), which is also the phosphatase for GP and PK. At least one glycogen-binding protein, G M, targets PP1 to glycogen and has been shown in mice to be required for exercise-stimulated GS activation (Aschenbach et al. 2001). It is tempting to speculate that the co-localization between PP1–G M and glycogen regulatory enzymes like GS is linked to the well described inverse correlation between in vitro GS activity and glycogen content in muscle (Danforth, 1965; Nielsen et al. 2001). Worth mentioning, PP1 is also known to be regulated by phosphorylation of endogenous inhibitors of PP1 like inhibitor-1 and -2, both of which are expressed in skeletal muscle (for refs see Nicolaou et al. 2009). In addition, GS activity shows a partial resistance to phosphatase treatment in vitro, suggesting that other described covalent modifications of GS such as glycolysation (Parker et al. 2003) may contribute to GS regulation in skeletal muscle. In vivo, allosteric activation by G-6-P is probably an all-important point of regulation. This is evidenced by recent data in mice where muscle-specific replacement of wild-type GS with a G-6-P insensitive mutant GS protein potently reduced insulin and prevents AICAR-stimulated glycogen synthesis (Bouskila et al. 2010; Hunter et al. 2011), suggesting that sensing of G-6-P from transported glucose is required for most of the stimulation of glycogen synthesis. Interestingly, the stimulatory effect of AICAR on glycogen synthesis occurred despite the fact that direct AMPK-dependent phosphorylation of GS at sites 2+2a causes a moderate reduction of GS activity in vitro (Jørgensen et al. 2004), arguing that allosteric regulation can override covalent regulation, at least on sites 2+2a.

In relation to the recently re-emphasized distinct subcellular depots of muscle glycogen, GS seems located to different compartments depending on its phosphorylation state. Hence, GS phosphorylation on site 1b, presumably by adrenaline-activated PKA, is located intramyofibrillarly following roughly 2 h of exhaustive human knee-extension exercise, while GS phosphorylation on the AMPK sites 2+2a is located with subsarcolemmal and intermyofibrillar glycogen depots (Prats et al. 2009). The details of how GS re-localizes to these compartments are not clear but have been suggested to depend on the actin cytoskeleton in as much as GS and GP assemble with β-actin, but not γ-actin, into spherical structures after glycogen-depleting electrical rabbit tibialis anterior muscle stimulation (Prats et al. 2005).

Figure 1. Glucose utilization in the working muscle is increased through increased delivery and uptake of plasma glucose and increased glycogenolysis
Transport of glucose across the sarcolemma and T-tubular membranes is determined by the amount of contraction- and insulin-responsive glucose transporter 4 (GLUT4) proteins in the outer membrane. This magnitude of glucose transport response with contraction correlates with work intensity with evidence suggesting the involvement of kinases like AMPK, p38 MAPK and SNARK whereas Ca^{2+} activated proteins are probably required but likely to be insufficient to stimulate glucose transport. Allosteric and covalent regulation increases both glycogen mobilization by glycogen phosphorylase (GP) and resynthesis by glycogen synthase (GS) simultaneously during exercise by altering enzyme activity and/or location. GP may also be regulated by the availability of its substrates glycogen and inorganic phosphate (P_i). Depending on the work intensity and duration, glucose-6-phosphate (G-6-P), an important allosteric inhibitor of GP and stimulator of GS, may increase.
Glycogen resynthesis post-exercise – the role of increased insulin-stimulated glucose uptake

Mechanistically, while increased microvascular recruitment may play a role in insulin sensitization in vivo by increasing glucose delivery (for refs see Wojtaszewski & Richter, 2006), prior contraction also sensitizes glucose transport and GLUT4 translocation ex vivo independently of the capillary network (Fisher et al. 2002; Geiger et al. 2005), suggesting that part of insulin sensitization by prior exercise stems from an effect on GLUT4-mediated glucose transport. It is worth noting, however, that if exercise involves muscle damaging eccentric components, then insulin sensitivity may in fact be decreased in the days after exercise due to decreased GLUT4 expression and impaired insulin signalling (for refs see Maarbjerg et al. 2011).

What might be the molecular mechanisms behind the exercise effect on insulin-stimulated glucose transport when no muscle damage is induced? One study has shown that AMPK activation in incubated rat epitrochlearis by AICAR or hypoxia, in conjunction with one or more unknown serum proteins >10 kDa (Gao et al. 1994), can increase submaximal insulin-stimulated glucose transport ex vivo 3.5 h after removal of the AICAR stimulus (Fisher et al. 2002). Importantly, this occurred without a measurable potentiation of proximal steps of insulin signalling like PI3K activity and Akt phosphorylation, consistent with previous observations in humans (Wojtaszewski & Richter, 2006). This effect could speculatively be relayed by downstream phosphorylation of TBC1D4, an emerging regulator of GLUT4 trafficking, which shows an increase lasting many hours post-exercise at certain residues including known AMPK sites in rats and humans (Sakamoto & Holman, 2008; Maarbjerg et al. 2011). Low muscle glycogen content correlates with high AMPK activity (Jorgensen et al. 2004) and glycogen has been shown to directly bind and inactivate AMPK through the carbohydrate-binding domain of the AMPK β-subunit (McBride et al. 2009). This makes it tempting to speculate about a connection between the release of AMPK from glycogen during exercise and the ensuing increase in insulin sensitivity. Supporting a regulatory role of glycogen is the finding that the increased post-exercise insulin sensitivity correlates significantly with the amount of glycogen broken down during the preceding exercise bout ($r^2 = 0.53$; Richter et al. 2001). However, if a serum-factor is required for contraction to cause insulin sensitization ex vivo (Gao et al. 1994) but not for contraction-stimulated glycogen breakdown, then the relationship between contraction-stimulated glycogen use and insulin sensitivity is probably non-causal.

Both exercise and the protein synthesis inhibitor anisomycin acutely increased p38 MAPK activation in incubated rat soleus and epitrochlearis and increased submaximal insulin-stimulated glucose transport 3 h after...
cessation of either stimulus (Geiger et al. 2005). The effect of anisomycin was prevented by the selective p38 MAPK inhibitor SB202190, whereas the exercise-effect was not, suggesting that contraction could utilize redundant signalling pathways to increase insulin sensitivity after exercise. Interestingly, resting p38 MAPK phosphorylation 3 h post-exercise has been observed to remain 50% higher in previously exercised leg muscles compared to controls (Thong et al. 2003).

Studies in incubated rat muscles have indicated that any glucose transport-increasing stimulus including insulin itself may enhance insulin-stimulated glucose transport some hours after stimulation, possibly by re-location of GLUT4 to a more easily recruitable pool, thereby allowing a larger GLUT4 mobilization by an unaltered insulin signal (Geiger et al. 2006). Worth mentioning, this attractive hypothesis is not supported by a recent study showing no insulin-sensitizing effect of doing sequential insulin clamps in humans (Lucidi et al. 2010). However, muscle insulin sensitization of prior stimulation may have been overridden by the anti-hyperglycaemic hormonal and lipolytic responses that are activated in the period between the two clamps. A direct examination of the hypothesized altered location of insulin-responsive GLUT4 pools by insulin-sensitizing stimuli in rat and human muscle will be needed to directly test this hypothesis. The effects of prior exercise on insulin-stimulated glucose transport and glycogen resynthesis are recapitulated in Fig. 2.

Conclusion

Carbohydrates in the form of plasma glucose and muscle glycogen are important fuels during exercise. The increase in muscle glucose uptake during exercise is dependent upon the delivery of glucose (capillary perfusion and plasma glucose concentration) and the permeability of the muscle membrane to glucose. The latter is regulated by a plethora of molecular signalling thought to include calcium, stretch and energy stress signalling and probably others. Muscle glycogen is utilized as a function of exercise intensity and duration and is controlled by the activity of the enzyme glycogen phosphorylase as well as the concentration of both of its substrates (glycogen and inorganic phosphate). In the post-exercise recovery period, muscle glucose uptake displays an increased sensitivity to insulin in this way increasing glucose uptake after a meal in the muscles that have performed the exercise and therefore are in need of rebuilding their glycogen stores. Whereas the molecular mechanisms involved in post-exercise increased insulin sensitivity are not fully understood, they could involve repackaging of the GLUT4 vesicles in more easily recruitable pools post-exercise. Furthermore, exercise-induced phosphorylation of proteins such as TBC1D4 and p38 MAPK, which remain phosphorylated for hours after exercise, may contribute to insulin-sensitization.

References


Regulation of glucose and glycogen metabolism in exercise


**Acknowledgements**

This work was supported by grants to E.A.R. by the Danish Medical Research Council, The Lundbeck Foundation, NovoNordisk Foundation and the research program of the UNIK: Food, Fitness & Pharma for Health and Disease (see www.foodfitnesspharma.ku.dk). The UNIK project is supported by the Danish Ministry of Science, Technology and Innovation. T.E.J. was supported by a postdoctoral fellowship from the Danish Medical Research Council.
Physiological adaptations to low-volume, high-intensity interval training in health and disease

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Abstract  Exercise training is a clinically proven, cost-effective, primary intervention that delays and in many cases prevents the health burdens associated with many chronic diseases. However, the precise type and dose of exercise needed to accrue health benefits is a contentious issue with no clear consensus recommendations for the prevention of inactivity-related disorders and chronic diseases. A growing body of evidence demonstrates that high-intensity interval training (HIT) can serve as an effective alternate to traditional endurance-based training, inducing similar or even superior physiological adaptations in healthy individuals and diseased populations, at least when compared on a matched-work basis. While less well studied, low-volume HIT can also stimulate physiological remodelling comparable to moderate-intensity continuous training despite a substantially lower time commitment and reduced total exercise volume. Such findings are important given that ‘lack of time’ remains the most commonly cited barrier to regular exercise participation. Here we review some of the mechanisms responsible for improved skeletal muscle metabolic control and changes in cardiovascular function in response to low-volume HIT. We also consider the limited evidence regarding the potential application of HIT to people with, or at risk for, cardiometabolic disorders including type 2 diabetes. Finally, we provide insight on the utility of low-volume HIT for improving performance in athletes and highlight suggestions for future research.

(Received 16 November 2011; accepted after revision 23 January 2012; first published online 30 January 2012)

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Abbreviations  HIT, high-intensity interval training; PGC-1α, peroxisome-proliferator activated receptor γ coactivator; PPO, peak aerobic power output.

Introduction

High-intensity interval training (HIT) describes physical exercise that is characterized by brief, intermittent bursts of vigorous activity, interspersed by periods of rest or low-intensity exercise. HIT is infinitely variable with the specific physiological adaptations induced by this form of
training determined by a myriad of factors including the precise nature of the exercise stimulus (i.e. the intensity, duration and number of intervals performed, as well as the duration and activity patterns during recovery). When compared on a matched-work basis or when estimated energy expenditure is equivalent, HIT can serve as an effective alternate to traditional endurance training, inducing similar or even superior changes in a range of physiological, performance and health-related markers in both healthy individuals and diseased populations (Wisloff et al. 2007; Tjonna et al. 2009; Hwang et al. 2011). Less is known regarding the effects of low-volume HIT, but growing evidence suggests this type of training stimulates physiological remodelling comparable with moderate-intensity continuous training despite a substantially lower time commitment and reduced total exercise volume (Gibala & McGee 2008). These findings are important from a public health perspective, given that ‘lack of time’ remains one of the most commonly cited barriers to regular exercise participation (Stutts 2002; Trost et al. 2002; Kimm et al. 2006). Moreover, recent evidence suggests that HIT is perceived to be more enjoyable than moderate-intensity continuous exercise (Bartlett et al. 2011). Here we review some of the mechanisms responsible for improved skeletal muscle metabolic control and changes in cardiovascular function in response to low-volume HIT, as well as the potential health-related implications for patients with chronic diseases including type 2 diabetes and cardiovascular disease. We also speculate on the practical application of low-volume HIT for elite performance. Although it is recognized that the underlying mechanisms are probably different compared with less-trained subjects (Iaia & Bangsbo 2010), responses in elite athletes may help our understanding of why low-volume HIT is such a potent exercise stimulus.

Physiological remodelling after low-volume HIT

The most common model employed in low-volume HIT studies has been the Wingate test, which consists of a 30 s ‘all out’ cycling effort against a supra-maximal workload. Subjects typically perform four to six work bouts separated by ~4 min of recovery, for a total of 2–3 min of intense exercise during a training session that lasts ~20 min. As little as six sessions of this type of training, totalling ~15 min of all out cycle exercise over 2 weeks, increased skeletal muscle oxidative capacity as reflected by the maximal activity and/or protein content of mitochondrial enzymes (Burgomaster et al. 2005; Gibala et al. 2006). We have also directly compared 6 weeks of Wingate-based HIT with traditional endurance training that was designed according to current public health guidelines (Table 1) (Burgomaster et al. 2008; Rakobowchuk et al. 2008). We found similar training-induced improvements in various markers of skeletal muscle and cardiovascular adaptation despite large differences in weekly training volume (~90% lower in the HIT group) and time commitment (~67% lower in the HIT group). In addition to an increased skeletal muscle oxidative capacity (Fig. 1), other endurance-like adaptations have been documented after several weeks of low-volume HIT including an increased resting glycogen content, a reduced rate of glycogen utilization and lactate production during matched-work exercise, an increased capacity for whole-body and skeletal muscle lipid oxidation, enhanced peripheral vascular structure and function, improved exercise performance as measured by time-to-exhaustion tests or time trials and increased maximal oxygen uptake (Burgomaster et al. 2005, 2008; Gibala et al. 2006; Rakobowchuk et al. 2008).

Wingate-based HIT is, however, extremely demanding and may not be safe, tolerable or appealing for some individuals. We therefore sought to design a more practical model of low-volume HIT that is time efficient while also having wider application to different populations including people at risk for chronic metabolic diseases. To accomplish this goal we decreased the absolute intensity of the work bouts, but increased their duration and shortened the rest intervals. Our new practical HIT model consists of 10 × 60 s work bouts at a constant-load intensity that elicits ~90% of maximal heart rate, interspersed with 60 s of recovery. The protocol is still time efficient in that only 10 min of exercise is performed over a 20 min training session. Importantly, this practical, time-efficient HIT model is still effective at inducing rapid skeletal muscle remodelling towards a more oxidative phenotype, similar to our previous Wingate-based HIT studies and

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Table 1. Summary of protocols in studies from our laboratory that directly compared 6 weeks of either high-intensity interval training (HIT) or traditional endurance training

<table>
<thead>
<tr>
<th>Variable</th>
<th>HIT group</th>
<th>Endurance group</th>
</tr>
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<tbody>
<tr>
<td>Protocol</td>
<td>30 s × 4–6 repeats, 4.5 min rest (3 sessions per week)</td>
<td>40–60 min cycling (5 sessions per week)</td>
</tr>
<tr>
<td>Training intensity (workload)</td>
<td>‘All out’ maximal effort (~500 W)</td>
<td>65% of ( V_{\text{O2peak}} ) (~150 W)</td>
</tr>
<tr>
<td>Weekly training time commitment</td>
<td>~10 min (~1.5 h including rest)</td>
<td>~4.5 h</td>
</tr>
<tr>
<td>Weekly training volume</td>
<td>~225 kJ</td>
<td>~2250 kJ</td>
</tr>
</tbody>
</table>

From Burgomaster et al. (2008). \( V_{\text{O2peak}} \), peak oxygen uptake.
high-volume endurance training (Little et al. 2010b). Both types of low-volume HIT protocols are also effective for improving functional performance, as shown by cycling time trials that resemble normal athletic competition (Gibala et al. 2006; Little et al. 2010b).

The molecular mechanisms underlying skeletal muscle metabolic adaptations to low-volume HIT have recently been investigated. Given the potency of HIT to increase mitochondrial capacity, it is perhaps not surprising that investigations have examined the influence of low-volume HIT on the activation of peroxisome-proliferator activated receptor γ coactivator (PGC)-1α, which is regarded as the ‘master regulator’ of mitochondrial biogenesis in muscle (Wu et al. 1999). Evidence suggests that exercise intensity is the key factor influencing PGC-1α activation in human skeletal muscle (Egan et al. 2010). In this respect, acute low-volume Wingate-based HIT increases PGC-1α mRNA by several-fold when measured 3 h post-exercise (Gibala et al. 2009; Little et al. 2011b). This is comparable with the acute increase in PGC-1α mRNA expression observed after a bout of continuous endurance-type exercise (Norrbom et al. 2004; Egan et al. 2010). Similar to endurance exercise (Wright et al. 2007; Little et al. 2010a), acute Wingate-based HIT may activate PGC-1 by increasing its nuclear translocation (Little et al. 2011b). The increase in nuclear PGC-1 following low-volume HIT coincides with increased mRNA expression of several mitochondrial genes (Little et al. 2011b), suggesting that a program of mitochondrial adaptation is engaged with these short bursts of intensity exercise (Fig. 2).

The upstream signals that activate PGC-1α and mitochondrial biogenesis in response to low-volume HIT have not been clearly elucidated but probably relate to robust changes in intramuscular ATP:ADP/AMP ratio following exercise (Chen et al. 2000) and the concomitant activation of 5'-adenosine monophosphate-activated protein kinase (AMPK) (Gibala et al. 2009; Little et al. 2011b). Activation of p38 mitogen-activated protein kinase (MAPK), possibly via increased generation of reactive oxygen species (ROS) (Kang et al. 2009), may
also be involved (Gibala et al. 2009; Little et al. 2011b). Elevated levels of PGC-1 protein also accompany increased markers of mitochondrial content following a period of low-volume HIT. Six weeks of Wingate-based HIT increased the protein content of PGC-1 by ∼100% in young, healthy individuals (Burgomaster et al. 2008) and 2 weeks of 10 × 1 min HIT resulted in a ∼25% increase in nuclear PGC-1 protein (Little et al. 2010b). Collectively, these results indicate that PGC-1α is probably involved in regulating some of the metabolic adaptations to low-volume HIT. Given the positive effects that a modest increase in muscle PGC-1α appears to have on oxidative capacity, anti-oxidant defence, glucose uptake, resistance to age-related sarcopenia and anti-inflammatory pathways (Sandri et al. 2006; Benton et al. 2008; Wenz et al. 2009), the increase in PGC-1α following low-volume HIT may highlight potential widespread health benefits for this type of exercise.

The impact of interval types of training programs on cardiovascular structure and function has also been investigated (Wisloff et al. 2009), but few studies have utilized low-volume HIT models. However, as little as 2 weeks of Wingate-based HIT has been reported to increase cardiorespiratory capacity as reflected by changes in peak oxygen uptake (V\textsubscript{O\textsubscript{peak}}) (Whyte et al. 2010) although this is not a universal finding (Burgomaster et al. 2005). Another study showed that 6 weeks of Wingate-based HIT increased V\textsubscript{O\textsubscript{peak}} to the same extent as traditional endurance training despite a markedly reduced time commitment and total training volume (Burgomaster et al. 2008). We have also shown in young healthy men and women that low-volume HIT increases compliance in peripheral but not central arteries (Rakobowchuk et al. 2008). The protocol also increased endothelial function in the trained legs to an extent that is comparable to changes observed after a much higher volume of continuous moderate-intensity training (Rakobowchuk et al. 2008). The mechanisms regulating cardiovascular adaptations to various forms of low-volume HIT have yet to be comprehensively examined.

Potential application of HIT in people with or at risk for cardiometabolic disorders

While much of the work conducted to date has involved relatively high-volume protocols that are comparable in volume to traditional endurance training, HIT has been shown to improve cardiorespiratory fitness in a range of populations including those with coronary artery disease, congestive heart failure, middle age adults with metabolic syndrome and obese individuals (Warburton et al. 2005; Wisloff et al. 2007; Moholdt et al. 2009; Munk et al. 2009). In many cases, the increase in cardiorespiratory fitness after HIT was superior to after continuous moderate-intensity training (Wisloff et al. 2007; Tjonna et al. 2008, 2009; Moholdt et al. 2009). Endothelial function, assessed using flow-mediated dilatation of the brachial artery, is improved to a greater extent following HIT compared with continuous moderate-intensity training (Wisloff et al. 2007; Tjonna et al. 2008, 2009; Moholdt et al. 2009). Other studies have documented beneficial changes in various components of resting blood pressure (Rognmo et al. 2004; Schjerve et al. 2008; Whyte et al. 2010) and left ventricular morphology (Wisloff et al. 2007). It appears that this type of cardiac remodelling requires a longer duration of training and greater exercise volume than the load required to alter cardiorespiratory fitness or peripheral vascular structure and function. It could be that the short intense bursts of activity with low-volume HIT induce large-magnitude increases in cellular and peripheral vascular stress, while effectively ‘insulating’ the heart from those stresses due to the brief duration of the exercise bouts. This relative central insulation permits individuals to train at much higher intensities than they would otherwise, but may also result in different timelines and effective stimulus loads between the central and peripheral components of the cardiovascular system.

Low-volume HIT studies in persons who might be at risk for cardiometabolic disorders or patients with chronic disease are very limited. However, recent work has shown that as few as six sessions of either Wingate-based HIT and the more practical constant-load model over 2 weeks improve estimated insulin sensitivity in previously sedentary, overweight individuals (Whyte et al. 2010; Hood et al. 2011). Insulin sensitivity in these studies was calculated based on either single fasting glucose and insulin measurements (Hood et al. 2011) or the

Figure 3. The effect of varying the intensity of interval training on changes in 40 km time-trial performance

Well-trained male cyclists were randomly assigned to one of five different doses of high-intensity interval training (HIT): 12 × 30 s at 175% of peak sustained power output (PPO), 12 × 1 min s at 100% PPO, 12 × 2 min at 90% PPO, 8 × 4 min at 85% PPO, or 4 × 8 min at 80% PPO. Cyclists completed six HIT sessions over a 3 week period in addition to their habitual aerobic base training. Redrawn from Stepto et al. (1999) with permission.

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response to an oral glucose tolerance test (Whyte et al. 2010) and therefore primarily reflects hepatic as opposed to peripheral (skeletal muscle) insulin sensitivity. Peripheral insulin sensitivity following exercise training may be improved by increased skeletal muscle glucose transport capacity, mediated in part by the protein GLUT4. Skeletal muscle GLUT4 content after short-term HIT is increased by a comparable magnitude (∼2-fold) to that observed after high-volume endurance training (Hood et al. 2011). We also recently demonstrated that low-volume HIT was well tolerated and rapidly improved skeletal muscle GLUT4 content in eight patients with type 2 diabetes (Little et al. 2011a). This small pilot study also showed that six sessions of HIT over 2 weeks reduced average 24 h blood glucose concentration and post-prandial glucose excursions, measured via continuous glucose monitoring under standardized diet but otherwise free-living conditions (Little et al. 2011a). These beneficial adaptations were realized even though the weekly training time commitment was much lower than common public health guidelines that generally call for at least 150 min of moderate to vigorous exercise per week to promote health. While the preliminary evidence from these small, proof-of-principle studies are intriguing, large-scale investigations into the effects of interval training in diverse free-living conditions (Little et al. 2011a) would be required to resolve whether low-volume HIT is a realistic, time-efficient exercise alternative to reduce the risk of cardiometabolic disease or improve health and wellbeing in patients with chronic disease.

**HIT and athletic performance**

HIT has been an integral part of training programs for the enhancement of athletic performance since the beginning of the 19th century. Yet despite being a core component of competition preparation, the unique effect of specific training interventions on the performances of well-trained individuals is sparse. This, perhaps, is understandable for several practical reasons. First, exercise physiologists have found it difficult to convince elite athletes that it could be worthwhile to experiment with their normal training programs. Second, even if athletes (and their coaches) were willing to modify their training practices, conventional approaches to investigate the response to different doses of a treatment (i.e. interval training) using repeated-measures design in which each athlete receives all the different doses is totally impractical for studies of physical training; the long-lasting effects of any given dose of training prevent athletes from receiving more than one dose of the treatment.

Over a decade ago we embarked on a series of investigations into the effects of interval training in competitive endurance athletes using a standardized training protocol, namely, replacing a portion (∼15–20%) of an athletes’ aerobic base training with six to eight sessions of continuous (5 min) high-intensity (90% of $\dot{V}_{\text{O}_{2}\text{peak}}$) work bouts undertaken twice a week throughout a 3 week intervention period (see Hawley et al. 1997 for review). We systematically examined the effect of this interval training protocol on a variety of outcome measures including performance (Lindsay et al. 1996; Stepto et al. 1999), skeletal muscle metabolism (Westgarth-Taylor et al. 1997; Stepto et al. 2001), cell signalling (Yu et al. 2003; Clark et al. 2004) and the interaction of HIT with various diet manipulations (Stepto et al. 2002; Yeo et al. 2008). Stepto et al. (1999) employed a novel approach to determine the effects of divergent interval training protocols on performance lasting ∼1 h by fitting polynomial or other curves to the responses for each interval training dose for individual athletes. As we originally hypothesized, training sessions that employed work bouts that were closely matched to race-pace (8 × 4 min at 85% of peak aerobic power output (PPO)) significantly enhanced performance (2.8%, 95% CI = 4.3–1.3%). Yet, somewhat surprisingly, short-duration, supra-maximal work bouts (12 × 30 s at 175% of PPO) were just as effective in improving performance (2.4%, 95% CI = 4.0–0.7%). Consistent with this observation, Psilander et al. (2010) recently reported that a single bout of low-volume HIT (7 × 30 s ‘all out’ efforts) stimulated increases in mitochondrial gene expression that were comparable to or greater than the changes after more prolonged (3 × 20 min bouts at ∼87% of $\dot{V}_{\text{O}_{2}\text{peak}}$) endurance exercise in well-trained cyclists. Given the lower volume of work and the fact that mitochondrial transcription factor A, the downstream target of PGC-1α, was only increased after the 30 s protocol, the authors concluded that brief intense interval training might be a time-efficient strategy for highly trained individuals.

Guellich and colleagues (2009) have recently extended our early findings (Stepto et al. 1999, Fig. 3) that ‘polarized training’ enhanced endurance performance. These workers reported that elite endurance athletes from a range of sports including rowing, running, cycling and cross-country skiing perform only a small portion of their training at competition/race-pace intensities, with the bulk of their workload comprising low-intensity, high-volume workouts, and exposure to extreme HIT sessions. In a recent review Laursen (2010) proposed that a polarized approach to training, in which ∼75% of total training volume be performed at low intensities, with 10–15% performed at supra-maximal intensities may be the optimal training intensity distribution for elite athletes who compete in intense endurance events. We suggest that the unique genetic and/or molecular signature resulting from polarized training is a fertile area for future research. Indeed, directly linking exercise-induced signalling cascades in skeletal muscle to defined metabolic responses and specific changes in gene and protein expression that occur after diverse interval training
regimens may provide clues as to why HIT is such a potent intervention for promoting both health outcomes and enhancing athletic performance and exercise capacity.

**Conclusion and directions for future research**

Considerable evidence currently exists to support a role for low-volume HIT as a potent and time-efficient training method for inducing both central (cardiovascular) and peripheral (skeletal muscle) adaptations that are linked to improved health outcomes. Limited work has examined the application of low-volume HIT in people with, or at risk for, cardiometabolic disorders, and at present the potential benefits of this type of training are unclear. Regardless of the group studied, the majority of low-volume studies have utilized relatively short intervention periods (i.e. lasting up to several weeks). Future work involving long-term (i.e. months to years) interventions in a variety of clinical cohorts (i.e. individuals with insulin resistance, obesity, type 2 diabetes and cardiovascular disease) are urgently needed to better understand how manipulating the exercise stimulus impacts on cardiovascular and musculoskeletal remodelling in these populations. One aspect that is unclear from the present literature is the precise intensity and minimal volume of training that is needed to potentiate the effect of the stimulus-adaptation on outcomes such as mitochondrial biogenesis and relevant health markers. To answer such questions, a complex series of studies needs to be undertaken that systematically ‘titrate’ levels of the ‘training impulse’ and determine subsequent cellular, performance and clinical responses after divergent training interventions. In this regard, the perspectives gained from the use of supra-maximal interval-based training in well-trained athletes may aid in understanding why and how low-volume HIT improves health and functional performance in the general population and in many chronic disease states. Information derived from future studies will need to provide practical, evidence-based recommendations for novel exercise prescription that can be incorporated into daily living and form an integral component in the development of future combinatorial therapies for the prevention and treatment of chronic inactivity-related diseases. If achieved, these goals will simultaneously reduce the economic burden associated with an inactive lifestyle.

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Acknowledgements

Work cited from the authors’ laboratories has been supported by the Natural Sciences and Engineering Research Council of Canada, the Canadian Institutes of Health Research, the Canadian Diabetes Association and The Australian Research Council.
Title: Exercise Therapy—The Public Health Message

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Key Words: Health, sedentary, physical activity, disease, medicine

Manuscript

Abstract: (200)

Words: (2668)

References: (24) Tables and Figures: (1)

This is an Accepted Article that has been peer-reviewed and approved for publication in the Scandinavian Journal of Medicine & Science in Sports but has yet to undergo copy-editing and proof correction. Please cite this article as an "Accepted Article"; doi: 10.1111/j.1600-0838.2012.01462.x
Abstract

Non-communicable chronic diseases (NCDs) such as cardiovascular disease, diabetes and cancer are currently responsible for 65% of all deaths worldwide and are projected to cause over 75% of all deaths by 2030. A substantial accumulation of epidemiological and experimental evidence has established a causal relationship between NCDs and well-known yet preventable risk factors (e.g., physical inactivity and obesity). Given that physical activity has both direct and indirect effects on the mortality and morbidity of NCDs via other risk factors (e.g., obesity, diabetes and hypertension), it is now undeniable that sedentary lifestyles are one of the most significant public health problems of the 21st century.

In 2007, the American College of Sports Medicine (ACSM) and American Medical Association (AMA) launched the Exercise is Medicine® (EIM) initiative in recognition of the fundamental importance of physical activity to health and well-being. EIM is on the forefront of a global movement to reduce sedentary lifestyles, foster implementation of exercise counseling into clinical practice and disseminate exercise therapy on a global scale. If the devastating human losses and financial burden of inactivity-induced chronic disease are to be ameliorated, the wide-ranging cost-effective health benefits and financial feasibility of physical activity interventions must be appreciated and promoted.
There are currently nearly 59 million deaths/year occurring in the world and according to the World Health Organization (WHO) approximately 65% of them are due to non-communicable chronic diseases, such as cardiovascular disease, type 2 diabetes, cancer, and chronic respiratory diseases (WHO, 2010). The portion of deaths attributable to non-communicable chronic disease has steadily increased over the past several decades, and is expected to increase to more than 75% of all deaths by 2030 (WHO, 2009). Non-communicable diseases frequently cause death over prolonged periods after initial diagnosis, and require extensive and expensive treatments. In addition to the human suffering and family burdens associated with these diseases, there also are profound economic consequences for families, local communities, and countries (Beaglehole et al., 2011).

Causes of Non-communicable diseases. According to the WHO, more than one third of all deaths can be attributed to a relatively small number of risk factors (WHO, 2009). The five leading risk factors are high blood pressure, tobacco use, high blood glucose, physical inactivity, and obesity, which cause more than 25% of all deaths. The substantial and growing evidence on the importance of non-communicable diseases around the world has generated considerable interest at the highest levels of governments. In September 2011, top officials from the WHO and health ministries of many countries met at the United Nations to discuss the problem. The General Assembly formally recognized that the spread of non-communicable diseases represents a global crisis, and that women, men, and children in all countries and all income groups are at risk (UN, 2011).

Physical Inactivity as a Health Problem. Physical inactivity has direct effects on the development of non-communicable chronic disease, and also has a substantial contribution to all
of the other top five risk factors except tobacco use. Thus it is reasonable to assume that physical inactivity is one of the leading health problems in the world. In the United States, obesity and physical inactivity account for nearly 20% of all deaths (Danaei et al., 2009), and a substantial portion of disability and mortality of some cancers, diabetes, and cardiovascular disease are directly attributable to inactivity-induced low levels of cardiorespiratory fitness (CRF) and obesity (LaMonte et al., 2005; Sui et al., 2010; WHO, 2000). There have been profound declines in physical activity over the past several decades, due to declining energy expenditure at work and home care, and increasingly sedentary leisure time (Archer & Blair, 2011; Church et al., 2011).

**Do We Underestimate the Hazards of Inactivity?** The data briefly reviewed above clearly illustrate a health emergency resulting from inactivity. However, we postulate that these data actually underestimate the harmful effects of not being active. First, the risks associated with inactivity begin in childhood and increase throughout the life span (Booth et al., 2011; Charansonney, 2011). As such, measures of fitness in adulthood may reflect a reduced capacity for fitness induced via a sedentary childhood. Secondly, as mentioned earlier, inactivity is an important contributor to the risk factors of high blood pressure, high blood glucose, and obesity. Finally, nearly all of the data reviewed by the WHO and others are based on self-reported leisure time physical activity. There are major problems with these data. Self-report of physical activity is notoriously inaccurate (Troiano et al., 2008). Investigators may not ask the right questions, people may not remember accurately, and some will exaggerate. This results in a misclassification of activity habits, and this leads to an underestimate of the true effect of inactivity.
We recently illustrated the effect of misclassification of activity in a large cohort of 10,555 women and 31,818 men who reported their activity and also had cardiorespiratory fitness assessed by a maximal exercise test in a laboratory (Lee et al., 2010). Participants were followed for an average of >12 years, during which time 230 women and 1,492 men died. Compared to inactive individuals, women reporting the recommended level of activity had a 17% lower risk of dying, and active men had a 13% lower risk of dying. When we examined risk of dying in those who were moderately fit as determined by the treadmill test, we saw a 39% lower risk in women and 36% lower risk in men, as compared with unfit individuals. Moderate fitness can be achieved by meeting current physical activity recommendations of 150 minutes of moderate intensity activity/week. High fit women and men had an even lower risk of dying. These findings held after adjusting for age and other risk factors (e.g., high blood glucose, high blood sugar).

We also have examined the attributable fractions of deaths in the Aerobics Center Longitudinal Study population (Blair, 2009). Attributable fractions are based on the strength of a risk factor with mortality and on the prevalence of the risk factor in the population being studied. Figure 1 shows the results of these analyses. The attributable fractions are the estimated number of deaths in the population that are due to a particular risk factor, and each of these is adjusted for possible confounding factors, including each of the other risk factors in the figure. Note that low fitness is estimated to cause ~16% of deaths, which is far higher than any other risk factor, with the possible exception of hypertension in men, and is greater than the combined deaths due to obesity, diabetes, and smoking.

<Insert Figure 1>
Integrating exercise counseling in clinical practice; the Kaiser Permanente experience.

Kaiser Permanente (KP) is the largest Health Maintenance Organization (HMO) in the world, with close to 7 million patients in California and another 3 million in various smaller regions around the United States. It is described as a staff model HMO, because patients pay a specific amount each month and in turn receive all their health care within this integrated system. Unlike traditional health insurance, KP is most successful when patients are healthy and do not require expensive procedures. So there is a strong incentive to keep patients healthy and avoid unnecessary procedures. By the same token, if essential preventive measures are delayed or ignored, KP must pay the full cost that will accompany more severe or advanced illness.

Because of the overwhelming evidence documenting the extensive health benefits of exercise, Kaiser Permanente has put forth a strong effort to encourage patients to be more physically active.

Exercise as a vital sign. Since October of 2009, KP in Southern California has been using an exercise vital sign (EVS) to assess patient exercise habits at every visit. This is recorded in the KP electronic medical record (EMR). As each patient is being brought into the examination room for an evaluation, and after measuring traditional vital signs (i.e., blood pressure, pulse and temperature), the medical assistant (MA) asks the patient two questions about their exercise habits. The first is “on average, over the past month, how often do you engage in moderate physical activity, like a brisk walk?” This is followed by a second question “on those days, on average, how many minutes do you engage in such physical activity?” With this information, the MA is able to calculate the average minutes per week of reported moderate or greater exercise for each patient. Each patient’s EVS is then displayed in the chart header next to traditional vital signs and is printed into their electronic note for that visit. The EVS has been
very successful at KP in Southern California (with over 81% of patients having their EVS recorded in the first year of use (Sallis & Coleman, 2011) and it is currently being implemented at KP in Northern California and Colorado, with plans to implement in all the KP regions around the country in the near future.  

The exercise prescription. KP Physicians and other healthcare providers are encouraged to review each patient’s EVS and make a comment; either to congratulate them for doing 150 minutes or more of PA each week, or provide them with an exercise prescription that encourages them to meet that goal. One of the easiest ways for physicians to prescribe exercise in the office setting is by following the “FIT” pneumonic. The “F” stands for frequency, with a recommendation of 5 or more (most) days of the week. The “I” stands for intensity, which is recommended to be at least moderate in nature (50-70% of maximum predicted heart rate by 220-age). The easiest way to gauge intensity is by using the sing-talk test, whereby patients are instructed to exercise at a level intense enough they cannot sing while exercising, but that it is not necessary for the exercise to be so intense they cannot talk. The first “T” stands for type of exercise and it is recommended that patients engage in any activity that works large muscle groups, increases heart rate and causes them to lightly perspire. The second “T” stand for time and it is recommended that patients exercise for 30 minutes.  

In addition to the standard exercise prescription, it is recommended that physicians, when time permits, provide patients with some key exercise messages, such as the fact that three 10 minute bouts of exercise provide similar benefits do doing 30 minutes all at once. That there is no amount of exercise that is insignificant and doing even 15 minutes per day of walking has proven to significantly lower mortality rates (Wen et al., 2011). Also, it is never too late to start exercising and the benefits of exercise are similar for people of all sizes and shapes, regardless of
weight change. The greatest benefits are seen when someone who is sedentary begins doing just
moderate levels of activity.

**The KP Thrive Campaign and Everybody Walk! Campaign.** In 2004, KP launched a
massive campaign called “Thrive”. It embodies KP’s commitment to *Total Health*, which
includes mind, body and spirit, since it is well known that all three of these factors play an
integral role in what we would define as being “healthy”. This campaign has included print,
radio and TV ads with a central theme being the importance of exercise (and other lifestyle
factors) to health. At the same time there has been a similarly focused internal campaign to get
all physicians and staff to walk the talk and really live out the *Thrive* brand. The KP *Thrive*
campaign has been amazingly successful and resonated with both patients and KP staff, and in
fact has been the highest rated advertising campaign in the history of healthcare advertising.

Earlier this year, KP Chairman and CEO George Halvorson, announced sponsorship of
an unbranded campaign to get America walking. This campaign, called *Everybody Walk!*, is a
reflection of Mr. Halvorson’s realization that inactivity has a tremendous effect on the business
of healthcare and more importantly, patients’ health and longevity. The campaign is
disseminated primarily through a website (everybodywalk.org) that includes a series of videos
(both inspirational and instructional), maps of walking trails, calendars of walking events and
smart phone applications that are all designed to get America walking.

*Exercise is Medicine ®: Translating Exercise Therapy into Practice on a Global Scale*

During the past decade, several leading international organizations have recognized the
ability of physical activity to ameliorate the growing burden of non-communicable diseases
(NCDs) and improve health, and have issued calls to action to make physical activity a priority.
Efforts also are being made to connect physical activity with health care, including the (World
In an effort to make physical activity an integral part of health care, first in the United States and later internationally, the American Medical Association and the American College of Sports Medicine (ACSM) co-launched what rapidly evolved into a multi-organizational, multi-national initiative called Exercise is Medicine® (EIM), coordinated by ACSM. Some preliminary survey work by ACSM found that 60% of patients reported that they would be more likely to start a physical activity program if advised to do so by their health care provider (HCP). Coupled with such compelling evidence for the benefits of physical activity, this provided the impetus to launch EIM in the United States in 2007. EIM’s primary goal is to make physical activity an integral part of the US health care system and include it as a vital sign that is addressed at every HCP-patient interaction. One way to achieve this is to integrate a physical activity assessment into electronic medical records (EMR) and to this end, EIM is currently engaged in such efforts in the USA to attempt to persuade EMR vendors in the US to integrate physical activity into their systems. Indeed, this sort of systemic level effort to bring physical activity into health care is likely to be needed to effect change on a population level. Other systemic level levers that EIM is calling for in the US include securing the classification of physical inactivity as a “disease” through an International Classification of Diseases code (ICD-10-CM code)(WHO, 2012) and the classification of physical activity as a Healthcare Effectiveness Data and Information Set (HEDIS) measure for the adult population. HEDIS is a benchmark by which US health care systems are assessed by the US Federal Government when evaluating payments for patients over 65 years of age care.
EIM’s long term goal is for physical activity assessment, prescription and referral to become part of every patient-HCP interaction. At the launch of the initiative, EIM learned from HCPs that barriers to physical activity counseling included lack of time, lack of training in physical activity counseling and lack of reimbursement for physical activity counseling. An EIM Health Care Providers’ Action Guide was developed to teach HCPs how to provide patients with a physical activity “prescription” within the 30 second time window that an HCP typically has available. There is increasing attention to the problem, and additional efforts need to be made to encourage physicians to counsel patients about physical activity (Khan et al., 2011).

As important as it is to bring physical activity counseling into the HCP’s office, work from the Karolinska Institute in Sweden (Kallings et al., 2008; Kallings et al., 2009) and the Green Prescription movement in New Zealand (Elley et al., 2003) shows that advice from an HCP is likely to be only the first step and that sustained behavior change will probably require community based support. To meet this need, EIM encourages HCPs to refer patients to community health and fitness professionals who can help guide patients through the behavior change strategies essential for sustained patient behavior change and is currently developing an infrastructure to provide a link to a network of qualified health and fitness professionals to whom they can refer a patient whose needs surpass their counseling expertise or time available.

Although EIM began in the United States in 2007, international demand led to it being launched as a global initiative in June, 2010. To support this globalization, five additional EIM Regional Centers were launched in 2010 and 2011, located in Colombia (Latin American Region), Germany (European Region), South Africa (African Region), Singapore (Southeast Asian Region) and Australia (Australian-Pacific Region). These Regional Centers support and encourage the development of a National Task Force (NTF) in each of the countries in their
region, using a multi-sectorial approach that brings representatives from leading primary care and sports medicine organizations together with leaders from academia, the government (where there is the interest), and industry to work within the unique systems and resources of the country to make physical activity an integral part of the country’s health care system.

Conclusions

We now have overwhelming evidence that physical inactivity and low levels of fitness are two of the leading causes of morbidity and mortality in the world. This applies not only to non-communicable diseases but to preserving function as we age, improving quality of life, and enhancing mental function. We can no longer afford to ignore inactivity in clinical, public health, and educational settings. We call for action from groups around the world to promote physical activity for all individuals.
References


Figure 1. From (Blair, 2009). Attributable fractions (%) for all-cause deaths in 40,842 (3333 deaths) men and 12,943 (491 deaths) women in the Aerobics Center Longitudinal Study. The attributable fractions are adjusted for age and each other item in the figure. *=cardiorespiratory fitness determined by a maximal exercise test on a treadmill. (Reprinted by permission of the Br J Sports Med)
Training and Bone – from Health to Injury

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This is an Accepted Article that has been peer-reviewed and approved for publication in the Scandinavian Journal of Medicine & Science in Sports but has yet to undergo copy-editing and proof correction. Please cite this article as an "Accepted Article"; doi: 10.1111/j.1600-0838.2012.01461.x
Abstract

Mechanical load through physical activity has been shown to be one of the best stimuli to increase the bone strength. This effect of mechanical load accounts for both the accrual of bone mineral and structural skeletal adaptations. Exercise prescription also includes a “window of opportunity” in the late pre- and early peri-pubertal period, where exercise is supposed to insert the most obvious beneficial effects, even if physical activity provides recordable skeletal benefits during all growth. There is also evidence that benefits in bone mass and bone structure obtained by mechanical load during growth may be maintained at advanced age. The notion that former male athletes have lower fracture risk than expected by age, support this view. Physical activity could therefore be recommended at growth and adolescence as one possible strategy to reduce the future burden of fragility fractures.
Adaptive response of bone to loading

The skeleton is a metabolic active organ that responds to mechanical stimuli by initiating or inhibiting bone modelling and remodelling in order to keep peak strains within a safe physiological range load (Frost & Schonau 2000). The feedback system, where the skeletal response depends on the type and duration of the load, is referred to as the mechanostat (Frost 1987). Key features for osteogenic stimuli include load that is dynamic, have high magnitude, high frequency and unusually distributed strains (Cullen et al. 2001; Hsieh & Turner 2001; Turner 1998). In addition, the required mechanical load necessary to stimulate osteogenesis decreases as the frequency of the load increases (Cullen, Smith 2001; Hsieh & Turner 2001; Turner 1998). But the osteogenic response to mechanical load becomes saturated after a few loading cycles (Rubin & Lanyon 1984) so that additional load provides no further benefit (Umemura et al. 1997). However, the bone cell mechanosensitivity recover following rest. Therefore the most effective load is when separating loading into short bouts followed by periods of rest (Robling et al. 2002; Robling et al. 2002; Srinivasan et al. 2002).

This has been shown in animal studies when four hours of rest between loads doubles the osteogenic response and the sensitivity to loading is almost completely restored after eight hours of recovery (Robling et al. 2001). That is, the loading characteristics most beneficial for bone strength are very specific, making general prescription of physical activity for cardiovascular health or weight reduction non-optimal for skeletal health. Exercise prescription with the aim to improve cardiovascular function or reduce weight should include training with lower intensities as jogging and cycling but for an extended duration. Similar training modalities are not the most effective for
skeletal strength. If the aim is to improve skeletal status, exercise like soccer, volleyball, tennis and squash are most effective as these sports includes the types of loads that is most effective for skeletal strength. But, in elderly fracture preventive training should probably more address the risk of falling and therefore include less intensive and other types of activities such as Tai Chi, in randomised controlled trials (RCT) shown to reduce the fall frequency (Fiatarone et al. 1990; Li et al. 2004; Province et al. 1995). The musculoskeletal effectiveness of training has been reported in a variety of original reports, systematic reviews and meta-analyses (Hamilton et al. 2010; Karinkanta et al. 2010; Kelley & Kelley 2006; Martyn-St James & Carroll 2006; Martyn-St James & Carroll 2006; Martyn-St James & Carroll 2008; Martyn-St James & Carroll 2009; Martyn-St James & Carroll 2010; Nikander et al. 2010). This report is however not a structured review, instead a summary from the symposium Biomedical Basis of Elite Performance in London, March 2012

**Maturity dependent response of bone to loading**

osteogenic response are also maturity and gender dependent (Daly 2007; Kannus, Haapasalo 1995) so that the strongest response to mechanical stimuli occur during growth, especially in the pre- or early peri-pubertal period (Bass, Naughton 2007; Blimkie et al. 1996; Bradney, Pearce 1998; Daly 2007; Detter, Nilsson 2010; Fuchs, Bauer 2001; Haapasalo, Kannus 1998; Heinonen, Sievanen 2000; Hind & Burrows 2007; Iuliano-Burns, Saxon 2003; Kannus, Haapasalo 1995; Linden, Alwis 2007; Linden, Gärdsell 2005; Lofgren, Stenevi-Lundgren 2009; MacKelvie, Khan 2003; MacKelvie, Petit 2004; McKay, Petit 2000; Morris, Naughton 1997; Nichols, Sanborn 2001; Petit, McKay 2002; Valdimarsson, Linden 2006; Van Langendonck, Claessens 2003). The skeletal response to mechanical load in young adults is far less than during growth and in elderly, physical activity seems to reduce age related bone loss or at best produce increments in BMD of a few percentage points (Berard et al. 1997; Heinonen et al. 1996). These benefits may be of less clinical significances for fracture reduction. The reported lower fracture incidence in physically active elderly is therefore probably not the result of exercise induced skeletal benefits but probably the result of other effects such as increased muscle strength and/or improved neuromuscular function, traits possible to influence by training also in the oldest (Fiatarone, Marks 1990; Karinkanta et al. 2010). Physical training in old people should therefore be focused as to reduce the risk of falling, more than in preventing osteoporosis when trying to reduce the fracture risk (Jarvinen et al. 2008). But as the topic of this report is “training and bone”, from now on the review focus on the skeletal effects of physical activity during growth, as the most obvious exercise
induced skeletal benefits are found in this period, and if exercise induced skeletal effects in young years are retained in old ages.

**Exercise and bone mass in athletes**

Physical activity increases the accrual of bone mineral at growth. This was reported already four decades ago when Nilsson and colleagues showed that athletes had 1-1.5 standard deviations (SD) higher BMD than being expected by age (Nilsson & Westlin 1971). Following reports have verified this and it is now considered general knowledge that high impact sports such as triple-jumping, tennis, squash, gymnastics and soccer in both genders is associated with higher BMD than being expected by age (Karlsson et al. 2001). BMD has for example in young female gymnasts shown to be substantially higher than in sedentary children (Bass et al. 1998) and young tennis players have a 10-15% arms side-to-side difference in BMD in favour of the dominant arm (Bass et al. 2002; Haapasalo, Kannus 1998; Kannus, Haapasalo 1995). The difference is also more obvious if the exercise is initiated before than after puberty (Bass, Saxon 2002; Kannus, Haapasalo 1995). In contrast, endurance sports such as running, cycling and swimming, has in several studies been associated with less beneficial effects than impact sports (Karlsson et al. 2001). That is, studies in athletes, as a model of high exercising individuals, have provided us with knowledge as regard the adaption of the skeleton when exposed to increased mechanical load. But, it is then imperative to realize that these data provide us with information on what is possible to reach in very dedicated individuals, more than what is probable to reach in the general physical active child.
Bone mass in general physical active children

But also children with a moderate level of physical activity may reach skeletal benefits with increased training (Bass, Naughton 2007; Blimkie, Rice 1996; Bradney, Pearce 1998; Daly 2007; Detter, Nilsson 2010; Fuchs, Bauer 2001; Heinonen, Sievanen 2000; Hind & Burrows 2007; Iuliano-Burns, Saxon 2003; Kannus, Haapasalo 1995; Linden, Alwis 2007; Linden, Gärdsell 2005; Lofgren, Stenevi-Lundgren 2009; MacKelvie, Khan 2003; MacKelvie, Petit 2004; McKay, Petit 2000; Morris, Naughton 1997; Nichols, Sanborn 2001; Petit, McKay 2002; Valdimarsson, Linden 2006; Van Langendonck, Claessens 2003). The benefits should however not be underestimated as even a small increase in BMD may generate a markedly increase in bone strength (Robling, Hinant 2002; Warden et al. 2007). Published exercise intervention studies in pre- and peri-pubertal children has predominantly used extra physical education classes or supplementary leisure time training and few studies have followed these children with an intervention that exceed 12 months (Blimkie, Rice 1996; Bradney, Pearce 1998; Cheng et al. 2002; Fuchs, Bauer 2001; Heinonen, Sievanen 2000; Iuliano-Burns, Saxon 2003; Linden, Alwis 2007; Linden, Gärdsell 2005; McKay, Petit 2000; Morris, Naughton 1997; Valdimarsson, Linden 2006; Van Langendonck, Claessens 2003). The longer intervention studies published infer previous reported short-term benefits to remain with long-term extra physical training in the school curriculum (Dencker et al. 2006; Detter, Nilsson 2010; Linden, Gärdsell 2005; Lofgren, Stenevi-Lundgren 2009; MacKelvie, Khan 2003; MacKelvie, Petit 2004; Nichols, Sanborn 2001) (Figure 1).
Similar interventions could also be initiated without increasing the risk of sustaining childhood fractures (Detter, Nilsson 2010; Lofgren, Stenevi-Lundgren 2009), an adverse effect of exercise that has been reported to be associated with high level of physical activity, probably as a result of increased rate of trauma in conjunction with the activity (Clark et al. 2008; Tobias et al. 2007; Tveit et al. 2010).

**Gender discrepancies in the response to physical activity**

The explanation for the maturity- and sex-dependent response to mechanical load (Daly 2007; Kannus, Haapasalo 1995) is probably the result of exercise preferentially affecting surfaces of bone that is undergoing apposition and hormonal discrepancies (Ruff et al. 1994). The pre-pubertal skeleton seems also to have the capacity to respond to loading by adding more bone on the periosteal surface than would normally occur through growth (Bass, Saxon 2002; Ducher & Bass 2007; Dyson et al. 1997). Or in other words, exercise may induce a larger skeleton. But studies also infer that there exists an endosteal apposition in pre-pubertal boys as a response to mechanical load (Bradney, Pearce 1998; Ducher & Bass 2007; Ward et al. 2005) whereas such a response seems less likely in pre-pubertal girls, possibly as the result of oestrogen inserting inhibition of periosteal expansion (Bass, Saxon 2002; Ward, Roberts 2005). Exercise in late puberty is associated with bone apposition on the endosteal surface, as shown in female tennis players (Bass, Saxon 2002), and the enlargement of bone size in response to loading has been reported to increase from pre- to peri-puberty in male but not in female tennis players (Bass, Saxon 2002;
Ducher & Bass 2007). These gender discrepancies in response to mechanical load confer a more beneficial exercise response in boys than in girls.

**Physical activity and bone structure**

The increased bone size also induces a greater increase in bone strength than an increase in BMD alone (Bass, Saxon 2002; Haapasalo et al. 2000; Kontulainen et al. 2002). Three-dimensional techniques such as the peripheral quantitative computed tomography (pQCT) and magnetic resonance imaging (MRI) have identified regional periosteal expansion at mechanically loaded sites. For example, bone size was approximately 10% higher in arms of pre-pubertal gymnasts than in general active children (Dyson, Blimkie 1997; Ward, Roberts 2005). The arms side-to-side difference in bone size was also obvious in pre-pubertal tennis players (Bass, Saxon 2002; Ducher & Bass 2007). But, if bone also is laid down on the endosteal surface, cortical thickness will increase. For example, there are reports that infer cortical cross-sectional area to be up to 12% greater in the lower limbs of young runners or gymnasts compared to controls in spite of having the same bone width (Duncan et al. 2002; Greene et al. 2005; Ward, Roberts 2005). For creating a strong bone, endosteal apposition is however less beneficial than periosteal apposition, this due to the fact that bone resistance to bending increases by the forth power of the radius of a tubular structure (Ahlborg et al. 2001; Ahlborg et al. 2003; Turner & Burr 1993).

**Region specific response to physical activity**
The osteogenic response in the arms and legs are also site-specific (Heinonen et al. 2002; Ward, Roberts 2005). For example, endosteal apposition has in tennis players been found at the 60-70% distal humerus but not at the 40-50% mid humerus (Bass, Saxon 2002; Ducher & Bass 2007) and there is a different response to mechanical load in the anterior-posterior and in the medial-lateral direction and different in the proximal, mid-diaphysis and distal part of the long bones (Haapasalo, Kontulainen 2000; Heinonen, Sievanen 2002; Jones et al. 1977; Robling, Hinant 2002; Ward, Roberts 2005). If these discrepancies are the result of different type of loads in different regions, different thresholds for osteogenic response in different regions, or different load magnitudes relative to bone size in different regions remains to be proven.

Redistribution of bone mass from less loaded regions to areas submitted to high mechanical strains may also regional specific increase bone strength. Thus, changes in the shape of the bone could increase bone strength without increasing bone mass or bone size. This adaptive model has been shown to occur in both animal (Carlson & Judex 2007; Hiney et al. 2004; Robling, Hinant 2002; Warden, Fuchs 2007) and humans (Cheng, Sipila 2002; Jones et al. 2002; Macdonald et al. 2007).

**Adverse effects of physical activity**

Most skeletal changes associated with physical activity are beneficial and there are only sparse descriptions of adverse effects. However, in some individuals the bone mass remains unchanged or even decreases in spite of having high level of physical
activity, something that has been seen as a factor behind both stress fracture and shin splints (Magnusson et al. 2001). But the most serious adverse skeletal side effect from exercise is seen in individuals with high intensity activity. Menstrual and hormonal alterations are frequently connected with dieting, low body mass and eating disorders in addition to strenuous training. A long duration of hard training can lead to decreased oestrogen levels and a training-induced amenorrhoea, often accompanied by reduced bone mass (Pearce et al. 1996). Several studies in females within different sports have verified that this menstrual dysfunction leads to lowered bone mass, even lower than in controls (Pearce, Bass 1996). If the menstrual dysfunction is normalised, the bone mass slowly increases but is not fully restored (Micklesfield et al. 1998). Interestingly, a similar negative metabolic effect after very hard exercise has also been seen in men (Malm et al. 1993). Other sports injuries could also insert an indirect effect of the skeleton as an muscle injury that requires immobilization is usually followed by bone loss due to less mechanical load and fracture by posttraumatic fracture induced osteopenia (Karlsson et al. 1996; Karlsson et al. 1996; Karlsson et al. 2001).

Are exercise induced high bone mass retained into old ages?

But, before physical activity at growth can be recommended as a preventive strategy for low BMD and osteoporosis, it must be shown that the exercise-induced skeletal benefits are retained into old ages. It must also be shown that exercise at growth is associated with low fracture incidence in old ages. Hypothetically this seems less likely as the mechanostat theory indicates that reduced level of physical activity ought to be followed...
by decreased bone strength (Frost 1987; Gafni & Baron 2007). However, prospective studies that have followed former physical active individuals with reduced level of activity report a higher BMD in the retired athletes but at a lower level than during the physical active period (Bass, Pearce 1998; Gunter et al. 2007). This remaining effect has been shown to depend on greater bone size, cortical area and trabecular volumetric density in the upper limbs (Bass, Pearce 1998) and greater cortical area and trabecular volumetric density in the tibia (Eser et al. 2007) in former athletes. Former male and female soccer players have also been shown with residual greater BMD a decade after retirement from sports in spite of having increased BMD loss following cessation of active exercise career. But, the benefits with 5-10 years of retirement were only half of the benefits found when they were in their active career (Nordstrom et al. 2005; Valdimarsson et al. 2005). Former tennis players have also arm side-to-side difference after reduction in physical activity level but at a lower level than during active career (Kontulainen et al. 1999). But there is now also prospective controlled data presented that infer exercise induced benefits in BMD to remains with 3 decades of retirement from sports (Tveit, Ahlborg 2010) and cross sectional reports infer that also structural benefits are retained in former old athletes (Karlsson et al. 2002). These benefits could be of clinical significance as the fragility fracture risk has been shown to be only half in former athletes compared to the controls (Tveit, Ahlborg 2010) (Figure 3). Taken the discussed studies into account, it seems possible that the faster loss in BMD that has been shown in close conjunction with termination of active exercise career is transient, so that there remains long-term benefit with exercise that is transferred into reduced fracture risk in old ages.
But, there is also reports which refute the notion that exercise induced skeletal benefits are retained after active career. This would not be unexpected, as Wolff’s law suggests the skeleton to adapt to the current level of mechanical load. Cessation of running was in one report associated with increased bone loss whereas there was no loss in those who continued with the running (Michel et al. 1992). Unilateral leg presses four times a week during 12 months was associated with non-significant increased bone mass but all these benefits was lost with 3 months of detraining (Vuori et al. 1994). Cross sectional studies show similar results when reporting that former male soccer players have a higher residual BMD during the first two decades after retirement, but not four to five decades after retirement (Karlsson et al. 2000) (Figure 2) and similar data has been shown in former female soccer players (Duppe et al. 1996), former male weight lifters (Karlsson et al. 2003; Karlsson, Hasserius 1996; Karlsson et al. 1993; Karlsson et al. 1995) and former male and female ballet dancers (Karlsson et al. 1993; Khan et al. 1996). However, all these cross sectional evaluations has now been challenged by the just cited prospective controlled data (Tveit, Ahlborg 2010).

Are exercise induced benefits in skeletal structure retained into old ages?

As the mature skeleton is thought to lose bone mass essentially through remodelling on the endosteal envelope, and to a much lower extent on the periosteal envelope (Riggs et al. 2004), the increased bone size obtained by physical activity during growth (Linden et al. 2006; Linden et al. 2006; Specker & Binkley 2003) may be better preserved than the amount of acquired bone mineral (Karlsson, Bass 2001). This could be of clinical
importance as bone structure is an independent predictor of fracture risk (Ahlborg, Johnell 2003). Exercise-associated enlargement in bone size has also been shown to be retained with short-term retirement from tennis playing (Haapasalo, Kontulainen 2000). Former male tennis players retired for 1 to 3 years had in this report arm side-to-side difference of 20% in bone mineral content, 18% in cross-sectional area, 15% in cortical wall thickness and 30% in bone strength index. Also children aged 3 to 5 years retained their benefits in bone structure gained by exercise with 1-2 years of reduced activity level (Specker et al. 2004). There is however limited data in old retired athletes. The few studies that have evaluated bone structure in old athletes suggest that the exercise-induced benefits in BMD may be eroded in those who have substantially decreased their training volumes (Karlsson, Linden 2000) while structural benefits may persist (Karlsson, Alborg 2002). In recent cited cross-sectional study, former male athletes above age 50 and retired from sports for up to 65 years, had in mean larger femoral neck area and wider lumbar vertebrae spine width than sedentary controls (Karlsson, Alborg 2002).

**How important is recreational exercise?**

Not only the amount of physical activity in young years is of importance, also the level of recreational training after high intense training at growth predicts bone strength in old ages. For example, current training was more importance than training level in young years when old male soccer players were evaluated (Karlsson, Linden 2000). In old tennis players, there was also arm side-to-side difference of 4-7 % in old tennis players if they continued with tennis after career but on recreational level (Huddleston et al. 1980). These notions once more emphasise the importance of Wolff’s law when inferring that
recreational exercise after a period of high intense training during young years, may at least partly preserve exercise-induced bone mass benefits.

Could exercise at growth be a strategy to reduce the fracture risk?

Current knowledge when using surrogate end points as BMD or bone structure thus indicate that exercise at growth may be associated with long-term benefits as regard fracture reduction. A reduced fracture risk has also been reported in retired athletes. 663 former athletes above age 50 years, and retired from sports for up to 65 years had lower fracture risk than 943 age- and gender matched controls, 8.9% in the former athletes and 12.1% in the controls (Nordstrom, Karlsson 2005) (Figure 4). The proportion of individuals with low energy fragility fractures was also lower in the former athletes, 2.3% versus 4.2%. Similar conclusions was drawn in one study that included 400 former male soccer players and 800 controls (Karlsson, Alborg 2002) and there are now also a report that evaluate 2075 former male athletes and controls aged 50-91 years, a study that report a lower incidence of all type of fractures as well as fragility fractures and distal radius fractures in the former sportsmen (Tveit, Ahlborg 2010) (Figure 3). But there are also studies refute this view. In one study that included 2622 former female college athletes and 2776 controls now aged 20-80 years, there was no different fracture rate in the two groups (Wyshak et al. 1987). However, as this study includes individuals as young as 20 years, individuals on different level of physical training during active career, individuals with different retirement period from active training and individuals with different level of current level of recreational exercise, data is difficult to interpret.
Conclusions and perspective

Mechanical load has been shown to be one of the best stimuli to enhance not only bone mass but also the structural skeletal adaptations, both contributing to bone strength. Childhood and adolescence, and then more specifically in the late pre- and early peri-pubertal period, are the periods when physical activity may exert the most obvious skeletal benefits. There are evidence supporting the notion that skeletal gains obtained by mechanical load during growth are maintained at advanced age and the notion that former male athletes have a lower fracture risk than expected by age support the view that exercise at growth may be associated with long-term benefits of clinical importance.
Figure legends

**Figure 1.** Mean annual changes in lumbar spine bone mineral density (BMD) in boys and girls during the 5-year intervention with daily school physical education provided in the intervention group and 1-2 times per week in the control group presented as mean with 95% confidence interval (95% CI). Group comparisons are adjusted for Tanner stage at follow-up. Adapted from Detter et al. 2010.

**Figure 2.** Bone mineral density (BMD) of the lower extremity, femoral neck and upper extremity in active and retired male soccer players and controls in relation to age. BMD in the active and retired athletes is presented as Z-scores (number of standard deviations (SD) difference compared to age- and gender-matched controls) in groups with advancing age and increased time since retirement from active exercise career. Bars represent SD, * p<0.05, ** p<0.01. Adapted from Karlsson et al. 2000.

**Figure 4.** Fracture free survival in 709 former male athletes with a mean age of 69 years (range 50–93), retired from sports a mean 34 years (range 1–63) ago after retirement from sports and in 1368 matched controls. Fragility fracture was defined as fractures of proximal humerus, distal radius, spine, pelvis, hip, and tibial condyles sustained after age 50 years. The evaluation was done through Kaplan Meier survival analyses and significance-tested by Log Rank test. Adapted from Tveit et al. 2010.
Figure 4. Proportion of individuals with one or more fractures in 663 former male athletes now aged 50 to 94 years and in 943 age- and gender-matched controls. The figure includes the lifetime risk of sustaining a fracture, the risk of sustaining a fracture after age 35 (after retirement) and the risk of sustaining a fragility fracture, a wrist fracture and a hip fracture after age 50 due to a low-energy trauma. Adapted from Nordstrom et al. 2005.
Annual Changes Per Year During the 5-Year Exercise Intervention

Boys

Girls

Spine BMD

Cases Controls

Cases Controls

p<0.001

p<0.001
Figure 3

Fragility fractures after age 50 years

Fracture free survival

Athletes

Controls

RR=0.50 (0.27 - 0.89)

Age (years)
Figure 4

Proportion of Individuals with Fractures in Former Male Soccer Players and Controls Aged 60-94 Years

(Percent)
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GH/IGF-I axis and matrix adaptation of the musculotendinous tissue to exercise in humans

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Running head: GH/IGF-I axis, matrix, and exercise

This is an Accepted Article that has been peer-reviewed and approved for publication in the Scandinavian Journal of Medicine & Science in Sports but has yet to undergo copy-editing and proof correction. Please cite this article as an "Accepted Article"; doi: 10.1111/j.1600-0838.2012.01459.x
Abstract

Exercise is not only associated with adaptive responses within skeletal muscle fibres, but also with induction of collagen synthesis both in muscle and adjacent connective tissue. Additionally, exercise- and training leads to activation of the systemic growth hormone/insulin like growth factor I axis (GH/IGF-I), as well as increased local IGF-I expression. Studies in humans with pathologically high levels of GH/IGF-I, and in healthy humans who receive either weeks of GH administration or acute injection of IGF-I into connective tissue, demonstrate increased expression and synthesis of collagen in muscle and tendon. These observations support a stimulatory effect of GH/IGF-I on the connective tissue in muscle and tendon, which appears far more potent than the effect on contractile proteins of skeletal muscle. However, GH/IGF-I may play an additional role in skeletal muscle by regulation of stem cells (satellite cells), as increased satellite cell numbers are found in human muscle with increased GH/IGF-I levels, despite no change in myofibrilar protein synthesis. Although advanced age is associated with both a reduction in the GH/IGF-I axis activity, and in skeletal muscle mass (sarcopenia) as well as in tendon connective tissue, there is no direct proof linking age related changes in the musculo-tendinous tissue to an impaired GH/IGF-I axis.
The growth hormone/insulin-like growth factor I (GH/IGF-I) axis plays a vital role for the growth and maturation of children and adolescents, and is also a major regulator of substrate metabolism and insulin sensitivity (Moller & Jorgensen, 2009). Several studies have focused upon the importance of the GH/IGF-I axis in the human locomotor system, for e.g. bone and skeletal muscle development with regard to growth and metabolism. However, only very few studies have tried to investigate the role of the GH/IGF-I axis in relation to connective tissue matrix adaptation within tendon and skeletal muscle. The matrix of tendon and skeletal musculature plays an essential role in the transmission of force from the individual contracting muscle cell to the skeletal structures, resulting in limb and body movement. Thus the investigation of matrix regulation in the musculotendinous tissue is of importance for understanding its structure and function. This review will focus on the role of the GH/IGF-I axis in matrix biology in tendon and skeletal muscle.

**Exercise-induced regulation of GH/IGF I in blood**

Both circulating GH and IGF-I will increase in response to exercise (ROTH et al., 1963; Bang et al., 1990). Circulating GH responds to exercise in tight relation to the exercise intensity, and the GH level is related to the central effort in the brain to perform exercise. This view is supported by an experiment where the use of partial neuromuscular blockade, in order to raise the central effort to complete a certain exercise bout, resulted in a greater GH response compared to that of control individuals without blockade (Kjaer et al., 1987). The IGF-I response to exercise is always less pronounced than the GH response (Wallace et al., 1999), and is not necessarily just a direct result of GH release. This is indicated by the fact that the rise in IGF-I sometimes occurs earlier than is predicted from the time of GH release.
(Schwarz et al., 1996). In addition it has been shown that circulating IGF-I can rise in response to exercise in patients with pituitary insufficiency (Bang et al., 1990).

Repeated exercise and regular training leads to a chronic elevation in circulating levels of GH and IGF-I, and it has been demonstrated that training is associated with an increased capacity to secrete GH from the pituitary gland. This has been shown both in athletes who have trained for many years (Kjaer et al., 1984), and in subjects who carried out intense training over a period of one year (Weltman et al., 1992). Similarly, other hormonal responses, e.g. epinephrine release capacity from the adrenal medulla, are enhanced by training (Kjaer & Galbo, 1988).

**Local changes in IGF-I in response to mechanical stimulus**

Several studies indicate that not only circulating levels of IGF-I increase in response to exercise, but also local levels of IGF-I can be induced in tissues subjected to loading. Thus, increased expression of IGF-I mRNA in human skeletal muscle has been shown in response to both long and short-term loading (e.g. (Hameed et al., 2004; Heinemeier et al., 2011; Bamman et al., 2001), and animal data indicate that the loading-induced IGF-I expression in muscle happens independently of pituitary GH release (Yamaguchi et al., 2006). Similarly in tendon tissue, rat studies show that loading of the tendon leads to higher local expression of IGF-I on both protein and mRNA levels (Hansson et al., 1988; Heinemeier et al., 2007b; Olesen et al., 2006). In young men however, no elevation in IGF-I expression was found in the patella tendon in response to moderate endurance type kicking exercise (Heinemeier et al., 2011), indicating perhaps that loading was insufficient in this model and/or that human tendons might be less responsive to mechanical stimuli than rat tendons.
The induction of IGF-I expression in skeletal muscle in response to loading has traditionally been linked to the hypertrophic response of muscle fibres. However, there are several indications the GH/IGF-I system may be more related to the regulation of the connective tissue of the muscle-tendon unit (discussed below).

**Mechanical regulation of matrix synthesis**

The dominating component of tendon and muscle connective tissue is collagen, primarily type I and III fibrillar collagen. The total collagen content represents 80-90% of the tendon tissue organic mass, whereas in muscle the percentage is 5-10%, primarily located around the muscle fibres (endomysium, perimysium) or surrounding the entire muscle (epimysium) (Light & Champion, 1984). Type I and III pro-collagen molecules are composed of 3 poly-peptide chains (type I collagen of two α1(I) chains and one α2(I) chain; type III collagen of three α1(III) chains) that are coupled in a triple-helix. Following removal of C- and N-terminal pro-peptides from pro-collagen, the collagen molecules self-assemble extracellularly and form collagen fibrils (Mylläharju & Kivirikko, 2001).

Mechanical stimuli of cells/tissue appears to be a major regulatory factor in collagen homeostasis, and many in vitro studies demonstrate enhanced collagen expression and synthesis in response to mechanical loading (reviewed by (Chiquet et al., 2009)). Similar to this, mechanical loading through exercise/training can induce collagen synthesis in both the loaded tendon and muscle tissue of humans. This is shown by local changes in type I collagen pro-peptide levels (Langberg et al., 1999; Cramer et al., 2004), and by increases in incorporation of amino-acid tracers (Miller et al.,
2005). Additionally, short-term strength training in rats leads to increased mRNA expression of collagen I and III in both tendon and muscle tissue (Heinemeyer et al., 2007a). A number of in vitro studies suggest that this mechanical stimulation of collagen expression and synthesis depends on the auto/para-crine action of certain growth factors, including insulin like growth factor-I (IGF-I) (Hansson et al., 1988; Abrahamsson & Lohmander, 1996; Butt & Bishop, 1997; Yang et al., 2004; Nakama et al., 2006; Schild & Trueb, 2002). Thus, it may be speculated that mechanical loading via exercise leads to induction of GH/IGF-I systemically and locally, and that this is related to regulation of matrix production in tendon and muscle tissue.

**GH/IGF-I in stimulation of collagen synthesis**

A stimulatory action of IGF-I on collagen synthesis in connective tissue is supported by several in vitro and animal studies. Thus, in vitro studies on human fibroblasts and rabbit tendon explants show robust increases in collagen synthesis in response to IGF-I (Bird & Tyler, 1994; Goldstein et al., 1989; Abrahamsson et al., 1991; Abrahamsson & Lohmander, 1996), and over-expression of IGF-I in striated muscle in mice leads to elevated levels of collagen in heart muscle (Delauher et al., 1999). Furthermore, administration of rhGH to GH-deficient dwarf rats results in an up-regulation of local IGF-I and collagen (I/III) mRNA expression in skeletal muscle (Wilson et al., 1995), as well as a higher collagen turnover in knee tendon and ligaments (Kyparos et al., 2002).

Similarly in humans, considerable evidence for a connection between GH/IGF-I and collagen production exists. Thus, agromegalic patients who have high concentrations
of GH and IGF-I display increased formation of collagen rich tissues, such as bone (Ezzat et al., 1993). In line with this, a more recent study demonstrated higher levels of collagen and IGF-I mRNA in local musculotendinous tissue in adult acromegalic patients compared to adult GH-deficient patients (Doessing et al., 2010b), as well as a tendency towards a higher collagen protein synthesis rate (Doessing et al., 2010b). Furthermore, supplementation with GH in hypo-pituitary resulted in a rise in mRNA for both IGF-I and collagen in skeletal muscle (Sjogren et al., 2007). Similarly, in healthy young individuals subjected to 2 weeks of GH injections (with doubling of normal circulating IGF-I), it was found that IGF-I mRNA, collagen I mRNA, and collagen protein synthesis was elevated in both tendon and muscle, whereas muscle myofibrillar protein synthesis was unaffected (Doessing et al., 2010a). These results support a collagen-stimulating role of GH/IGF-I in human connective tissue and indicate that GH/IGF-I is more important in strengthening of the matrix tissue than for muscle cell hypertrophy in adult human musculotendinous tissue (further discussed below).

The above-mentioned studies do not clearly conclude whether GH or IGF-I plays a dominating role in stimulation collagen synthesis. However, both systemic and local administration of IGF-I in animals was shown to increase tendon collagen content, and also to improve the strength of the tendon (Dahlgren et al., 2002; Provenzano et al., 2007). In line with this a coupling between local IGF-I and healing of skin has been demonstrated (Dunaiski & Belford, 2002). The direct link between IGF-I and collagen synthesis is further supported by a recent human study, where IGF-I was injected directly into the patella tendons, resulting in an increase in local collagen synthesis relative to control tendons injected with saline (Hansen et al., 2012). These
observations indicate that IGF-I in itself is at least sufficient to induce collagen production, and it seems likely that IGF-I dominates over GH in regulation of collagen homeostasis.

The signalling pathway for IGF-I mediated induction of collagen synthesis has not been described in detail, although *in vitro* studies on human fibroblasts suggest that IGF-I induces collagen production through the IGF-I receptor (Bird & Tyler, 1994; Goldstein *et al.*, 1989). Data on fetal lung fibroblasts suggests that this signalling is propagated through the PI-3 kinase pathway (Chetty *et al.*, 2006), while results from human hepatic stellate cells indicate that IGF-I mediated collagen stimulation is dependent on both PI-3 kinase and ERK (Svegliati-Baroni *et al.*, 1999). Finally results from human dermal fibroblasts indicate that the IGF-I mediated stimulation of collagen expression may be a secondary result of and induction of TGF-β1 synthesis, which in turn leads to increased collagen synthesis (Ghahary *et al.*, 2000).

**GH/IGF-I as mediators of loading induced collagen synthesis**

Taking into account the evidence of loading-induced increases in circulating GH/IGF-I, and local IGF-I, combined with the stimulating effect of IGF-I on collagen synthesis, it seems that GH/IGF-I may well play a mediating role in mechanically induced stimulation of matrix production. As mentioned earlier, prolonged training leads to an increased capacity to secrete GH in humans (Weltman *et al.*, 1992; Kjaer *et al.*, 1984) and at the same time an increased Achilles tendon cross sectional area is found in long term runners (Rosager *et al.*, 2002). Although it is tempting to believe that increased circulating levels of GH/IGF-I could contribute to these training induced
adaptations, it is unlikely to be the full explanation. The most important determinant for tendon growth and size in adult humans is most likely the degree of mechanical loading on the individual tendon. In support of this, runners and jumpers not only had thicker Achilles tendons compared to untrained counterparts, but also compared to elite-trained kayakers who carried out a less weight bearing sports (Kongsgaard et al., 2005). Furthermore, studies of male athletes competing in sports with a pronounced side-to-side difference (badminton and fencing) showed a greater cross sectional area of the patella tendon in the leading leg compared to the non-leading leg (Couppe et al., 2008). Whereas these finding do not support an effect of circulating levels of GH/IGF-I, it seems likely that local concentrations of IGF-I could influence the adaptation. In support of IGF-I playing such a role, it has been shown that loading of the rat tendon-muscle unit leads to increased local expression of IGF-I mRNA in parallel with collagen mRNA in both muscle and tendon tissue (Heinemeyer et al., 2007b; Heinemeier et al., 2007a; Olesen et al., 2006).

**GH-IGF-I, skeletal muscle and its stem cells**

Although a positive correlation exists between circulating GH levels and whole body protein synthesis, studies on GH supplementation to both young and elderly healthy individuals have not been able to demonstrate any enhancing effect of GH on muscle mass or muscle strength either per se or as an addition to muscle strength training (Yarasheski et al., 1992; Lange et al., 2002). This view is further supported by data indicating that a functional IGF-I receptor is not crucial for overload muscle hypertrophy in mice (Spangenburg et al., 2008). In addition recent studies on young men found equal acute and long-term muscle anabolic responses to strength training whether this was done at high or low GH/IGF-I environments (West et al., 2010; West
et al., 2009). In contrast, GH administration has been shown to have an enhancing effect upon muscle growth, strength and performance in GH deficient children and adults (Cuneo et al., 1991; Lucidi et al., 1998; Mauras et al., 2000) and also in immature animals (Molon-Noblot et al., 1998). Importantly, it has recently been shown in mice that IGF-I only induces muscle hypertrophy in growth situations (Shavlakadze et al., 2010). This illustrates that in healthy adult skeletal muscle, no additional growth will be achieved with GH/IGF-I administration, and that GH/IGF-I in relation to skeletal muscle is mostly of importance for early development and growth. In support of this, 2 weeks of GH administration did not result in any rise in muscle myofibrillar contractile protein synthesis (Doessing et al., 2010a), nor could any difference in muscle protein synthesis be detected between acromegalic and adult growth hormone deficiency patients despite a 2 fold difference in circulating GH and IGF-I levels (Doessing et al., 2010b). Interestingly, in those two human studies, the detection of human pax-7 positive satellite cells (muscle stem cells) in skeletal muscle showed that the number of satellite cells per muscle fibre was elevated in GH treated young men vs. non-treated, and also in acromegalic patients vs. growth hormone deficient (Fig. 1). The association between IGF-I levels and stem cell activity is supported by a recent study on mice, in which an increase in the number of pax-7 positive cells observed in skeletal muscle in response to viral-mediated IGF-I gene transfer (Stevens-Lapsley et al., 2010). In addition, previous mouse data indicated that satellite cell activation was an important part of IGF-I mediated muscle hypertrophy in mice (Barton-Davis et al., 1999). In adult humans the stimulatory effect of GH/IGF-I on myofibrillar protein synthesis seems absent (Doessing et al., 2010a), however our data indicate that the stimulatory effect of GH/IGF-I on satellite cell activity does exist in human skeletal muscle (Fig. 1). This finding, in combination
with the enhancing effect of GH/IGF-I on matrix production, adds support to the suggestion that IGF-I is an important factor in regeneration of injured muscle tissue (Charge & Rudnicki, 2004).

**Matrix in tendon and muscle: Potential effect of GH-IGF I in ageing**

Ageing is associated by a decrease in both GH and IGF-I plasma levels (somatopenia) (Zadik et al., 1985), but whether this decline in the GH/IGF-I axis is directly related to any changes of matrix in tendon or skeletal muscle is unknown. The collagen content in human tendon has only recently been determined and compared in young and old individuals (Couppe et al., 2009). Here it was found that the collagen content was approximately 30% lower in old vs. young tendon (Couppe et al., 2009). This finding supports the possibility that reduced activity in the GH/IGF-I axis in older individuals could result in lower collagen content in elderly human tendon. In skeletal muscle very few studies have been carried out in relation the effect of age on the collagen rich connective tissue. Some studies find indications of increased relative amounts of collagen in skeletal muscle of animals with ageing (Kovanen & Suominen, 1989), as well as indications of higher collagen synthesis rates in skeletal muscle of old compared to young men (Babraj et al., 2005). On the other hand a more recent human study demonstrated no difference in collagen content between young and old skeletal muscle (Haus et al., 2007). Thus no clear picture exists, with regard to ageing associated changes in muscle collagen, and the existing data do not support the view that impaired IGF-I contributes significantly to the age associated changes in the matrix regulation.
In summary, current data show that elevated circulating GH/IGF-I, concomitant with increased local IGF-I, leads to stimulation of collagen expression and synthesis in the connective tissue of the muscle-tendon unit in both humans and animals. Given the induction of local IGF-I observed simultaneously with increased collagen expression in response to mechanical loading of the muscle/tendon tissue during exercise, it is suggested that IGF-I may mediate loading-induced collagen synthesis. However, no causal relationship linking IGF-I to the exercise induced regulation of connective tissue synthesis has yet been verified, and further studies are needed to confirm this connection. In addition, the association between high GH/IGF-I activity and satellite cell number could indicate a link between GH/IGF-I and stem cell function in adult human skeletal muscle.
Figure legends

**Figure 1:**
Differing numbers of satellite cells (Pax7+) under conditions of high and low growth hormone in, A) skeletal muscle biopsies from Acromegalic (Acro) and Growth hormone deficient (GHD), and B) Growth hormone (GH) and Placebo (Pl) treated individuals. Mean +/- SEM error bars; #p=0.06 Acro vs. GHD, * p<0.01 GH vs. PI; Mann-Whitney test.

**Figure 2: Effect of growth hormone (GH) supplementation on muscle and tendon tissue.**
GH supplementation (1) leads to increased circulating levels of IGF-I and to increased local expression of IGF-I in skeletal muscle and tendon tissue. This is concurrent with increased expression of fibrillar collagen in muscle (2) and tendon tissue (3) as well as increased satellite cell numbers (4). Myofibrillar protein synthesis appears unaffected by the increased levels of both circulating and local IGF-I levels (grey arrows). (Modified from Doessing & Kjaer, Physiology News, Autumn 2010).
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Rehabilitation of muscle after injury – the role of anti-inflammatory drugs

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This is an Accepted Article that has been peer-reviewed and approved for publication in the Scandinavian Journal of Medicine & Science in Sports but has yet to undergo copy-editing and proof correction. Please cite this article as an "Accepted Article"; doi: 10.1111/j.1600-0838.2012.01463.x
Running head:
Skeletal muscle injury
Abstract

Nonsteroidal anti-inflammatory drugs (NSAIDs) are widely consumed among athletes worldwide in relation to muscle injury and soreness. This review aims to provide an overview of studies investigating their effects on skeletal muscle, in particular the repair processes in injured muscle. Muscle injury occurs in diverse situations and the nature of muscle injuries varies significantly, complicating extrapolations between experimental models and ‘real life’. Classical muscle strain injuries occur at the interphase between the muscle fibres and connective tissue, most often in the myotendinous junction, whereas contusion or overload injury can damage both myofibres and intramuscular connective tissue. The role of NSAIDs in muscle repair is complicated by differences in injury models used, variables evaluated, and time point(s) selected for evaluations.

While the temporal pattern of the influence of NSAIDs on muscle repair is difficult to settle on, it appears that a potential beneficial effect of NSAIDs in the early phase after injury is not maintained in the long term, or even negated by a long term repair deficit. At the cellular level, evidence exists for a negative influence of NSAIDs on the muscle stem cell population (satellite cells). At a structural level, it is known that muscle connective tissue undergoes significant remodelling during muscle regeneration, but the potential of NSAID exposure to alter this response in humans needs investigation.

Key words:
Nonsteroidal anti-inflammatory drugs (NSAIDs), muscle damage, eccentric exercise, muscle regeneration, connective tissue, DOMS
Introduction
Nonsteroidal anti-inflammatory drugs (NSAIDs) are a class of analgesic medicines available over the counter worldwide and often employed in the early stages after muscle injury, despite a lack of clinical studies to evaluate their effectiveness. A growing awareness of the extent of NSAID consumption in the sporting world in particular has begun to emerge in the last decade, where reports now document widespread use among sports men and women from college to elite levels of performance, with youth players also being well represented (Alaranta et al., 2006; Tscholl et al., 2009; Tscholl et al., 2008; Warner et al., 2002). An interesting outcome of these reports is that the incidence of reported NSAID use generally exceeds the incidence of reported injury (Tscholl et al., 2009), leading to the conclusion that NSAIDs are not only consumed in the treatment of muscle injury, but also on a prophylactic basis (Warden, 2009; Warden, 2010). Despite this widespread use, whether NSAIDs are detrimental or beneficial for a muscle undergoing repair from injury, or indeed for muscle adaptation in general, remains unclear. The purpose of this review therefore is to provide an overview of the studies investigating the effects of NSAIDs on skeletal muscle, in particular how this medication may influence the repair processes in an injured muscle. Human studies have been cited, where available, and the relevant contributions in this context from animal and cell models are also included.

The nature of muscle injury
The treatment of muscle injury and the time it takes to repair varies according to the nature of the injury. Muscle injury can occur in many different situations, such as during explosive movement, direct impact to the muscle, and under controlled experimental conditions designed to study the mechanisms behind muscle injury and repair. Unfortunately the nature of these types of injuries is not the same and it is therefore often difficult to extrapolate findings from experimental studies on the role of NSAIDs in muscle repair to injuries sustained during more “real life” situations.

Strain and contusion injuries
Detailed descriptions of the types of muscle injury can be found elsewhere (Järvinen et al., 2007) so will only be briefly mentioned here. The classic muscle rupture is the strain injury, occurring at the interphase between the muscle fibres and connective tissue components of the muscle. Such sites include the fibre–aponeurosis and the fibre–tendon (myotendinous junction) connections. Hence, the terms “strain” or “rupture” refer more strictly to the point of attachment of the muscle fibres to these connective tissue structures, rather than an actual disruption of the muscle fibres themselves. This type of injury typically occurs in the hamstring, quadriceps or calf muscles as a result of explosive sprinting or kicking activity. Contusion injury, on the other hand, usually results from direct contact with another athlete, for example during a tackle when the deep muscles of the quadriceps are compressed against the femur by the opponent’s knee. In this situation the impact of the collision on the muscle can lead to vascular damage within the muscle, often resulting in a substantial haematoma. The extent of muscle fibre damage varies and the length of recovery time depends on how quickly the haematoma can be cleared (anything from 2 to 10 weeks). Muscle strain injuries are slower to heal (typically 8 to 12 weeks), due to the required reconstruction of the intricate muscle–matrix interphase (see Figure 1) and susceptibility to re-injury before healing is fully complete. The extent of injury (see Figure 2) and repair progress can be followed by ultrasound (Thorsson et al., 1993).

Figure 1 ‘Transmission electron micrograph of human myotendinous junction’ near here
Myofibre injury
Damage or rupture of individual muscle fibres can occur following unaccustomed exercise, especially when the muscle performs lengthening (eccentric) contractions, as in the case of the quadriceps muscles during the downward phase of the squat exercise. Delivery of a high level (pain threshold limit) of neuromuscular electrical stimulation to the muscle in a controlled experimental setup, with or without eccentric contractions, can also induce myofibre damage (Crameri et al., 2007; Mackey et al., 2008). Damage to muscle fibres as a result of these human models has been repeatedly documented by light and electron microscopy analysis of muscle biopsy specimens obtained from the affected muscle at varying time points after the activity (Crameri et al., 2007; Jones et al., 1986; Lauritzen et al., 2009; Mackey et al., 2008; Newham et al., 1983), and complete regeneration from such damage is known to occur in healthy individuals, although this may take longer than 3-4 weeks (Mackey et al., 2011; Paulsen et al., 2010). In experimentally-induced muscle damage, a significant drop (40-60% is not uncommon) in the force-producing capacity of the muscle has been reported, from which full functional recovery can take up to 3-4 weeks (Ebbeling & Clarkson, 1989; Mackey et al., 2004).

Delayed-onset muscle soreness
The development of muscle soreness is also a common outcome of performing unaccustomed exercise (Clarkson et al., 1986; Crameri et al., 2007; Mackey et al., 2004), and can occur in the presence or absence of actual damage to the muscle fibres (Crameri et al., 2007). The delay in onset, typically not reaching its peak until 2-3 days after the activity (Child et al., 1998; Crameri et al., 2007; Mackey et al., 2008), has given rise to the term delayed-onset muscle soreness (DOMS), the extent of which can range from minor irritation to being quite debilitating in severe cases, resulting in acute pain during lengthening actions of the affected muscles. Investigations into the potential of NSAIDs to alleviate DOMS in exercised muscles are numerous, and the outcomes are inconsistent, with roughly equal numbers finding no effect of NSAIDs on DOMS (Arendt-Nielsen et al., 2007; Bourgeois et al., 1999; Donnelly et al., 1990; Kuipers et al., 1985; Mikkelsen et al., 2009; Nieman et al., 2006; Trappe et al., 2002) or a significant attenuation of DOMS (Baldwin et al., 2001; Donnelly et al., 1988; Dudley et al., 1997; Hasson et al., 1993; O'Grady et al., 2000; Paulsen et al., 2010; Sayers et al., 2001; Tokmakidis et al., 2003). It is possible that differences in the experimental model, extent of damage and timing or dosage of medication contribute to these divergent outcomes. However, since DOMS is not necessarily indicative of true muscle injury, combined with the fact that DOMS only occurs with unaccustomed exercise and not following repeated bouts of the same exercise (Byrnes et al., 1985; Nosaka et al., 2001), it can be argued that the issue of whether NSAIDs are effective in treating DOMS is of lesser importance than their effect on repair of injury.

Evidence for the potential of NSAIDs to influence the repair of injured skeletal muscle
Comparison of the many reports in the literature examining the role of NSAIDs in muscle repair is difficult due to differences in the model of muscle injury and the type of medication used, the variables on which evaluation is based, and, most importantly, the time point(s) selected for these evaluations. For example, it is not possible to draw conclusions on the long
term outcome if only one early time point is included. (In general in this review, we define “early” as a few days, and “long term” as up to one or two months.) While some investigations have not found any effect of NSAID administration on muscle recovery (Donnelly et al., 1990; Paulsen et al., 2010; Tokmakidis et al., 2003), there are several reports supporting a protective effect of NSAID medication, typically characterised by a lesser degree of muscle damage and function deficit in the early period after injury (Baldwin et al., 2001; Bourgeois et al., 1999; Cheung & Tidball, 2003; Dudley et al., 1997; Hasson et al., 1993; Lapointe et al., 2003; Mishra et al., 1995; O’Grady et al., 2000; Sayers et al., 2001). However, the few studies that have followed the repair process over a longer period of time suggest that any apparent benefit of NSAID treatment in the short term is not maintained in the long term (Almekinders & Gilbert, 1986; Mikkelsen et al., 2009; Paulsen et al., 2010; Vignaud et al., 2005), or even negated by a long term deficit (Mishra et al., 1995; Obremsky et al., 1994; Shen et al., 2005) in force production and muscle repair. It should be noted though that the distinction made here with regard to the short and long term outcomes is based on a broad mixture of different experimental models from human and animal studies. While the relevance of the experimental models used in these studies is low for strain injuries (where the site of rupture is the myotendinous junction or aponeurosis rather than the muscle fibres themselves), they nonetheless indirectly provide insight into the influence of NSAID treatment on the intramuscular structures and cells involved in the repair process.

**NSAID effects on cells responsible for muscle repair**

A population of muscle stem cells, known as satellite cells, is well recognised as being indispensable for the repair of skeletal muscle (Lepper et al., 2011; Sambasivan et al., 2011). The interplay between satellite cells and other cell types, such as macrophages (Arnold et al., 2007; Sonnet et al., 2006), endothelial cells (Christov et al., 2007) and fibroblasts (Murphy et al., 2011), is currently a growing field in muscle regeneration research. Taken together, there is accumulating evidence from human and animal studies for an inhibitory effect of NSAID action on expansion of satellite cell and macrophage numbers (Bondesen et al., 2004; Bondesen et al., 2006; Mackey et al., 2007; Mikkelsen et al., 2009; Monda et al., 2009; Shen et al., 2005), underlining the potency of NSAIDs to influence the behaviour of cells playing a key role in muscle repair. In contrast to this however, there is one study reporting increased numbers of ED2 macrophages (cells associated with muscle repair) in rat muscle subjected to unloading followed by reloading, when Ibuprofen was administered (Cheung & Tidball, 2003). Support for no effect of NSAID administration on satellite cell or fibroblast proliferation, or capillarisation, has been reported in rat muscle recovering from contusion injury (Thorsson et al., 1998). While the literature is not entirely consistent, it does appear that the evidence for a negative influence of NSAIDs on cellular activity during muscle repair outweighs evidence for a beneficial effect and thus warrants further study of how NSAID action could affect the interaction between inflammatory and regenerative processes in injured skeletal muscle (see Figure 3).

**Figure 3 ‘Schematic illustrating the potential sites of NSAID action with relevance for the repair of muscle strain injury’ near here**

**NSAID effects on connective tissue healing**

In addition to the cells involved in muscle repair, it has also been shown that the connective tissue surrounding individual fibres (endomysium) and fibre bundles (perimysium) undergoes significant
remodelling during damage and regeneration in humans and likely plays an important role in providing protection against re-injury (Mackey et al., 2011; Mackey et al., 2004). While there are as yet no studies examining the effects of NSAIDs on the endo- and perimysium specifically, other connective tissues of the body have received some attention. The influence of NSAIDs on bone, ligament and tendon has been reviewed (Magra & Maffulli, 2006; Radi & Khan, 2005; Ziltener et al., 2010), where it appears that healing of these tissues is not improved by NSAID treatment. Furthermore, inhibitory effects of NSAID treatment have been observed on the long term healing of rat ligaments (Dahners et al., 1988; Elder et al., 2001), human joint sprains (Slatyer et al., 1997) and rat bone fractures (Endo et al., 2002). Interpretation of these outcomes however is clouded by the possibility that the medication may indirectly have facilitated a premature return to physical activity, rather than a direct effect of NSAID on the tissue.

**NSAID effects on connective tissue adaptation to exercise**

It was recently demonstrated that 12 weeks of resistance training in older persons increased the stiffness and modulus of the patellar tendon while cross-sectional area was unaltered. Interestingly, over-the-counter doses of Ibuprofen did not influence this resistance training response, while acetaminophen yielded an increased tendon cross-sectional area, but an unchanged stiffness and modulus (Carroll et al., 2011). With regard to short term responses, an inhibitory effect of NSAID exposure on the acute exercise-induced increase in collagen synthesis of healthy human tendons has been reported (Christensen et al., 2011). While this study only examines the outcome of the tendon response to a single bout of exercise, it raises the possibility that NSAID ingestion could also suppress the adaptive response of skeletal muscle connective tissue to exercise. This could take the form of hampering optimal regeneration of the complex myotendinous junction structure during recovery from a strain injury. Another possibility exists, however; that prolonged NSAID consumption could also prevent an ongoing strengthening of the myotendinous junction with long-term training, making this site more susceptible to strain injury. A recent study analysing homogenate of human muscle biopsies collected 8 days after a bout of unaccustomed high force lengthening contractions however did not find any effect of local infusion of NSAID into the working muscle on gene expression levels or fractional synthetic rate of collagen synthesis (Mikkelsen et al., 2011), despite an inhibitory effect of NSAID exposure on satellite cell proliferation in the same subjects (Mikkelsen et al., 2009). This study would appear to be in contrast to the tendon data mentioned above (Christensen et al., 2011), although collagen from two different tissues was investigated in these two studies. The adaptation of muscle connective tissue is known to be a multi-phase process though, with a strong anabolic response of the muscle extracellular matrix not occurring until relatively late in the regeneration process, preceded by an early phase where de-adhesion and disassembly of the matrix are prioritised (Mackey et al., 2011). Furthermore, muscle extracellular matrix is a complex structure, not only responsible for force transmission, but also capable of actively regulating cells in its environment (Gillies & Lieber, 2011; Kragstrup et al., 2011), and therefore represents an important, but so far, relatively neglected area of study. Further investigations are clearly required to elucidate the time course and role of the muscle connective tissue response during muscle repair following injury, and indeed the potential of NSAID exposure to alter this response, both in the context of acute muscle injury and in the ongoing training-induced fortification of muscle-matrix contact structures such as the myotendinous junction (see Figure 3).
NSAIDs and muscle adaptation without injury

While studies uncovering an effect of NSAID action at the cellular level are valuable in the overall understanding of how this medication may affect skeletal muscle, it can be argued that the cumulative outcome in terms of function is the most important variable to consider. Further evidence for the potency of NSAID treatment to influence muscle adaptation can be found in investigations into the development of muscle hypertrophy in response to long-term overload or resistance training. A 50% - 75% blunting of the muscle hypertrophy response to overloading has been reported in animals treated with NSAIDs (Novak et al., 2009; Soltow et al., 2006). While NSAID ingestion has been reported to suppress the protein synthesis response in young individuals to a single bout of exercise (Trappe et al., 2002), the outcome from human studies exploring the potential of NSAIDs to limit the hypertrophy response to 3 months of resistance training is not as clear (Petersen et al., 2011; Trappe et al., 2011).

NSAID treatment was observed to have no effect on gains in muscle size in elderly patients with osteoarthritis (Petersen et al., 2011), although maximal muscle strength increased slightly, but significantly, more in the NSAID group compared with the placebo group (Petersen et al., 2011). Another human study demonstrated a clear positive influence of NSAID consumption on hypertrophy in healthy elderly individuals, with the Ibuprofen group demonstrating a significantly greater increase in quadriceps muscle volume, combined with superior increases in strength, when compared with the placebo group (Trappe et al., 2011). While these findings may appear out of line with the animal studies presented here, it is possible that an age-associated elevation in systemic levels of inflammatory cytokines may explain the discrepancy. Indeed, it has been shown in old rats that Ibuprofen ingestion lowered the naturally-occurring age-associated systemic inflammation and resulted in a reduced loss of muscle mass, compared to control rats (Rieu et al., 2009). This explanation may also be behind the positive findings of NSAID treatment on recovery of force production in elderly individuals after a single bout of eccentric contractions (Baldwin et al., 2001). While this area of research is still in its infancy and requires further investigation, it can be speculated that NSAID treatment may have a positive effect on muscle injury in individuals with elevated circulating levels of inflammatory cytokines, such as might be the case in some elderly individuals. Further study is also required to evaluate potential differences between young and elderly in acute and chronic responses to exercise stimuli.

Perspectives

While there is evidence for and against a beneficial role of NSAIDs in muscle repair after injury, it appears on balance that reports favouring NSAID treatment may be outweighed by evidence pointing to a long term negative influence of NSAIDs on muscle recovery from injury and adaptation of muscle and connective tissue to exercise training. An exception to this may be certain individuals with elevated systemic levels of inflammatory cytokines. However, in light of the adverse side effects and health risks associated with consumption of this medication, the risk-benefit ratio needs to be carefully evaluated when considering NSAID ingestion. Given the importance of muscle connective tissue as the main site of strain injuries and its role in cell signalling, it is clear that studies investigating the time course and the role of the muscle connective tissue response during muscle repair following injury, and the potential of NSAIDs to alter this response, could contribute valuable knowledge to our understanding of how the repair of injured muscle could be optimised.
Acknowledgements
Michael R. Krogsgaard and Klaus Qvortrup are acknowledged for their help. Funding is gratefully acknowledged from MYOAGE (nr. 223576) funded by the European Commission under FP7, The Danish Medical Research Council (no. 10-094021) and from Nordea Foundation (Healthy Ageing grant).
References


Legends

Figure 1. Transmission electron micrograph of human myotendinous junction (MTJ).
Transmission electron micrograph of human MTJ where the interdigitations at the contact surface between the muscle and tendon are clearly visible.

Figure 2. Ultrasonography picture of an acute muscle strain injury.
Ultrasonography picture of an acute muscle strain injury in a young female judo athlete who experienced sudden pain in the right calf after landing. The ultrasonography picture is taken at day 7 after injury and demonstrates a rupture in the myo-tendinous transition from m. gastrocnemius and the Achilles tendon, as well as a defect between m. gastrocnemius and m.soleus. On the left picture regions in the injured right side and the healthy left side are displayed, and on the right picture, the injured region is shown in a more complete view. Note that the muscle itself on ultrasonography appears relatively intact.

Figure 3. Potential sites of NSAID action in repair of muscle strain injury.
Schematic (compare with Fig. 1) illustrating the potential sites of NSAID action with relevance for the repair of muscle strain injury. Effects have been reported for both muscle and tendon, while the actual site of strain injury, the myotendinous junction (MTJ), remains to be investigated. Satellite cells (SC), macrophages (M), and fibroblasts (F), as well as the signalling between these cells, are likely targets at the cellular level.
Fig 2
Fig 3