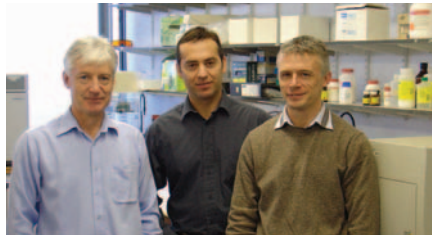


Adaptive responses to starvation in humans: important role for skeletal muscle PDK4

In humans, adaptations to fasting have evolved in order to survive periods of limited food resources and thus starvation. One important aspect of the adaptive response to fasting is a reduction in skeletal muscle carbohydrate utilization in order to spare glucose for those organs and tissues with an obligatory requirement for it (e.g. central nervous system). Muscle pyruvate dehydrogenase kinase (PDK4), the enzyme that impairs the rate-limiting step in glucose oxidation, is of major importance in mediating the starvation-induced shift in metabolic fuel utilization

The physiological and metabolic responses to fasting have been studied extensively in many species over the last 150 years. William North published his studies on the effects of starvation and exercise on nitrogen metabolism (in which he acted as his own subject and observed an increase in whole body nitrogen excretion under both conditions) in June 1878, in the second ever issue of *The Journal of Physiology* (North, 1878). In the same journal Pembrey and Spriggs (1904) observed that during fasting in rats the respiratory quotient (and thus glucose oxidation) decreased quickly within the first few days of fasting and remained constant during the prolongation of fast. In 1932, Goldblatt and his co-workers in St Thomas's Hospital, London presented evidence of carbohydrate (CHO) intolerance after starvation in healthy men (Goldblatt & Ellis, 1932). Specifically, in response to ingestion of a standardized glucose load following a 39h fast, as opposed to overnight fast, they observed an augmented blood glucose response and lower respiratory quotient and CHO oxidation rate. The starvation-induced impairment in CHO oxidation persisted even after injection of 10 units of insulin prior to glucose ingestion, prompting the authors to suggest that this may be an important adaptation which facilitates glycogen repletion in tissues (such as skeletal muscle and liver) that may have incurred a fall in glycogen content during the previous period of starvation. To our knowledge, this was the first experimental evidence of fasting-induced insulin resistance in humans.

Although these questions are still pertinent today, limited progress has been made since those pioneering studies in elucidating the precise



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mechanism(s) controlling the starvation-induced switch in substrate utilization and the development of insulin resistance in humans. Studies on humans from our laboratory in the 1990s using insulin clamps and stable isotopes demonstrated a reduction in whole body insulin sensitivity and a shift in basal (non-insulin) and insulin-stimulated substrate utilization from CHO to fat after 36-72h of starvation (Mansell & Macdonald, 1990; Webber *et al.* 1994). Importantly, starvation resulted in a marked reduction in glucose uptake by the forearm muscle both in the basal state and during insulin infusion, indicating profound muscle insulin resistance (Mansell & Macdonald, 1990).

What is the mechanism by which skeletal muscle downregulates its glucose oxidation? Some elegant animal studies performed by Mary Sugden and her coworkers in the late 1980s and early 1990s showed that suppression of skeletal muscle pyruvate dehydrogenase complex (PDC) activity plays a major role in the down-regulation of glucose oxidation in response to starvation (Sugden *et al.* 1993). Skeletal muscle PDC activity is inhibited by phosphorylation of the complex by pyruvate dehydrogenase kinase (PDK). The more recent identification of at least four different forms of PDK (PDK1-4) in skeletal muscle has intensified the research on

their role as potential molecular regulators of glucose oxidation under a number of nutritional and pathological conditions of altered glucose homeostasis, including starvation and refeeding.

Therefore, our most recent study using insulin tolerance tests and muscle biopsies attempted to elucidate the intramuscular mechanisms underlying the adaptive response to fasting for 48h and to subsequent refeeding with a CHO-rich diet for 24h in healthy humans (Tsintzas *et al.* 2006). Our findings confirmed the previously demonstrated starvation-induced development of insulin resistance (as whole body insulin sensitivity decreased by ~42% after fasting) and demonstrated that this persists even after 24h of refeeding (as insulin sensitivity recovered by only half of the reduction upon refeeding) (Fig. 1A). Similarly, starvation decreased and refeeding increased skeletal muscle PDC activity, although there was a tendency for the latter to be lower after refeeding when compared with the pre-starvation value (Fig. 1A). As PDK is a major regulator of PDC, we also measured the expression of all four isoforms of PDK identified in human skeletal muscle. Starvation increased muscle PDK4 gene and protein content (without affecting the other three isoforms), whereas refeeding reversed these responses (Fig. 1B). We concluded that the selective increase in protein content of PDK4 in human skeletal muscle is an important adaptation to starvation, which most likely contributes to the long-term control of PDC activity and thus reduction of CHO oxidation under those conditions. In addition to these changes in factors affecting glucose oxidation, we also observed a decrease

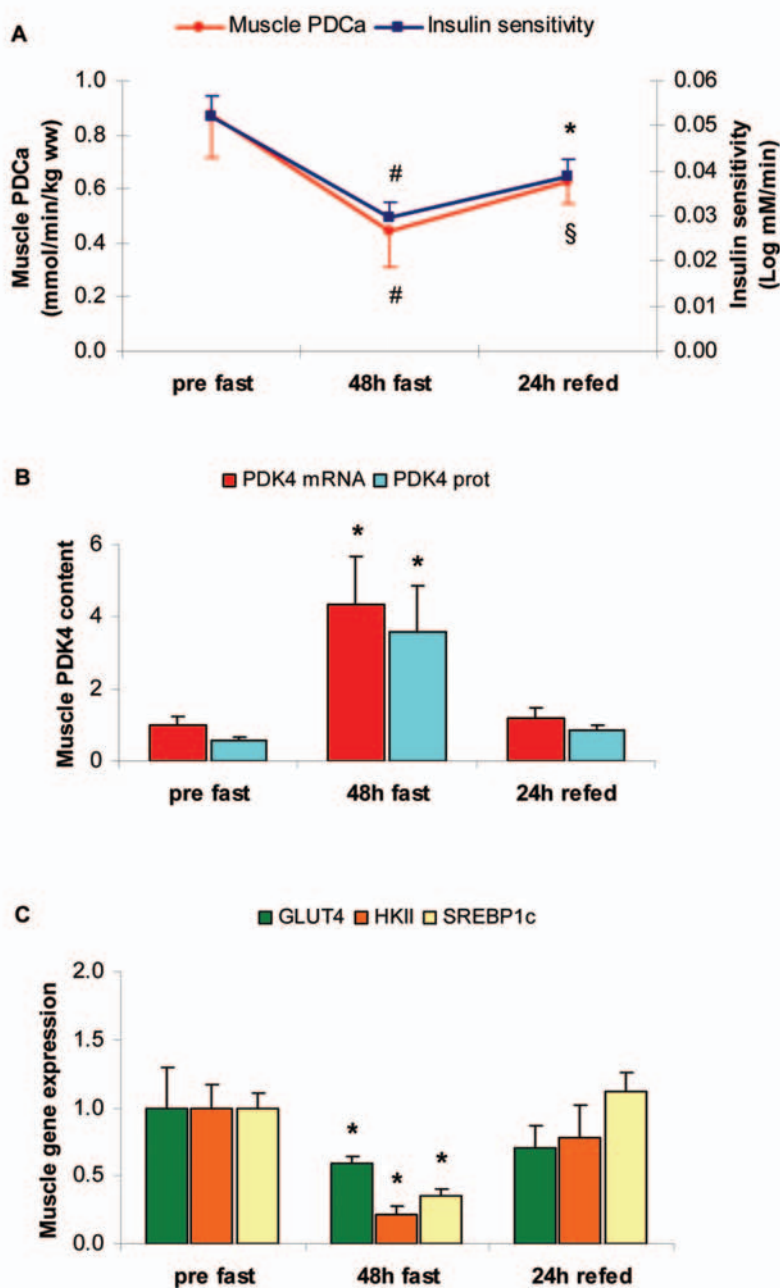


Figure 1. Whole body insulin sensitivity and skeletal muscle PDC activity (1A), PDK4 mRNA and protein (1B) and GLUT4, HKII and SREBP1c mRNA (1C) responses to 48 h starvation (Fast) and 24 h refeeding (Refed) with a high CHO diet. Values are mean \pm SEM; $n = 10$ for insulin sensitivity and all mRNA data and $n = 9$ for PDCa and PDK4 protein data. # $P < 0.01$ from pre-fast; * $P < 0.05$ from pre-fast; § $P < 0.10$ from pre-fast.

in the expression of key genes involved in muscle glucose uptake and phosphorylation: hexokinase II (HKII) and SREBP1c (a transcription factor that mediates the effects of insulin on HKII) were downregulated by 5- and 2.5-fold, respectively, after 48h of starvation whereas refeeding completely reversed these responses (Fig. 1C). Although muscle GLUT4 content also decreased by ~ 2 -fold in response to starvation, its reversal was incomplete by refeeding and closely

matched the slow recovery of insulin sensitivity (unpublished observation) (Fig. 1C).

What is the mechanism by which starvation increases PDK4 content in human skeletal muscle? We have recently shown for the first time in healthy humans that insulin can suppress PDK4 gene expression in human skeletal muscle (Chokkalingam *et al.* 2007) whereas elevated levels of plasma free fatty acids (FFA) can

elevate the muscle PDK4 content (unpublished observation). Since circulating insulin concentrations decrease whereas FFA levels increase during starvation, these may be important regulators of PDK4 expression in muscle during starvation.

However, in contrast to what one may expect, starvation or refeeding failed to alter the content and/or activate (by phosphorylation) important muscle insulin signalling proteins (including IRS1 and 2, Akt/PKB, FOXO1) (Tsintzas *et al.* 2006). Our findings also showed that starvation and the concomitant increase in circulating fatty acids did not affect the expression of transcription factors [peroxisome proliferator-activated receptors (PPARs) and their coactivator PGC1 α] and key genes involved in muscle fatty acid uptake and oxidation, namely fatty acid translocase (CD36), carnitine palmitoyltransferase 1 (CPT1) and long-chain acyl-CoA dehydrogenase (LCAD) (Fig. 2). Collectively, these findings suggest that, in contrast to results from animal and *in vitro* studies, an increase in skeletal muscle PDK4 content in fasted humans may occur in a novel manner distinct from the PPARs and insulin signalling pathways. Future studies should examine whether changes in intramuscular substrate availability/flux are responsible for the adaptive changes in glucose metabolism during fasting in humans.

In summary, it has been known for a long time that healthy humans adapt to fasting by increasing fat and reducing carbohydrate utilization in skeletal muscle in order to spare glucose for those organs and tissues (e.g. brain) with an obligatory requirement for it. We have shown for the first time that during starvation in humans, unlike rodents, regulation of fat metabolism does not require an adaptive response at the level of gene expression, implying a much greater capacity for fat oxidation than is utilized in the overnight fasted state. In contrast, changes in the content of key genes involved in glucose uptake (GLUT4), phosphorylation (SREBP1c and HKII) and oxidation (PDK4) are required to switch off glucose utilization by muscle

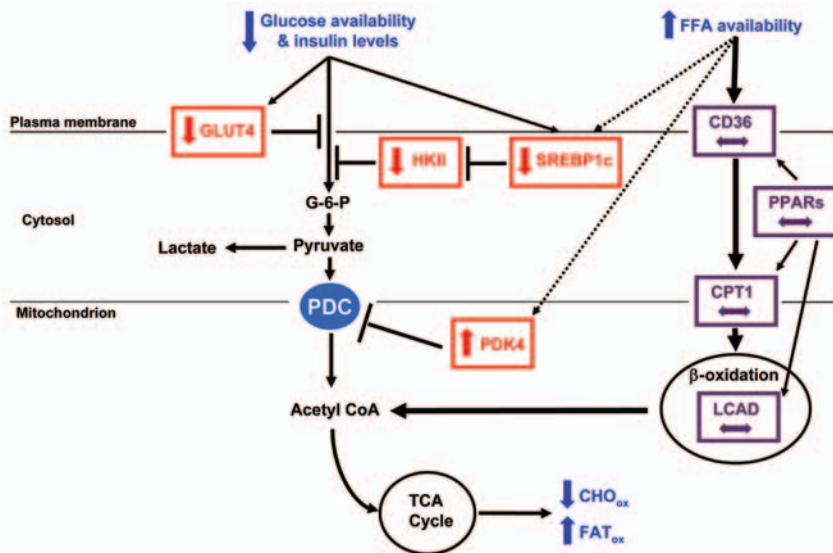


Figure 2. Simplified diagram of the adaptive response to starvation showing a shift in substrate utilization from CHO to fat in human skeletal muscle. The increase in fat availability and oxidation during starvation does not require changes in the expression of genes (CD36, CPT1 and LCAD) and transcription factors (PPARs) involved in fat metabolism (shown in purple). On the other hand, genes involved in glucose uptake (GLUT4), phosphorylation (HKII and SREBP1c) and oxidation (PDK4) are switched off (shown in red). Physiological and metabolic responses are shown in blue. Arrows indicate direction and magnitude of responses to starvation. Dashed lines indicate potential interactions.

tissue (Fig. 2). This may represent an important aspect of the molecular basis of the development of insulin resistance in metabolic conditions characterized by energy restriction.

Acknowledgments

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The antique equipment show

Have you ever noticed a pile of redundant equipment in a cupboard under the stairs or in that old store room that has been neglected for the last decade or so? Have you ever wondered why these old pieces of equipment are gathering dust in the corner? Were you thinking of putting them in the skip? All too likely as 'old-style' physiology departments merge into mega departments with relocation, refurbishment and redundancy. But someone at some time must have wanted to keep it so why not find out what it is for, who used it and whether it really is worth keeping?

One of the functions of the The Physiological Society's History and Archives Committee is the preservation of interesting pieces of equipment that may be significant in illustrating and archiving the history of physiology and of The Society. Pieces of equipment could be one of a kind or crucial to important experiments which advanced the subject. The Committee ensures that the existence of the equipment is documented and is currently making arrangements to collect and store important pieces of equipment.



Apparatus used in the 1960 Hodgkin & Horowitz paper on the effect of sudden changes in ionic concentrations on the membrane potential of single muscle fibres. *J Physiol* 153, 370-385.

So before you consign a dusty relic to rubbish please try and find out a little bit about it, who used it, and for what, and its approximate age. If you think it might be of historical interest please contact Simon Kellas (skellas@physoc.org) in the first instance and he will get in touch with a member of the History and Archives Committee who will take your enquiry further. Remember we need to preserve our history, and with a little bit of time and care we can all contribute to this process.

Saffron Whitehead
History and Archives Committee

Ernest M Wright, professor of physiology at the David Geffen School of Medicine at UCLA and a Society Member, has been elected to the German Academy of Sciences Leopoldina in recognition of his scientific achievements in the field of transport proteins which carry essential molecules in and out of cells.

Founded in 1652, and officially renamed after Emperor Leopold I in 1677, Leopoldina is the world's oldest society of scholars in the natural sciences. The number of members below the age of 75 is limited to 1,000. Three quarters of its members come from Germany, Austria and Switzerland, and the remainder from more than 30 other countries. There are currently 34 Nobel Prize winners and, in total, there have been 163 members awarded this honour. There are 28 sections of the Leopoldina ranging from mathematics to medicine.