KETONE BODIES

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Ketone bodies are water-soluble 4-carbon fuel substrates produced endogenously from fatty acids in the liver in response to low insulin and blood glucose concentrations. Ketone bodies D-β-hydroxybutyrate (βHB) and acetoacetate (AcAc) are always present in small amounts in our bloodstream (Robinson and Williamson, 1980), only increasing in response to energy deficit or some pathological conditions (Veech, 2004). Originally characterised in the odour of uncontrolled diabetes (Dreschfeld, 1886), the revelation of ketone metabolism as a vital evolutionary response to enhance survival duration in caloric deprivation took many decades to be recognised (Cahill and Owen, 1968). However, despite advances in clinical medicine, ketosis remains unfairly maligned as the 'grim reaper' of fuel substrates due to its association with poor clinical outcome in diabetic crisis (van Itallie and Nufert, 2003). This negativism, and the lack of practical utility to study ketone body metabolism outside starvation, high-fat diets, or ketone-salt intravenous infusion has meant that this fuel has, until recently, been largely ignored for its physiological potential.

Nutritional ketosis

Novel forms of edible ketone body are currently undergoing human trials (Clarke *et al.*, 2012; Cox, 2012) with promising results in exercise performance (Cox, 2012; Cox and Clarke, 2014). These dietary forms of ketone provide gut-absorbable βHB or AcAc as a novel dietary macronutrient. Delivered as a ketone body esterified to either fat (Hashim and van Itallie, 2014), or more usefully a ketone body precursor, such as the ketone monoester *R*-1,3,-Butanediol (Clarke *et al.*, 2012), blood ketone concentrations equivalent to that seen after weeks of total starvation are safely achievable in a matter of minutes (Clarke *et al.*, 2012).

Pleiotropic effects of ketone bodies on substrate metabolism

The conservation of carbohydrate reserves in the form of glycogen and gluconeogenic skeletal muscle protein is a hallmark of starvation-induced ketosis (Cahill, 1970), dramatically increasing survival duration in starving humans (Cahill and Owen, 1968; Felig *et al.*, 1969). Ketosis may also provide thermodynamic advantages over other carbon substrates by increasing the free energy conserved in ATP (ΔG_{ATP}) by the oxidation of ketones during mitochondrial oxidative phosphorylation (Veech, 2004; Sato *et al.*, 1995). The combination of improved energetic efficiency and fuel sparing is vitally important not only during famine, but may also provide a new method of sustaining

physical performance.

Athletes are ideally placed to use alternative energy sources

Athletic training increases the oxidative and enzymatic capacity of skeletal muscle for substrate combustion; in particular, blood borne nutrients (Holloszy and Coyle, 1984). While the athletic adaptations to harness greater energy requirement from fatty acids in endurance exercise are well known, ketolytic capacity also increases, with several-fold increases in ketolytic enzymes (Winder *et al.*, 1974) and reduced ketone levels in athletes vs. controls during and after exercise, suggesting greater utilisation (Johnson *et al.*, 1969). Furthermore the monocarboxylate transporters (SLC16 family) responsible for the transport of ketones through sarcolemmal and mitochondrial membranes (Halestrap and Meredith, 2004) are significantly upregulated by exercise.

Implications of ketosis for exercise performance

By their very nature as a substrate and signal in starvation, ketone bodies are oxidised by most body tissues to ease the use of carbohydrates and gluconeogenesis via proteolysis of muscle mass (Robinson and Williamson, 1980). The demands of endurance exercise place a premium on carbohydrate reserves, as skeletal muscle fuel selection during heavy exercise, such as during competitive sport, relies almost exclusively on glycogen and blood glucose for its energy requirements (Romijn *et al.*, 1993; van Loon *et al.*, 2001). In many ways, the metabolic demands of exercise parallel (albeit on a much more rapid scale) the metabolic conditions pertinent to survival in starvation.

Fuelling the engine: limitations to endurance performance

Intramuscular glycogen content is well known as a powerful determinant of endurance capacity (Bergström *et al.*, 1967), with glucose oxidation during heavy exercise often exceeding 5–8g/min; depletion of this fuel store coincides with volitional fatigue. For decades, nutritional strategies for high-performance sport have focused on finding methods to spare glucose reserves, either by providing alternative energy sources to compete with glucose for respiration, or to enhance the contribution of exogenous fuels to total energy requirements (Jeukendrup, 2004). However, under conventional metabolic conditions, muscle fuel preference during high-intensity exercise remains stubbornly fixed to carbohydrates (Romijn *et al.*, 1995; van Loon *et al.*, 2001).

Ketosis alters muscular carbohydrate metabolism

Ketone bodies not only provide an alternative substrate for oxidative respiration, but also compete with pyruvate as a preferred substrate for mitochondrial oxidation (Robinson and Williamson, 1980). Strong evidence for the importance of ketosis in altering working muscle metabolism dates back in excess of 60 years; most notably the work of Randle and co-workers (1963), which demonstrated that ketone bodies inhibit glycolytic flux and increase glycogen in rat cardiac muscle

and diaphragm. Maizels and colleagues (1977) subsequently demonstrated an increased glycogen concentration following exercise in rat soleus muscle when metabolising acetoacetate. This finding was repeated in an isolated perfused rat heart by Kashiwaya and colleagues (1997), who demonstrated that ketones in the perfusate have the ability to replicate the action of insulin in promoting glycogen synthesis. Perhaps most striking of all is the finding by Laughlin and colleagues (1994) that the addition of 1 mM D-β-hydroxybutyrate to a coronary artery perfusate of glucose and insulin in an *in vivo* anaesthetised dog heart raised glycogen synthesis rate six-fold. In humans, infusion of radiolabelled acetoacetate or βHB to ~5 mM during moderate exercise demonstrated a five- to eight-fold increase in $^{14}CO_2$ production from ketones, with an increased metabolic clearance (Fery and Balasse 1983). Such evidence suggests a sound rationale for the use of ketone bodies as a dietary supplement for exercise, as well as muscle recovery, and may challenge the accepted doctrine of fuel selection during exercise.

Duration vs. intensity

Effective oxidative respiration is clearly central to endurance exercise performance, of which the control of fuel homeostasis is an integral part. However, ketosis may not be advantageous to performance applications that rely almost solely on anaerobic glycolysis, or extremely high glycolytic flux for ATP production, such as sprint or middle distance events. Glucose is the only fuel which can be metabolised under anaerobic conditions to produce ATP, so ketone bodies would not be expected to contribute to energy transduction under these circumstances. Furthermore, highly glycolytic exercise may even be impaired if ketone body oxidation restricts glycolysis by negative feedback, either by an increase in $NADH/NAD^+$ or acetyl-CoA/CoA ratio (Randle *et al.*, 1963). Therefore the potential performance applications for ketosis appear more suited to sustained endurance, where incremental improvements in energy transduction/efficiency, or carbohydrate preservation, may translate to significant increases in performance.

Conclusion

While it is too soon to make conclusive statements regarding the role of supplemental forms of ketone bodies as a fuel in exercise, or their impact on performance, there is significant potential in exercise science for the considered application of this novel substrate. Preliminary studies on the safety of ketone body products, such as the ketone monoester R-1,3-butanediol, show it is generally well tolerated when consumed in the quantities needed to create significant increases in blood ketone concentrations. Both tests of efficacy and commercial production of such ketone bodies are required before they can be considered as useful for athletic performance. Multiple studies are underway to elucidate the potential of nutritional ketones in exercise, as well as their therapeutic utility in conditions of high energetic demand, or dysregulated substrate selection.

Competing interests Professor Kieran Clarke is a non-executive director of TdeltaST, a spin out company of the University of Oxford who own the

intellectual property rights to a D-3-β-hydroxybutyrate-1,3-butanediol ketone monoester.