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Review

Lactate – a signal coordinating cell and systemic function

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Summary

Since its first documented observation in exhausted animal muscle in the early 19th century, the role of lactate (lactic acid) has fascinated muscle physiologists and biochemists. Initial interpretation was that lactate appeared as a waste product and was responsible in some way for exhaustion during exercise. Recent evidence, and new lines of investigation, now place lactate as an active metabolite, capable of moving between cells, tissues and organs, where it may be oxidised as a fuel or reconverted to form pyruvate or glucose. The questions now to be asked concern the effects of lactate at the systemic and cellular level on metabolic processes. Does lactate act as a metabolic signal to specific tissues, becoming a metabolite pseudo-hormone? Does lactate have a role in whole-body coordination of sympathetic/parasympathetic nerve system control? And, finally, does lactate play a role in maintaining muscle excitability during intense muscle contraction?

The concept of lactate acting as a signalling compound is a relatively new hypothesis stemming from a combination of comparative, cell and whole-organism investigations. It has been clearly demonstrated that lactate is capable of entering cells *via* the monocarboxylate transporter (MCT) protein shuttle system and that conversion of lactate to and from pyruvate is governed by specific lactate dehydrogenase isoforms, thereby forming a highly adaptable metabolic intermediate system. This review is structured in three sections, the first covering pertinent topics in lactate's history that led to the model of lactate as a waste product. The second section will discuss the potential of lactate as a signalling compound, and the third section will identify ways in which such a hypothesis might be investigated.

In examining the history of lactate research, it appears that periods have occurred when advances in scientific techniques allowed investigation of this metabolite to expand. Similar to developments made first in the 1920s and then in the 1980s, contemporary advances in stable isotope, gene microarray and RNA interference technologies may allow the next stage of understanding of the role of this compound, so that, finally, the fundamental questions of lactate's role in whole-body and localised muscle function may be answered.

Key words: lactate metabolism, signalling mechanism, exercise, mammalian function.

Introduction

If the words exhaustion or fatigue are introduced into a general conversation regarding exercise, more often than not someone will relate lactic acid or lactate as a primary cause. (Note: at physiological pH, lactic acid almost completely dissociates to lactate and hydrogen ions, which is why lactate, as opposed to lactic acid, is commonly used.) The perception of the general public, the athletic community and the majority of students is that the 'waste' product lactate accumulates in muscle during intensive activity and is therefore the primary reason why exercise is forced to cease. This logic is rational, as our perception of lactate for the majority of the past 200 years has been as a metabolic waste end product. Research dating back beyond the last century has clearly demonstrated that (1) lactate accumulates in muscle and blood during exercise of increasing intensity and (2) blood and muscle lactate is observed to be at its highest at, or just following, volitional exhaustion. Subsequently, lactate has become assumed by many to be a muscular waste product serving to reduce muscle contractile function, thereby acting as a precursor or instigator of fatigue.

The formulation of the traditional lactate paradigm – intense exercise, lack of oxygen and fatigue

The exploration of intermediatory metabolism is not a new field. Interpretation of the role of lactic acid can be traced back to its identification in 1808 by Berzelius (Berzelius, 1808) and then later by Araki (1891), who showed that lactic acid concentrations in exhausted animal muscle were proportional

to the activation of the exercised muscle, the extent of which was thought to be associated with O_2 availability. In 1907, Fletcher and Hopkins conducted a series of experiments expanding this knowledge to the examination of isolated amphibian muscle (Fletcher and Hopkins, 1907). Through progressive investigations, the authors demonstrated that lactic acid appeared in response to muscle contraction, continuing in the absence of oxygen. A secondary observation was that, following the stimulated muscle contraction, the accumulated lactate disappeared when oxygen was present.

During the 1920s, work by three predominant research groups, A. V. Hill's group in London, the Heidelberg group of Otto Meyerhof, and Dill and Margaria's group at Harvard (Margaria et al., 1933), provided much of the basis for our understanding of lactate metabolism in exercise physiology. In 1923, Hill and Meyerhof combined their research observations and many of the accepted or hypothesized theories at the time in a historical review article (Hill and Meyerhof, 1923). The two main theoretical constructs to emerge from this paper were the identification and naming of the 'lactic-acid-cycle' (describing the processes utilizing the cyclical conversion of glycogen to lactic acid back to glycogen) and the recognition that 'two' distinct pathways supplied the energy required for muscle contraction, which were deemed aerobic (in the presence) and anaerobic (in the absence) of oxygen.

Whilst Meyerhof's research concerned the lactic acid cycle on non-circulated amphibian hemicorpus preparations, Hill et al. (1924a,b) subsequently sought to investigate this phenomenon in humans during exercise. From a series of experiments and observations, the authors determined the rise in lactic acid at the onset of exercise to be as a direct result of an O_2 deficit (hypoxia) in exercising skeletal muscle. The 'oxygen debt model' that Hill et al. (1924a,b) postulated, supported by subsequent work from Hill's laboratory (Hill, 1932), became the primary explanation for the increased appearance of lactic acid during exercise and ensuing fatigue. Subsequent recognition of these researcher's contributions (Hill and Meyerhof were jointly awarded the Nobel Prize for science in 1922) saw the O_2 debt hypothesis accepted as a leading theory in the physiological understanding of prolonged human exercise, whilst providing the paradigm for the body of further human research that ensued (Bassett, 2002).

The interpretation of research conducted during this period is, in many regards, the reason why lactate has received its label as an end product. Apart from research such as that generated by Cori's laboratory, which demonstrated that lactate could be converted back to glucose in the liver (Cori and Cori, 1929, 1933), research during the next 20 years sought to prove lactate as the cause of fatigue, rather than to question its function.

A prime example of lactate's suggested role during exercise in the years that followed was the introduction and widespread acceptance of the anaerobic threshold (AT) concept (Davis, 1985). It was observed that during exercise, an increase in blood lactate accumulation occurred at a standard relative exercise intensity (~60–75% $\dot{V}_{O_2}$ max) in individuals with varying fitness profiles. Combined with the appearance of lactate in the circulation was an increase in ventilatory drive and energy expenditure. This transition was seen as the turning point at which the anaerobic system became the predominant source of energy provision, with a concomitant increase in lactate concentrations beyond this transition a consequence of this metabolic switch. Hill et al.'s O_2 debt hypothesis (Hill et al., 1924a,b) seemed to explain the mechanism behind this increase, as well as progressive recruitment of glycolytic fibres and changes in substrate utilisation (Davis, 1985).

So is lactate only an anaerobic product?

During the 1980s, other research groups set out to question whether or not lactate was a waste product (Brooks, 1985; Connet et al., 1986). Initially, work by Jobsis and Stainsby (1968), and later by Connet et al. (1986), demonstrated that stimulated canine muscle was capable of producing and oxidising lactate at conditions equivalent to moderate intensity exercise, at which there was, seemingly, an adequate supply of oxygen. In light of this, Brooks (1986) postulated that for oxygen deficit (anaerobiosis) to be the primary cause of lactate accumulation, muscle anoxia must exist, since this was thought to be the stimulus for lactate production. Numerous research studies had previously demonstrated that at exercise intensities of 50–75% $\dot{V}_{O_2}$ max, where the AT supposedly occurred, sufficient reserves of cardiac output, localised blood flow and lactate and glucose arterial–venous differences existed for muscle to remain suitably perfused for aerobic metabolism to continue, thus providing conflicting information to that of the AT theoretical construct (Brooks, 1985, 1986).

Richardson et al. (1998) utilised phosphorous magnetic resonance spectroscopy (MRS) and myoglobin saturation, as measured by ^{1}H nuclear MRS, to address whether lactate increase during progressive exercise to exhaustion was due to muscle hypoxia. They observed that net blood lactate efflux was unrelated to intracellular oxygen partial pressure (P_{O_2}) across work intensities but was linearly related to O_2 consumption and intracellular pH. Therefore, the data provided by Richardson et al. (1998) support the notion that lactate efflux during exercise is unrelated to muscle cytoplasmic P_{O_2}, effectively dissociating lactate production and hypoxia.

Comparative examination of the glycolytic pathway across the animal kingdom has provided evidence that anaerobic conditions are not essential for lactate to be produced, demonstrating that energy systems work in unison as opposed to switching on and off, whilst duly confirming the dissociation between lactate and hypoxic or anoxic conditions. The tailshaker muscle of the western diamondback rattlesnake (*Crotalus atrox*) has provided a model that clearly demonstrates that aerobic metabolism can meet a high ATP demand. Species such as the rattlesnake are able to alter the energy requirement of muscle contraction so that glycolysis may continue. Tailshaker muscles are capable of sustaining high-frequency contractions in the region of 20–100 Hz for several hours with an ATP cost per twitch of 0.015 mmol l^{-1}

ATP per gram of muscle (Conley and Lindstedt, 1996). Utilising the same model, this time in ischemia and normoxic situations, Kemper et al. (2001) demonstrated that such elevated rates of glycolysis could happen independently of O_2 levels. Such muscle was capable of exercising without fatigue due to high blood flow levels allowing the rapid turnover of H^+ and lactate (and presumably other metabolites that might themselves be involved in a fatigue process) within the cells. Recent research suggests that mechanical trade-offs between twitch tension and duration and between joint force and displacement explain how the tailshaker muscle can alter rattling frequency rates without increasing the metabolic cost of activity (Moon et al., 2002).

These data allow for two considerations. Firstly, they allow for the acceptance that lactate is not only produced as a result of anoxic or hypoxic conditions but also that it is a metabolite produced during adequate oxygen provision. Secondly, aerobic ATP provision is a highly adaptable process, with skeletal muscle possessing an inherent ability to adapt to the energy requirements of the organism. It appears that many animal species are able to minimise the cost of muscle contraction so that cellular ATP production can meet ATP demand and sustain high contractile rates (Conley and Lindstedt, 2002) with lactate formed as an integral part of this working system, not as an end product *per se*.

A metabolite on the move…

During conditions of lactate production, at rest and during submaximal exercise, substrate concentrations support the conversion of pyruvate to lactate *via* the lactate dehydrogenase (LDH) reaction. Until relatively recently, our understanding, was that lactate moved from the cellular compartment to the blood *via* simple diffusion. Increased lactate concentrations were deemed a consequence of increased glycolytic flux rates, with cellular function inhibited when lactate was unable to leave the cytosolic compartment.

This understanding began to change following initial observations in rodent studies by Donovan and Brooks (1983), which demonstrated that endurance training reduced post-exercise lactate concentrations by enhancing lactate clearance, strongly suggesting that the major fate of lactate during or following exercise was probably oxidation. Further research demonstrated that lactate transport was sensitive to pH, specific transport inhibitors and temperature (Juel, 1988; Watt et al., 1988; Roth and Brooks, 1990). To directly measure lactate kinetics in humans, Mazzeo et al. (1986) used the stable isotope tracer [1-^{13}C]lactate to demonstrate that the rate of lactate disposal (R_d) was directly related to metabolic clearance rate (MCR). That oxidation, as determined by the appearance of ^{13}C enrichment in CO_2, was the major fate of lactate during exercise, and, subsequent to this, that the interpretation of lactate kinetics by way of concentrations was inappropriate, as circulatory endpoint values could not reflect lactate turnover in muscle (rate of production minus rate of removal). Donovan's findings were supported in humans (MacRae et al., 1992), whilst subsequent animal research in giant sarcolemmal vesicle and perfused hindlimb preparations added support to a carrier-mediated process for lactate transport in and out of skeletal muscle, as well as the stimulatory effects of contraction, pH and blood flow on both processes (Juel et al., 1991; Watt et al., 1994; Gladden et al., 1995).

Previous research in erythrocytes suggested three pathways for lactate transport. First, carrier-mediated transport by a H^+-coupled transporter; second, exchange with inorganic anions mediated by the band 3 protein Cl^-/HCO_3^- exchange; and third, passive diffusion of lactic acid across the lipid bilayer. Under physiological conditions, it was believed that the transport pathway mediated up to 90% of observed lactate flux (Deuticke et al., 1982). In the early 1990s, Kim et al. (1992) sequenced a membrane protein (Mev) from met-18b-2 hamster ovarian cells that exhibited an unusually high uptake of the 6-carbon branched dihydroxymonocarboxylate mevalonate. When a plasmid expressing a cDNA for Mev (pMev) was introduced by transfection into wild-type Chinese hamster ovary cells, an mRNA that hybridizes to the Mev cDNA was identified. Following cloning and sequencing of the wild-type version of Mev, coupled with the observation that the cloned protein did not facilitate mevalonate transport, it was concluded that the wild-type Mev transported other substances, independently of mevalonate. Further examination identified that this protein was related to the previously characterised transport system found in erythrocytes (Garcia et al., 1994).

Subsequently, an entire family of monocarboxylate transport (MCT) proteins (now with 14 isoforms) has been cloned, and their individual roles have been characterised (for detailed topological characteristics and processes, see Halestrap and Price, 1999; Halestrap and Meredith, 2004). The predominant MCTs in human skeletal muscle are MCT1 and MCT4, whilst MCT2 has been identified in the liver (McClelland et al., 2003). McCullagh et al. (1996) suggested that MCT1 facilitated uptake of lactate into muscle cells for oxidative metabolism, as such being coordinately expressed with the heart isoform of lactate dehydrogenase (LDH), with both being found in higher concentration in type I fibres. At a similar time, Wilson et al. (1998) showed that the low-affinity transporter MCT4 could be responsible for the net export of lactate from the cell, and as such was predominantly expressed in glycolytic type IIA fibres, which are known to be the major physiological producers of lactate when they are contracting.

With the increased knowledge of MCT-facilitated lactate transport, further evidence in support of the lactate shuttle hypothesis became available. Brooks (1986) postulated the framework of the lactate shuttle hypothesis prior to the discoveries of MCT or their distribution (Fig. 1). This hypothesis proposed that lactate was able to transfer from its site of production (cytosol) to neighbouring cells and a variety of organs (e.g. liver, kidney and heart), where its oxidation or continued metabolism could occur. Of key importance to this hypothesis was the appreciation that for lactate shuttling to

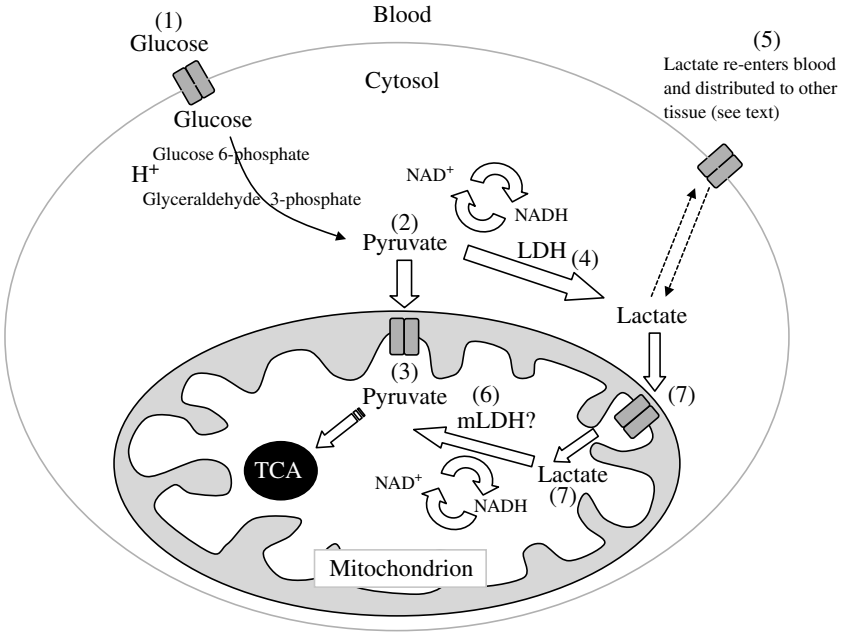

Fig. 1. The processes involved in the lactate shuttle hypothesis (Brooks, 1986). The pathway proposes that (1) glucose enters the cell, where it is sequentially broken down to pyruvate (2). Pyruvate enters the mitochondrion, allowing respiration to continue in the tricarboxylic acid (TCA) cycle (3). Lactate is subsequently formed *via* the lactate dehydrogenase (LDH) reaction (4) and is then exported from the cytosolic compartment *via* monocarboxylate transporter (MCT) transport (5), where it is redistributed to a variety of functional sites. Note the suggested presence of mitochondrial lactate dehydrogenase (mLDH) (6), which forms the construct of the intracellular shuttle system (7) (see text for description).

occur, as suggested, a cellular protein transport system would be implicated.

The original lactate shuttle hypothesis has since seen a number of revisions, with an intracellular component introduced (Brooks et al., 1999; Fig. 1). The extension to an intracellular shuttle system has not been without its controversy. The principle depends upon the presence of mitochondrial LDH (mLDH) for the re-conversion of lactate, once it enters the mitochondrion, to pyruvate and for mitochondrial located MCTs (Brooks et al., 1999). This component has been strongly challenged by two independent investigations (Rasmussen et al., 2002; Sahlin et al., 2002). The principal flaw to the Brooks model, detailed by these authors, was that lactate entering the mitochondria would create a futile cycle by which pyruvate is reduced to lactate in the mitochondria and *vice versa* in the cytosol. It was suggested that this would induce a situation compromising energy production, as both the redox state of the cell and the required direction of substrate flow would be reversed.

This suggested scenario, however, seems unlikely. Firstly, in conversion of pyruvate to lactate, lactate accepts an H^+ ion from NADH, thereby allowing increased availability of NAD and maintenance of the redox state of the cell. Secondly, within the intracellular model there would not be a futile cycle formed, as lactate entering the mitochondria would be converted to pyruvate and oxidised. Lactate acts as an alternative pathway for substrate to enter the mitochondria, competing with pyruvate for MCT transport. The intracellular shuttle (Fig. 1) does not suggest that pyruvate is not present in the intracellular compartment; instead it suggests that the LDH conversion of lactate to pyruvate is more than a cytosolic reaction alone. Data provided by Laughlin et al. (1993) utilising MRS in working canine hearts have proven that infusion of [^{13}C]pyruvate labelled cytosolic lactate and alanine pools whereas [^{13}C]lactate did not label cytosolic pyruvate or alanine. However, the TCA cycle substrate α-ketoglutarate was labelled, suggesting that infused lactate by-passed the cytosolic LDH reaction and was converted to pyruvate in the mitochondria. Brooks (2002b) questioned the methods used by Rasmussen et al. (2002) and Sahlin et al. (2002) in obtaining mitochondria, suggesting that mLDH could easily have been lost during this subfractionation process and was the main reason for the discrepancies in results. The controversy over mitochondrial-located MCTs might have been resolved by two recent studies (Butz et al., 2004; Hashimoto et al., 2005), with the latter using immunohistochemical analysis in combination with confocal laser scanning microscopy (CLSM) to clearly demonstrate the co-localisation of MCT1 and cytochrome oxidase (COX) at both interfibrillar and subsarcolemmal cell domains. These data would indicate that MCTs and associated proteins are therefore positioned specifically to facilitate functions of the lactate shuttle system. For detailed applications of the lactate shuttle hypothesis, see recent reviews by Brooks (2002a,b) and Gladden (2004).

Lactate has been suggested to play an important role in cellular and organelle redox balance, a function demonstrated in the proposed peroxisomal lactate shuttle (McClelland et al., 2003). It has long been known that long-chain β-oxidation of fatty acids occurs in mammalian peroxisomes (Lazarow and de Duve, 1976); however, for β-oxidation to continue, both $FADH_2$ and NADH must be reoxidized. McGroarty et al. (1974) first suggested the presence of LDH in rat liver peroxisomes, however it was not until the study of Baumgart et al. (1996) that LDH was identified in the peroxisomal matrix. McClelland et al. (2003) recently confirmed the findings of Baumgart et al. (1996) identifying the presence of LDH; further, peroxisomal β-oxidation was stimulated by pyruvate, with lactate generated when pyruvate was added to

peroxisomes. MCT1 and MCT2 were identified as facilitating the entry of pyruvate into the peroxisomal matrix and lactate efflux from the organelle, thus forming the basis for a peroxisomal lactate shuttle and explaining how lactate and its efflux can regulate specific cellular and organelle redox balance (Brooks et al., 1999).

MCT expression seems to be rapidly modulated to respond to changes in muscle activity. Many studies have demonstrated increases in MCT content following a single exercise bout (Green et al., 2002) or periods of endurance training (Baker et al., 1998; Bergman et al., 1999; Pilegaard et al., 1999; Dubouchaud et al., 2000). Recent research suggests that MCT increases may occur rapidly following exercise. Zhou et al. (2000) provided evidence that MCT4 mRNA was transiently increased during exercise. Further to this, Green et al. (2002) showed an increase in MCT1 (121%) and MCT4 (120%) protein expression taken from skeletal muscle biopsies 2 and 4 days after a 5–6 h 60% $\dot{V}_{O_2}$ peak exercise bout in humans. Most recently, Coles et al. (2004) have shown that 2 h exercise (21 m min^{-1}, 15% grade) in rats increases MCT1 and MCT4 mRNA 2–3-fold, peaking 10 h post exercise. These responses, however, were observed to be tissue specific [different responses found between soleus and extensor digitorum longus (EDL) muscles] and, in some cases, transiently upregulated so that protein levels had returned to pre-exercise levels 24 h post exercise. Subsequently, these authors suggested that the MCT family of transporters belong to a group of metabolic genes, rapidly activated following exercise (Hildebrandt et al., 2003). These gene products (mRNA) are present in small amounts in cells; however, they have rapid induction times, suggesting that small quantities of each are required for metabolic function to be supported (Hildebrandt et al., 2003). It does, however, remain to be seen whether such rapid induction of MCTs following exercise is repeated in human skeletal muscle. By contrast, denervation (Pilegaard and Juel, 1995) and inactivity (Wilson et al., 1998) lead to a decline in MCT expression.

These discoveries have been important in the recognition of lactate acting as a mobile metabolite, able to move within cellular compartments and adjacent muscle fibres and distributed widely across systemic circulation to inactive tissue and organs. Thus, lactate has the capacity to act as a metabolic signal at the cellular, localised and whole-body level, either directly or through its effects on H^+ or other metabolic regulators. Further, the rapid induction of MCT following repeated muscle contraction means that the mechanisms of lactate transport can quickly adapt to an exercise stimulus, resulting in the notion of lactate as a signal to a rapid adaptable process maintaining cell homeostasis.

Lactate as the cause or consequence of fatigue?

There is a host of research suggesting an association between increased lactate concentration and fatigue during exercise. Initial work by Hill (1932) indicated that the contraction force of isolated fibres declined at the same time as lactate accumulation increased. Later work by Fabiato and Fabiato (1978) and Allen et al. (1995) demonstrated that the likely mechanism for reduction in force production, by intracellular lactate acidification, was *via* reduced sensitivity of the sarcoplasmic reticulum Ca^{2+} pump to Ca^{2+}. For some time, the release of lactate and hydrogen ions was thought to occur at similar rates, inducing lactate acidaemia. However, evidence for dissociation between lactate and hydrogen ion release was demonstrated *in vivo* in humans by Bangsbo et al. (1997). This study showed that the release of protons can occur, to a large extent, through mechanisms other than diffusion of undissociated lactic acid ions during submaximal exercise. The non-lactate-related release of protons was estimated to account for approximately 75% of the total efflux of protons during an exercise bout, leading to the question as to what role lactate may therefore play during muscle contraction. Posterino and Fryer (2000) further demonstrated *in vitro* that elevated myoplasmic lactate concentrations had negligible effects on voltage-dependent Ca^{2+} handling and muscle contraction at the level of the contractile proteins. General acceptance now is that lactate ions themselves have little effect on muscle contraction (Lindinger et al., 1995; Posterino et al., 2001), whilst the importance of acidosis in muscle fatigue has also become questioned and may not be such a major factor (Westerblad et al., 2002). Recently, Robergs et al. (2004) reviewed evidence to suggest that there is no biochemical support for lactate production causing all of the intracellular acidosis, with lactate production actually retarding it, perhaps delaying the onset of muscle fatigue, whilst acidification resulted from other biochemical processes such as ATP breakdown and the earlier stages of glycolysis.

Some of the methods employed by Robergs et al. (2004) to illustrate their argument have been questioned by subsequent papers (Boning et al., 2005; Kemp, 2005); however, the general consensus from a variety of experimental approaches appears to be that lactate has minimal involvement in the onset of fatigue. Instead, recent research suggests an increase of inorganic phosphate (P_i) produced during contraction as the leading contender responsible for initiating muscle fatigue at the level of muscle function (see review by Westerblad et al., 2002). Contemporary explanation of fatigue certainly points to a combination of effects, as opposed to one mechanism, causing fatigue, certainly in whole-organism function. Accordingly, it is probably premature to also accept the P_i hypothesis as the sole cause of fatigue until further research is carried out, particularly *in vivo* (Gladden, 2004), just as care should be taken when dismissing H^+ accumulation from the aetiology of fatigue until our overall understanding of fatigue is improved (Fitts, 2003; Boning et al., 2005).

It would now seem that lactate ions may in fact have a protective effect on contraction force, as first demonstrated by Nielsen et al. (2001). In their experiments, it was observed that a reduction in tetanic force of intact isolated muscle fibres caused by elevated potassium (K^+) could be almost completely reversed when incubated in lactate (20 mmol l^{-1}). The substrate concentration used within this experiment led the authors to hypothesise that at high exercise intensities, where

intra-muscle lactate is known to range between ~15 and 25 mmol l^{-1}, lactate acts to increase force, counteracting the force-depressing effects of high extracellular K^+ whilst having no effect on the membrane potential or Ca^{2+} handling of the muscle. Further research has shown that at a K^+ incubation of 11 mmol l^{-1} and a temperature of 30°C, a 16% decline in force production of intact rat soleus or EDL can be seen compared with controls. At the same K^+ concentration, the previously observed force decrement was restored to control values when temperature (30–35°C), lactate (10 mmol l^{-1}) and catecholamine concentrations were all elevated, suggesting involvement of each of these factors in force restoration (Pedersen et al., 2003). Further, Karelis et al. (2004) have shown that maximum dynamic and isometric *in situ* force production of electrically stimulated rat plantaris muscle is elevated during intravenous lactate infusion (12 mmol l^{-1}) compared with controls. The authors attributed this observation to increased maintenance of M-wave characteristics during electrical stimulation and lactate infusion trials compared with controls.

Nielsen et al.'s original lactate protection hypothesis (Nielsen et al., 2001) has recently been supported by further work from this group. Pedersen et al. (2004) reported that, in the presence of chloride (Cl^-), intracellular acidosis increased the excitability of the T system in depolarized muscle fibres, counteracting fatigue at a critical phase in the excitation–contraction coupling process. Acidification reduced Cl^- permeability, thereby reducing the stimulus needed to generate a propagating action potential. This view is not recognised by all. By contrast, Kristensen et al. (2005) questioned whether this phenomenon can be extended to a whole-system model during exercise. These authors reported that muscle preparations *in vitro* were unable to produce a similar amount of force compared with controls when incubated in a 20 mmol l^{-1} Na-lactate, 12 mmol l^{-1} Na-lactate + 8 mmol l^{-1} lactic acid or a 20 mmol l^{-1} lactic acid solution and stimulated to fatigue. It was concluded that, although lactate regenerates force in passive muscle, this process is not apparent when muscle is exercised. The authors suggest that the depolarizing effect of lactate incubation observed by Nielsen et al. (2001) was not replicated, as K^+ depolarisation was less pronounced *in vivo* when muscle was stimulated. These data seem to suggest that the extension of the Nielsen et al. (2001) hypothesis to a full-system model is difficult due to the number of confounding systems that operate during exercise *in vivo*. It appears that lactate may delay the onset of fatigue by maintaining the excitability of muscle and that this situation may occur during extremely intensive exercise. The basis and understanding of this role, however, still remains poorly understood, whilst the methods to transfer isolated muscle research into full-system physiology are currently lacking. Clearly, further approaches to investigate this topic are warranted to establish whether Nielsen's hypothesis can be extended to whole-muscle function *in vivo*.

Peripheral or localised fatigue is characterised by metabolic change in specific skeletal muscle or muscle groups, whether it be a reduction in pH or an increased accumulation of a compound such as P_i. The classical theory of exercise-induced fatigue proposes that exercise is limited only after oxygen delivery to the exercising skeletal muscle becomes inadequate, inducing anaerobiosis (Mitchell and Blomqvist, 1971; Bassett and Howley, 2000). Noakes and colleagues have suggested an alternative hypothesis, implicating a 'central governor' (CNS), which regulates the mass of skeletal muscle recruited during exercise through motoneurone pool recruitment, a consequence of which would be to protect the heart from ischaemia during maximal exercise (Noakes, 1998; Noakes et al., 2001, 2004; St Clair Gibson et al., 2003). This model predicts that the ultimate control of exercise performance resides in the brain's ability to vary the work rate and metabolic demand by altering the number of skeletal muscle motor units recruited during exercise (Noakes et al., 2004). Some attempts have been made by this group to address physiological parameters in peripheral tissue that may act as the signal to the CNS to regulate exercise intensity (Rauch et al., 2005); however, this mechanism still remains unclear.

So could lactate have a role as a peripheral signal to the CNS during exercise? We now know that lactate is a mobile metabolite capable of cell and intracellular shuttling, with the circulation able to shift this metabolite to a number of facultative sites for oxidation or recycling. There is also mounting evidence in support of lactate utilisation in the brain (Ide and Secher, 2000) *via* the astrocyte–**neurone** lactate shuttle, a system clearly capable of affecting substrate delivery and neurone function (Pellerin et al., 1998; Pellerin and Magistretti, 2003). So could lactate be one of the peripheral exercise signals that might be incorporated into Noakes' model (Noakes et al., 2004)? Certainly, lactate's production characteristics allow it to perform such a role. It is elevated during exercise and reaches maximal levels at or just following the termination of exercise. Further, shuttling mechanisms would allow for an influence of lactate, centrally and peripherally, again fulfilling roles as part of the central governor hypothesis. It will be of interest to see whether the peripheral signal for the central governor is identified in future research and whether lactate has a role to play in this scenario.

Lactate as a signal?

It appears that we still do not fully understand all of the roles for lactate *in vivo*. Whilst much of the data presented so far have been gleaned from isolated muscle, or cell culture, understanding how these observations transfer to the whole organism is perhaps the next important question to be addressed.

Suggestion of a role for lactate as a metabolic signal at the whole-organism level has been postulated by Brooks (2002a), who proposed that lactate may operate as a pseudo-hormone. Within this model, blood glucose and glycogen reserves in diverse tissues are regulated to provide lactate, which may then be used within the cells where it is made or transported through the interstitium and vasculature to adjacent or anatomically

distributed cells for utilization. In this role, lactate becomes a quantitatively important oxidizable substrate and gluconeogenic precursor, as well as a means by which metabolism in diverse tissues may be coordinated. Lactate has the ability to regulate cellular redox state, *via* exchange and conversion into its more readily oxidized analogue, pyruvate, and effects on NAD^+/NADH ratios. Lactate is released into the systemic circulation and taken up by distal tissues and organs, where it also affects the redox state in those cells.

Further evidence for lactate acting as something more than a metabolite or metabolic by-product comes from wound repair research, where lactate appears to induce a biochemical 'perception' effect (Trabold et al., 2003). It had been suggested that the elevated acidosis associated with wound regeneration was a result of localised hypoxia. However, Trabold et al. (2003) provided evidence that lactate may act as a stimulus similar to hypoxia without any compromise to O_2 levels. Green and Goldberg (1964) demonstrated that collagen synthesis rose ~2-fold in lactate-incubated (15 mmol l^{-1}) fibroblasts, whilst Constant et al. (2000) showed that increased lactate was capable of upregulating vascular endothelial growth factor (VEGF) in similar proportions. To examine this apparent relationship, Trabold et al. (2003) elevated extracellular lactate in the wounds of male Sprague-Dawley rats by implanting purified solid-state, hydrolysable polyglycolide. This substance raised localised lactate to a maintained 2–3 mmol l^{-1}. Elevating lactate resulted in elevations in VEGF and a 50% increase in collagen deposition over a 3-week period. These data suggest that lactate is capable of inducing responses characteristic of O_2 lack, operating to instigate a pseudo-hypoxic (as far as concentration of lactate is concerned) environment. In combination with this action, the continued presence of molecular oxygen (as the tissue was not hypoxic) allows endothelial cells and fibroblasts to promote increased collagen deposition and neovascularization.

The possibility that lactate acts as a metabolic signal is important to take research further. Based on the hypotheses of Trabold et al. (2003) and Brooks (2002a), can a working model of lactate signalling be extended to systemic and localised exercise function?

Firstly, lactate could, potentially, influence local and central blood flow during exercise. Hypoxia is known to stimulate systemic vasodilation *via* a host of neural, hormonal and local factors (Skinner and Marshall, 1996). Fattor et al. (2005) have recently used the lactate clamp method to demonstrate an autoregulatory loop in sympathetic drive that is governed by lactate release. Circulatory norepinephrine was reduced during exercise at 65% $\dot{V}_{O_2}$ peak when lactate was maintained at 4 mmol l^{-1} compared with controls (2.115±166 pg ml^{-1} to 930±174 pg ml^{-1}, respectively), with epinephrine concentrations displaying a similar trend (EX; 262±37 pg ml^{-1} to LC; 113±23 pg ml^{-1}). This lends evidence to the possibility of modulatory control of catecholamines by lactate. The infusion of lactate had no effects on other glucoregulatory hormones (i.e. insulin and glucagon) or cortisol. The authors suggest that the lactate anion was sensed by either the ventromedial hypothalamus (VMH) or elsewhere *via* neuronal metabolism signalling abundant fuel supply; however, this theory remains to be tested. Therefore, the release of lactate into the circulation at the onset of exercise could promote vasodilation, allowing oxygenated blood to reach active muscle, acting in an additive or modulatory manner to the demands of tissues during exercise.

A role for lactate in fuel selection?

In many vertebrate species so far examined, fuel selection has been shown to correlate closely with exercise intensity (Roberts et al., 1996; Bergman and Brooks, 1999; Richards et al., 2002; Conley and Lindstedt, 2002). At rest and moderate exercise intensities, fat oxidation is the predominant source of ATP production. As exercise intensity rises, a proportional increase in carbohydrate (CHO) oxidation occurs, with lactate production following this trend (Fig. 2). This coordinated control was first identified and named the glucose–fatty acid cycle by Randle et al. (1963). In short, elevated glucose

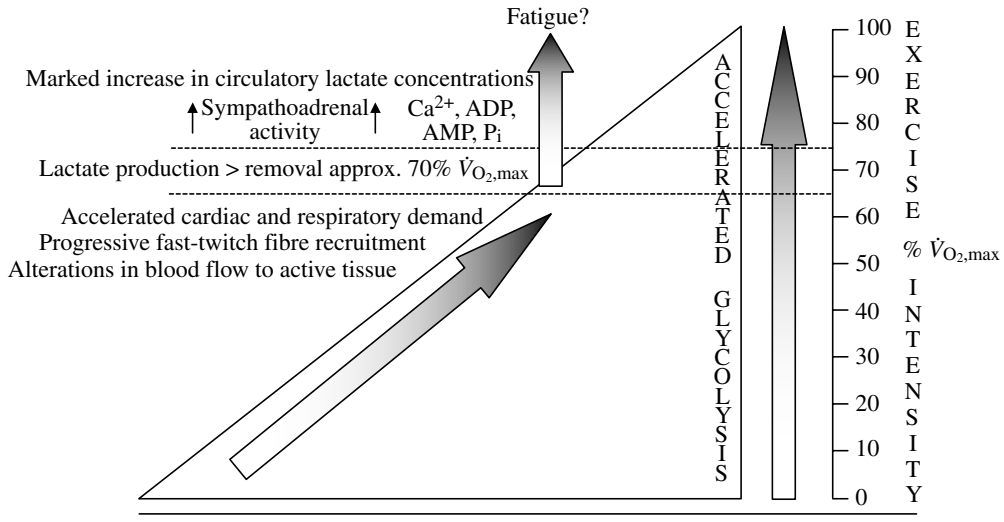

Fig. 2. Interacting processes suggested to be involved with increased lactate accumulation during exercise.

concentrations stimulate the secretion of insulin, which suppresses non-esterified fatty acid (NEFA) release from adipose tissue, altering fuel use and supply and leading to the preferential use of CHO. In the reverse situation, when plasma NEFA concentrations are elevated (e.g. during starvation, exercise or low insulin levels), fatty acids are predominantly released and oxidised, and glucose levels are observed to be low.

Brooks and Mercier (1994) recognised that a clear crossing point where fuel utilisation came from fat and CHO equally was observable in fuel selection. The 'crossover concept' suggests that the proportion of substrate utilization in an individual at any point in time depends on a trade-off between exercise-intensity-induced responses (which increase CHO utilization) and endurance-training-induced responses (which promote lipid mobilisation and oxidation). The crossover point may be taken as the power output at which energy from CHO-derived fuels predominates over that from lipids, with increases in power eliciting further increments in CHO utilization and decrements in lipid oxidation.

The exercise intensity at which a transitional shift in substrate supply might occur was originally examined in dogs and goats by Roberts et al. (1996) through calculated rates of fat and CHO oxidation from respiratory exchange ratio (RER) data. Maximal fat oxidation rates were observed at 40% of maximal exercise intensity in both species, with fat oxidation shown to provide around 77% of total energy requirements. Bergman and Brooks (1999) studied this in humans and found the highest lipid oxidation rate in the fed state at 40% $\dot{V}_{O_2}$ peak. Taken together, the data provided by Roberts et al. (1996) and Bergman and Brooks (1999) would suggest that humans and other mammals, regardless of differences in aerobic capacities, genotype and training adaptation, demonstrate similar substrate utilization patterns when relative exercise intensity is considered (Bergman and Brooks, 1999). Van Loon et al. (2001) utilised a continuous infusion of [U-^{13}C]palmitate and [6,6-$^{2}H_2$]glucose to provide direct measures of whole-body fat oxidation, which were increased from rest at approximately 8 kJ min^{-1} up to a maximum rate of 32±2 kJ min^{-1} at 55% maximal workload (W_{max}) or approximately 60–75% maximal oxygen consumption. As exercise intensity increased to 75% W_{max} fat oxidation declined by 34% to 19±2 kJ min^{-1}. Free fatty acid (FFA) concentrations and blood flow were maintained at the highest exercise intensity, suggesting ample FFA arterial availability.

Three possibilities have been suggested to explain the decline in FFA acid oxidation in the face of sufficient supply. Firstly, gradual depletion or limited turnover of the cytosolic free carnitine pool could alter long-chain fatty acid (LCFA) transport across the mitochondrial membrane (Harris and Foster, 1990). Secondly, reduced transport of FFAs by escalating cellular or systemic acidosis may limit FFA uptake due to downregulation of the fatty acid transporter, carnitine palmitoyl-transferase 1 (CPT1) (Sidossis et al., 1998; Bonen et al., 1999). Finally, changes in glucose flux and energy expenditure may regulate the amount of available malonyl-CoA, an allosteric inhibitor of CPT1, which has been shown to regulate fat oxidation (Ruderman and Dean, 1998; Roepstorff et al., 2005). To date, the exact mechanism regulating the relative contribution of CHO and fat to energy provision during exercise still remains unknown. The most recent examination of fuel balance during exercise was conducted by Roepstorff et al. (2005) who utilised high or low CHO diets to influence glycogen stores and substrate utilisation during 60 min bicycle exercise at 65% $\dot{V}_{O_2}$ peak in eight healthy male subjects. The authors observed a decline in muscle malonyl-CoA concentrations from rest to moderate intensity exercise; however, there was no change observed when fat oxidation rates were altered by the pre-exercise meal. Thus, the authors concluded that malonyl-CoA may have a role in increasing absolute levels of fat oxidation; however, it would not appear to play a major part in fine-tuning the shifts in CHO and fat oxidation during the rest-to-exercise transition or during sustained exercise. By contrast, the availability of free carnitine to CPT1 appears to participate in regulating fat oxidation during exercise, as muscle carnintine and fat oxidation rates were both lower during exercise with high compared with low glycogen conditions (Roepstorff et al., 2005).

So, is there potential for lactate to play a role in effecting this transition? Previous research has shown that, in isolated mitochondria, a reduction in pH decreases the activity of CPT1 by increasing the K_m of CPT1 for carnitine (Mills et al., 1984). Starritt et al. (2000) have shown that a decrease in pH from 7.0 to 6.8 reduces CPT1 activity by 40% *in vitro*, thereby offering a potential mechanism for extracellular acidosis to inhibit fat oxidation by reducing supply to the mitochondria or reducing the rate of fat oxidation at lower exercise intensities where a fall in pH of approximately 0.1–0.3 units is common (Starritt et al., 2000). There is a host of research suggesting a direct effect of lactate on inhibition of lipolysis and increased re-esterification of FFA (Issekutz et al., 1975; Ahlborg et al., 1976; Jeukendrup, 2002). Whilst it seems that this evidence supports a role for acidification in reducing fatty acid metabolism, it is not clear whether this can be attributed to an increase in H^+, lactate alone or a combination of each. Most recently, Corbett et al. (2004) have shown that as plasma lactate increases at progressive exercise intensities, so NEFA levels decline. If we put these data into a physiological context, it is known that the lactate threshold (a sustained increased in systemic lactate from resting levels) during exercise occurs in most subjects at 60–75% $\dot{V}_{O_2}$ max, with the accumulation of circulatory lactate known to increase non-uniformly beyond this exercise transition. This relationship could, of course, be chance, with lactate increase solely due to increased CHO oxidation or glycolytic flux. However, if we examine the increase in lactate in the context of a signalling hypothesis, lactate's role could be perceived as something very different. We know that ample tissue oxygenation is available in skeletal muscle at intensities of approximately 60–75% $\dot{V}_{O_2}$ max, allowing oxidative phosphorylation to proceed (Richardson et al., 1998), so lactate is not released as a result of tissue hypoxia.

Similarly, lactate will be maintained at a steady state beyond the lactate threshold, up to a maximal lactate steady state, indicating that lactate clearance capacity is not exceeded at these conditions (Billat et al., 2003). Could it be that lactate is released to signal a progressive switch in fuel utilisation from fat to CHO, reducing FFA substrate availability for the CPT complex whilst also acting, perhaps in combination with H^+ accumulation, to reduce pH, subsequently downregulating CPT1-facilitated FFA transport? This model may provide an efficient way of regulating fuel supply as lactate is produced, signals to its targets and is then re-used as a fuel, allowing continuation of glycolysis and oxidative phosphorylation.

As previously discussed, lactate is preferentially utilised, compared with glucose and pyruvate, in cardiac muscle (Laughlin et al., 1993). Further, Chatham et al. (2001) have reported a similar selectivity for [^{13}C]lactate to be preferentially oxidised ahead of [^{13}C]glucose, again in cardiac muscle preparations. Miller et al. (2002) extended this observation when they reported that infused lactate was preferentially oxidised in preference to glucose at rest and during whole-body exercise in humans. The authors concluded that lactate, provided by intravenous infusion, acted in a glucose sparing role, allowing glucose and glycogen stores to be maintained, to be utilized later in periods of increased exercise stress. Artificially elevating lactate concentrations, such as the lactate clamp method utilised by Miller et al. (2002), allows for the investigation of lactate's role in a variety of processes; however, it does provide a non-physiological situation, as lactate is added independently of glucose usage. The elevated lactate concentrations could therefore serve to stunt glycolysis, as opposed to sparing glucose concentrations. Infused lactate, if the intracellular lactate shuttle is indeed correct, will bypass glycolysis, becoming readily accepted into the mitochondria, where it is converted to pyruvate *via* mLDH. Therefore, lactate synthesis in the cytosol would be reduced, and an increase in H^+ would follow, since lactate production from pyruvate normally accepts an H^+ from NADH. This increased acidification could suppress glycolysis by inhibiting phosphofructokinase (PFK) activity whilst affecting the redox state of the cell. Glucose and glycogen would then be spared by lactate oxidation; however, this process cannot occur during regular exercise as, without the infusion, the only source of lactate production would be as a consequence of glycolysis (Fig. 3).

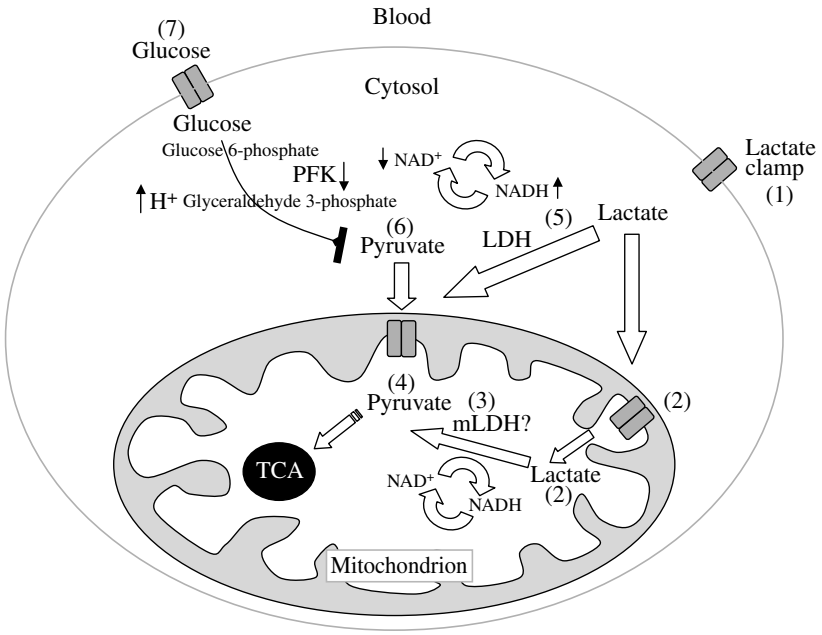

Fig. 3. The effect of artificially elevated lactate concentrations (lactate clamp) on metabolic processes. Increased circulatory lactate concentrations (1) result in lactate entering the cytosol, where it then enters the mitochondrion *via* MCT1 (2). Within the mitochondrion, lactate is converted to pyruvate *via* mLDH (3), which then progresses into the tricarboxylic acid (TCA) cycle (4). However, artificially raised cytosolic lactate concentrations (5) lead to suppression in glycolysis. Therefore, a resulting increase in H^+ and NADH occurs, and acidosis inhibits phosphofructokinase (PFK) activity (6). This suppression finally results in reduced glycolytic activation and a reduction, or sparing, of glycogenolysis.

Lactate and pain

The notion that lactate causes pain during exercise and may contribute to exercise cessation has been suggested and propagated by coaches, trainers and athletes for some time. There is no direct evidence available in the literature to warrant these claims; in fact, lactate infusion trials report no adverse effects of increased lactate on perceived effort of exercise (Miller et al., 2002, 2005) or in initiating the sensation of pain in muscle or joints.

Recent research could, however, implicate lactate as influential in the sensation of pain during exercise. Following the discovery of a receptor for protons in the nerve cell membrane (Krishtal and Pidoplichko, 1980), a family of receptor channel molecules has been identified and cloned (Waldmann and Lazdunski, 1998). These are the acid-sensitive ion channel family, or ASICs. Four ASIC isoforms have been identified in the human genome, each displaying a characteristic biophysical behaviour with respect to gating properties and pH dependence (see Krishtal, 2003 for a review). There has been a suggestion that lactate, in combination with extracellular H^+, may influence sensory mechanotransduction *via* an ASIC pathway, which in turn may modulate targeting of nociceptive sensation (Immke and McClesky, 2001).

ASICs are Na^+ channels. Immke and McClesky (2003) proposed that the ASIC channel is blocked at a site, near the external entry to the pore, by Ca^{2+}. Binding of hydrogen ions diminishes the affinity for Ca^{2+}, which promotes Ca^{2+} release, thus allowing Na^+ flow through the channel, where it will act to

depolarize the excitable tissue. At a pH of 7.4, Ca^{2+} affinity remains high (K_d=12 μmol l^{-1}) so that few channels can open; however, at pH 7.0 the affinity is low enough (K_d=100 μmol l^{-1}) that ASIC channels open. Lactic acid (it was not clarified whether it was lactate or H^+) seems to enhance the sensitivity of ASIC3, allowing the ASIC channel to open at lower H^+ levels and making the pore more sensitive to lactic acidosis (Immke and McClesky, 2001). This process has been implicated in the aetiology of stroke and seizure (ASICs have been detected throughout the CNS). The drop in pH and increased Ca^{2+} in both conditions are likely to affect CNS and peripheral nerve (e.g. nociceptor) function (Akaike and Ueno, 1994). Drew et al. (2004) recently utilised wild-type and ASIC2/3 double-knockout mice to conclude that the ASIC mechanism does not contribute to mechanically activated currents in mammalian sensory **neurone**s. It was suggested that an alternative ion channel type was the most likely source of mechanotransduction, with receptor classes of the transient receptor potential (TRP) channel family suggested as a potential candidate (Clapham, 2003).

The recent detection of ASIC isoforms in a cell line of skeletal muscle characteristics points to other roles for ASIC isoforms apart from pain sensation. Gitterman et al. (2005) demonstrated that the rhabdomyosarcoma cell line (SJ-RH30) possesses endogenous acid-gated currents, similar to the properties of currents arising from ASIC1α subunits (Gunthorpe et al., 2001). Further blocking of the acid-gated current was demonstrated firstly by 30 μmol l^{-1} of the known ASIC1α inhibitor amiloride and then secondly by a 1:1000 dilution of the ASIC1α antagonist psalmotoxin 1, found in *Psalmopoeus* venom (Escoubas et al., 2000). It was further demonstrated by these authors that the removal of extracellular Ca^{2+} enhanced channel conductance at pH 6.5 by ~250%. Preliminary investigation using TaqMan™ (Applied Systems, Warrington, UK) mRNA quantification provided evidence for expression of both ASIC1 and ASIC3 mRNA in adult human muscle (Gitterman et al., 2005). The question of whether human muscle is subject to quick fluctuations of pH of a magnitude capable of activating ASICs has been raised previously by Krishtal (2003) and is clearly paramount if lactate is involved in skeletal muscle ASIC activation *in vivo*. It has been suggested that blood lactate concentrations following strenuous exercise can rise to the region of ~20 mmol l^{-1} (Fitts, 1994); however, common levels range between 10 and 15 mmol l^{-1} in healthy active subjects. Concentrations of this magnitude alongside a pH change could be hypothesised to produce some degree of channel activation and increase in membrane Na^+ conductance or membrane depolarization. Such changes in membrane ion conductance and polarization could be a signal in themselves for changes in metabolite use and intracellular signalling pathways, either directly or through their modulation.

Microdialysis might allow further investigation of the Immke and McClesky (2001) hypothesis, having been used by a number of research groups (Rosdahl et al., 1993; Maclean et al., 1999; Green et al., 2000; Street et al., 2001; Rooyackers, 2005). Maclean et al. (1999) confirmed that a substantial increase in interstitial lactate occurs during the transition from rest to exercise, exceeding values seen in plasma. Street et al. (2001) added to these data by observing that interstitial pH declined in a near linear manner as intensity increased. The lowest pH observed 1 min after a 5 min bout of one-legged knee extensor exercise (70 W) was 6.93, with a mean of 7.04. A pH change of this nature could alter ASIC activation (Immke and McClesky, 2003) and act to increase muscle contractility, delay the onset of fatigue or act as a signal to cease exercise.

There clearly are discrepancies in research findings between whole-body and localised fatigue. Whilst our understanding of lactate action on ASIC function *in vivo* and the presence of ASIC protein in nerve and skeletal muscle is in its infancy, the revisiting of lactate's involvement in pain sensation is an interesting renewal of a long debate. It could be that, instead of pain, as such, lactate assists in the detection of severe exercise stress, signalling the termination or scaling down of exercise before muscle or other organ damage occurs. Lactate could potentially signal to nerve cells indicating the exercise stress, to which the sensation of pain would be produced and exercise would be reduced or cease.

Signalling with regard to *in vivo* processes – a working hypothesis

Accordingly, we would like to suggest the following signalling hypothesis (Fig. 4). In this scenario, lactate becomes mobilised at the onset of exercise (rate depending on mode and intensity of exercise). During this exercise transition, there is approximately a 2-fold elevation in circulating lactate (~1–2 min, increasing lactate to 1–2 mmol l^{-1}), and this concentration is greater still in muscle and interstitium. Lactate has a modulatory effect on vasodilation and catecholamine release, stimulating fat and carbohydrate oxidation. Lactate is then shuttled from its site of production in the cytosol to adjacent muscle fibres, where it is reconverted to pyruvate and enters the tricarboxylic acid cycle for oxidative phosphorylation, or is mobilised into the circulation, where it is reconverted to glucose by the liver, therefore providing an efficient signal-to-fuel process as lactate is recycled during gluconeogenesis or oxidised. In turn, lactate also promotes vasodilation of active musculature and stimulates ventilatory drive (Hardason et al., 1998; Gargaglioni et al., 2003).

As exercise progresses into the moderate to high exercise zone (65–85% $\dot{V}_{O_2}$ max; lactate 2–10 mmol l^{-1}), lactate production exceeds the removal capacity of the MCT transport system. Lactate and H^+ ions influence CPT1 function, thereby reducing fat oxidation and prompting a shift towards carbohydrate oxidation becoming the predominant fuel utilised. Availability of O_2 is still adequate for oxidative phosphorylation; however, the presence of lactate simulates conditions that may be recognised as hypoxic in nature, influencing angiogenesis, oxidative defence mechanisms and collagen synthesis, all serving to improve muscle function. Lactate has also previously been suggested to act as a

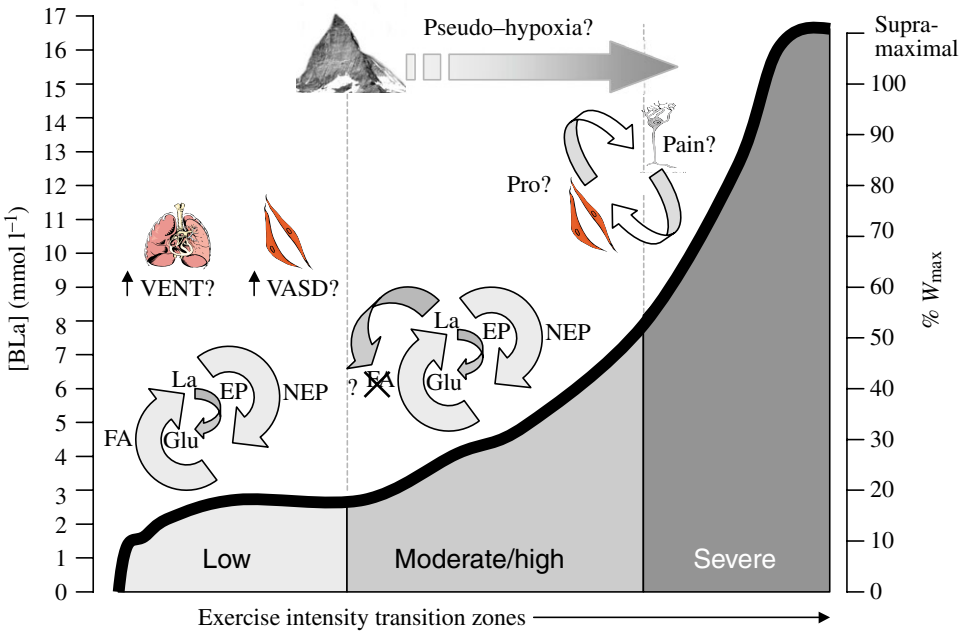

Fig. 4. Potential roles for lactate signalling during exercise of increasing intensity. At the onset of exercise, elevated lactate concentrations signal increased ventilatory drive and vasodilation, whilst sparing glucose and glycogen stores. As exercise intensity moves to the moderate zone, the increase in lactate mimics hypoxic conditions and triggers a number of adaptive responses. In this zone, lactate may also be involved with the transition to carbohydrate metabolism by inhibiting lipolysis. At severe exercise intensities, lactate acts as a peripheral signal to indicate exercise stress, whilst also maintaining the integrity of the muscle. High levels of lactate signal severe exercise stress and exercise is terminated, possibly through a central governor mechanism. Abbreviations: La, lactate; BLa, blood lactate; PRO, lactate protection; Glu, glucose; FA, fatty acid; EP, epinephrine; NEP, nor-epinephrine; VENT, increased ventilatory drive; VASD, vasodilation; W_{max}, maximal power output.

scavenger for free radicals released into the circulation (Groussard et al., 2000) and could potentially operate in this manner as exercise intensity increases.

As exercise progresses towards exhaustion, whole-body lactate levels continue to rise (detectable as 8–20 mmol l^{-1} in blood and higher in muscle). ATP provision in active muscle is approaching its maximal capacity and there is a gradual decline in cellular and systemic pH. Elevated lactate helps reduce glucose usage and glycogenolysis, minimising depletion of these stores as escalating acidosis reduces PFK function. Further, H^+ ions combined with lactate cause an opening in ASIC pores, signalling exercise termination. In this role, lactate is filling a dual purpose. Firstly, its release is indicating stress placed upon active muscle, whilst, secondly, high concentrations of intracellular lactate could potentially be acting in a protective manner. Acting as a peripheral signal, lactate could therefore provide a mechanism by which the CNS detects localised, at the level of muscle or muscle group, exercise stress and causes exercise to terminate (Noakes et al., 2004).

Future research

Lactate infusion studies have been used to artificially raise circulating lactate levels at rest and exercise, allowing examination of the effect of lactate on a variety of processes (see below). Whilst such methods have been routinely used in animal and human studies for a number of years, it is the combination of such infusion with improved stable isotope methods that has provided more mechanistic information.

The lactate clamp (LC) method (Gao et al., 1998) has been used to demonstrate that artificially elevated lactate levels during moderate exercise may increase lactate oxidation, spare blood glucose, reduce glucose production (Miller et al., 2002) whilst also increasing gluconeogenesis (Roef et al., 2003). Miller et al. (2005) have reported that the LC method allows an increase in lactate without causing acidosis; in fact, LC caused a mild alkalosis. LC did not increase ventilation or rating of perceived exertion, suggesting that the LC can be used to solely study the effect of lactate, rather than acidosis, on metabolic functions.

A number of exercise scenarios, as well as pathological conditions, exist that may also allow many of the ideas suggested in this paper to be scrutinised. Does lactate function in a signalling role during hypoxic stress? We know that following prolonged exposure to hypoxia, lactate levels have been shown to decline, in what is termed ‘the lactate paradox’ (Hochachka et al., 2002). This condition could test further the role of lactate during exercise. Also, what happens during exercise in myophosphorylase-deficient patients (McArdle’s disease), who are unable to increase their production of lactate during exercise, or during chronic hyperlactatemia such as that experienced by type II diabetes and HIV patients? Why might lactate be elevated in these scenarios?

As is evident from much of the research discussed in this review, improvements in gene analysis and manipulation technologies have occurred over the past decade. The sequencing of the human genome, in combination with RT-PCR and oligonucleotide array technology, now allows the rapid screening of a host of signalling pathways and novel ion channels from a relatively small tissue sample. There are also strategies for examining signalling pathways that may be involved if phosphorylation is a key event (Knebel et al., 2001; Haydon et al., 2002). Researchers also have the added benefit of gene knock-out technology and the use of transgenic approaches to allow proof of concept previously unattainable. Further to this, the introduction of siRNA approaches in whole body systems will allow researchers to examine and manipulate certain pathways and analyse a large variety of targets *in vivo*. The ability to artificially elevate lactate concentrations safely *in vivo*, and then limiting or removing pathways using siRNA manipulation, will allow direct assessment of lactate's role in a variety of local and whole-system processes whilst limiting the confounding influence of parallel energy systems and pathways that, to date, greatly restrict the scope of *in vivo* investigation.

Conclusions

The perception of lactate, acidosis and fatigue as being equivalent should no longer have a place in human physiology text or tutelage, and a conscious effort should be made to educate student and public perception of the multi-faceted role lactate may play at rest and during exercise.

Lactate should be appreciated not as a sink for glycolytic waste to accumulate or as an acidifier but as an effective mechanism for coordinated fuel sensing and tissue function. This fuel is shuttled to a variety of sites where it is directly oxidised, re-converted back to pyruvate or glucose and oxidised, allowing the process of glycolysis to restart and ATP provision maintained. The shuttling facilitators MCT1 and MCT4, and possibly others in the MCT family, are proteins with rapid induction capabilities and the ability to respond to a host of contraction and environmental stimuli. Lactate production and MCT transport characteristics could allow them to operate as a signal mechanism activating a variety of functions during exercise and recovery.

Research should now be directed towards understanding the function of lactate during exercise in humans. The idea of lactate signalling to a variety of targets has stemmed from data across a variety of research areas, from cell and organelle to whole-body and system based experiments. Lactate's potential role in a variety of processes has been clearly demonstrated; however, the mechanisms underlying these observations in many cases remain undetermined. Lactate's role in fuel selection should be clarified, with the LC method appearing to be a suitable method of investigating this process. Studies utilising siRNA application in combination with microarray analysis could be used to address signalling targets that lactate may influence during exercise, whilst examination of ASICs in skeletal muscle may provide a channel by which lactate acts to increase muscle contractility during *in vivo* function or to signal to nerve cells protecting against exercise damage. With these areas warranting investigation, it certainly seems feasible that lactate has a few tricks left to show us of its role in exercise function.

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