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Limits to sustainable muscle performance: interaction between glycolysis and oxidative phosphorylation

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Summary

This paper proposes a mechanism responsible for setting the sustainable level of muscle performance. Our contentions are that the sustainable work rate is determined (i) at the muscle level, (ii) by the ability to maintain ATP supply and (iii) by the products of glycolysis that may inhibit the signal for oxidative phosphorylation. We argue below that no single factor 'limits' sustainable performance, but rather that the flux through and the interaction between glycolysis and oxidative phosphorylation set the level of sustainable ATP supply. This argument is based on magnetic resonance spectroscopy measurements of the sources and sinks for energy *in vivo* in human muscle and rattlesnake tailshaker muscle during sustained contractions. These measurements show that glycolysis provides between 20 % (human muscle) and 40 % (tailshaker muscle) of the ATP supply during sustained contractions in these muscles. We cite evidence showing that this high glycolytic flux does not reflect an O_2 limitation or mitochondria operating at their capacity. Instead, this flux reflects a pathway independent of oxidative phosphorylation for ATP supply during aerobic exercise. The consequence of this high glycolytic flux is accumulation of H^+, which we argue inhibits the rise in the signal activating oxidative phosphorylation, thereby restricting oxidative ATP supply to below the oxidative capacity. Thus, both glycolysis and oxidative phosphorylation play important roles in setting the highest steady-state ATP synthesis flux and thereby determine the sustainable level of work by exercising muscle.

Key words: ^{31}P magnetic resonance spectroscopy, muscle, energetics, human muscle, rattlesnake.

Sustainable *versus* maximal performance

Muscle power outputs range from maximal values that can only be maintained for a few seconds to much lower power outputs that can be sustained for many minutes to hours. This relationship is illustrated by the power output of human cyclists in Fig. 1. Each data point represents the highest constant power that can be maintained for a given time. These data show that high power outputs can be sustained for only a short time and that the sustained power output declines as the exercise time increases. An output of approximately one-third of the maximal power is typically the highest that can be sustained for a prolonged period (Wilkie, 1985). The goal of this review is to focus on the determinants of this sustainable level of power output. Our contentions are that the sustainable work rate is determined (i) at the muscle level, (ii) by the ability to maintain ATP supply and (iii) by the products of glycolysis that may limit oxidative phosphorylation. Fig. 2 shows a diagram of the ATP, O_2 and H^+ balances in the muscle cells and illustrates the close ties among glycolytic flux, blood flow and oxidative phosphorylation in these balances. We argue below that no single factor 'limits' short-term (<1 h) sustainable performance, but rather that both glycolysis and oxidative phosphorylation determine the level of sustainable ATP supply.

Let us first consider the factors governing power output above the sustainable level. The rapid decline of power output with time shown in Fig. 1 is typically attributed to a fatiguing of the muscle as a result of the build-up of the by-products of ATP supply (see Fitts, 1994). The upper left diagram in Fig. 1 shows inorganic phosphate (P_i) and H^+ as the respective products of the breakdown of creatine phosphate (PCr), which is the storage form for high-energy phosphate in the cell, and of glycogenolysis, which generates H^+ and lactate if the pyruvate it produces is not oxidized. Muscle force often declines well before the ATP stores in the muscle are depleted, indicating that fatigue rather than ATP supply limits power output under these conditions. The result is that the high-flux pathways that generate ATP to meet the maximal power output demands also generate a high level of by-products that inhibit power output.

Sustained power output beyond a few minutes is

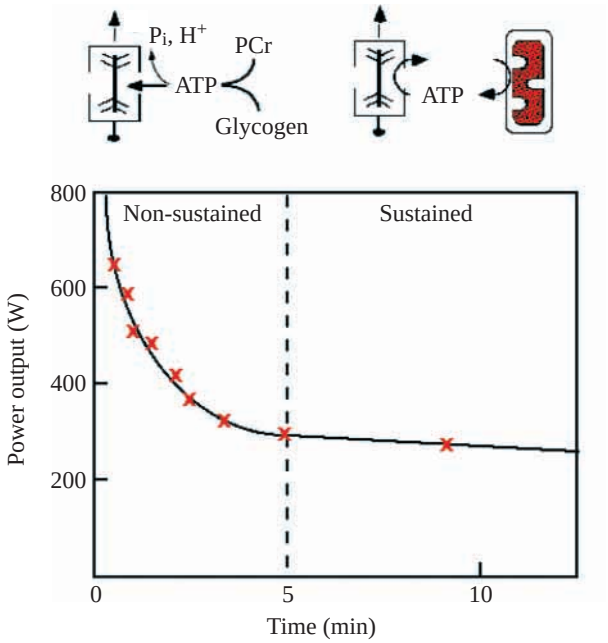

Fig. 1. Maximal power output of human legs as a function of time during cycling. The symbols represent the highest power output that can be sustained for a given time (adapted from Wilkie, 1985). The upper left diagram illustrates that high power outputs cannot be sustained because the source of ATP (phosphocreatine, PCr) generates by-products that inhibit contraction (inorganic phosphate, P_i and H^+). The upper right diagram illustrates that the continuous supply of ATP allows lower power outputs to be sustained.

characterized by a constant muscle force production and a continuous ATP supply, which both reflect the effects of mitochondrial oxidative phosphorylation. Recycling of the products of PCr breakdown and oxidation of the glycolytic products by the mitochondria ensures that the build-up of metabolites stabilizes at a level that permits sustained muscle force production. Mitochondrial oxidative phosphorylation also provides the longer-term supply of ATP needed to sustain muscle force production, as shown in the upper right diagram in Fig. 1. This dual role of the mitochondria shown in Fig. 1 illustrates our first and second contentions that sustainable power output is determined at the muscle level and that this power output is determined by ATP supply.

The simple scenario depicted in Fig. 1 (upper right) needs to be modified by two experimental findings. The first finding is that mitochondrial function alone does not determine the level of sustained performance, since sustained respiration is typically well below the maximal rate of oxygen uptake (Hammond and Diamond, 1997; Peterson et al., 1990). Sustainable muscle performance is also lower than that at the aerobic maximum. For example, human cyclists can maintain for 30 min approximately 80 % of the power output achieved in an aerobic capacity test (so-called $\dot{V}_{O_2max}$ test). This finding suggests that muscles have a higher capacity than the oxidative flux maintained during steady-state exercise (Hoppeler et al., 1985). Rodent and human exercise studies have shown a region of muscle work where there is no sign of fatigue, but where the

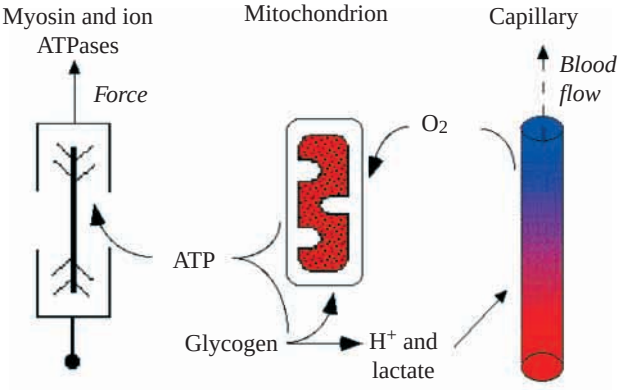

Fig. 2. ATP, H^+ and O_2 balances illustrating the integration of the major mass and energy fluxes in muscle.

muscle is not in metabolic steady state, as indicated by falling [PCr] and pH (McCully et al., 1993). Eventually, muscle force production drops as P_i and H^+ accumulate, but it is clear that the failure to achieve a steady-state [PCr] and pH precedes the decline in muscle force. This range of muscle work rates has been termed the transitional phase between steady-state and fatiguing contractions (Meyer and Foley, 1996). The paradox is that the muscle is not working at its oxidative capacity, yet the falling [PCr] indicates that ATP supply does not meet demand (hence the need to break down [PCr] to meet contractile needs).

The second finding modifying the upper right diagram in Fig. 1 may explain this paradox. Glycolytic pyruvate supply often exceeds oxidative needs, resulting in accumulation of H^+ and lactate in fully aerobic tissue (see Connett and Sahlin, 1996). The consequence of a higher than expected glycolytic rate is substantial non-oxidative ATP supply (so-called substrate-level phosphorylation) and the accumulation of inhibitory by-products during sustained contractions. We show below that this glycolytic flux contributes significantly to sustained ATP supply and generates H^+. We argue that this high glycolytic flux has the important consequence of restricting oxidative ATP supply to below the oxidative capacity during sustained contractions.

Partitioning of ATP supply

To determine how important glycolysis and H^+ accumulation are to sustained ATP supply, we use non-invasive ^{31}P magnetic resonance methods to partition ATP synthesis and determine the effect of glycolytic end-products on sustained performance. Quantifying the ATP sources and sinks involves the main storage form of energy in muscle, creatine phosphate (PCr). Fig. 3 shows the dynamics of [PCr] during exercise as a stack plot of separate ^{31}P spectra at rest, during exercise (drop in [PCr]) and during recovery (increase in [PCr]). The demand for ATP at the onset of exercise is met by the breakdown of [PCr] *via* the creatine kinase (CK) equilibrium:

$$[PCr] + [ADP] + [H^+] \leftrightarrow [ATP] + [Cr], \quad (1)$$

where [ADP] is the adenosine diphosphate concentration and

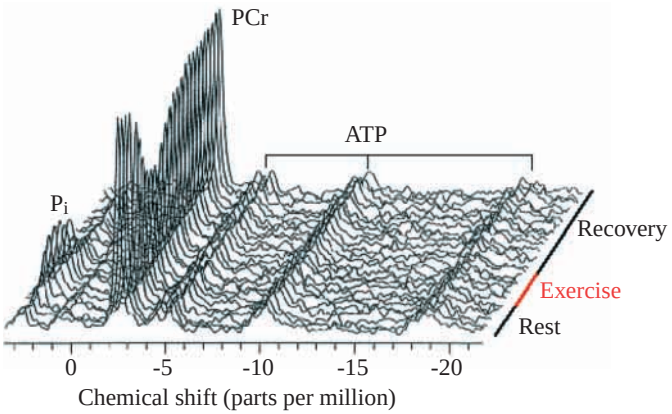

Fig. 3. Stack plot of magnetic resonance spectra showing inorganic phosphate (P_i), phosphocreatine (PCr) and ATP levels at rest, during exercise and through recovery in human quadriceps muscle. Reproduced with permission from The Physiological Society.

[Cr] is the free creatine concentration. We use the initial rate of PCr breakdown during exercise to quantify the muscle ATPase activity (Blei et al., 1993). Glycolysis is apparent as H^+ generation, from which we determine glycolytic ATP synthesis (Conley et al., 1997; Conley et al., 1998). After exercise has halted, [PCr] recovers to the resting level, and this recovery rate reflects mitochondrial ATP synthesis.

We partitioned ATP supply and demand using an extreme example of sustained performance: tailshaking by the rattlesnake, in which 50 Hz contractions can be maintained for hours (Martin and Bagby, 1973). The tailshaker muscle has many advantages over human muscle for the study of intracellular energetics. This muscle is uniform in its cell properties (Clark and Schultz, 1980; Schultz et al., 1980) and all its fibers appear to be recruited during rattling (Schaeffer et al., 1996), allowing unambiguous quantification of metabolic flux. To our surprise, glycolysis supplies more than one-third of the sustained ATP production during rattling (Kemper et al., 2001). The higher than expected fraction of ATP supplied by glycolytic flux during aerobic contractions is not restricted to the rattlesnake. We have reported that the glycolytic ATP contribution estimated for three human muscles studied in our laboratory is higher than the 8% contribution to ATP supply expected if (i) glycogenolysis is the sole source of pyruvate and (ii) pyruvate is the sole source of substrate for oxidative phosphorylation (Kemper et al., 2001). All the human muscles have contributions of 10–12% due to glycolytic flux, which generates H^+ and lactate. Including the 8% of ATP produced by glycolysis on the way to oxidation of pyruvate (with the above assumptions), this contribution of glycolysis to ATP supply can reach a total of up to 20%. It has long been recognized that glycolytic flux occurs during muscle exercise below the aerobic capacity (Connett and Sahlin, 1996), but these direct muscle measurements are the first to document the substantial fraction contributed by glycolysis to the total ATP flux.

We used the rattlesnake tailshaker muscle to validate our *in vivo* determinations using direct measurement of lactate generation and oxygen uptake (Kemper et al., 2001). This muscle has an exclusive blood circulation, allowing us to measure lactate generation by the tailshaker muscle alone during aerobic contractions. We found close agreement between the glycolytic ATP supply determined by *in vivo* magnetic resonance spectroscopy (MRS) and that determined from direct measurement of lactate generation during rattling (Kemper et al., 2001). These findings confirm the results from ^{31}P studies in human muscle (Conley et al., 1997; Conley et al., 1998) that glycolysis is a high-flux pathway that makes a significant contribution to ATP generation during muscle work.

Limits to oxidative phosphorylation

What is the consequence of this higher than expected glycolytic flux to sustained ATP supply and muscle performance? The substantial glycolytic flux that occurs during aerobic muscle exercise generates H^+ and lactate (see Connett and Sahlin, 1996). Accumulation of H^+ causes a decreased intracellular pH and, we argue, prevents the attainment of a metabolic steady state (Meyer and Foley, 1996). The result is that the highest sustained steady-state leg power output is often well below that achieved at the aerobic capacity (Hoppeler et al., 1985). The inability to elicit the aerobic capacity under steady-state conditions has led authors to conclude that muscle never operates at its oxidative capacity (Gollnick, 1985). The generation of H^+ and lactate has led some to suggest that this failure to achieve the aerobic capacity results from an O_2 supply shortfall (Wasserman and Koike, 1992). Oxygen delivery may limit respiration in whole-body exercise near the maximum oxygen consumption rate ($\dot{V}_{O_2max}$) in athletes (Wagner, 2000), but an O_2 limitation is unlikely at the much lower respiration rates of steady-state exercise. We present data below showing that oxidative phosphorylation rates approaching the mitochondrial oxidative capacity can be elicited in muscle. These results indicate that cellular metabolism itself, rather than O_2 supply, restricts oxidative phosphorylation during steady-state exercise. Our contention is that the interaction between glycolysis and oxidative phosphorylation in cellular metabolism keeps sustained ATP supply during steady-state exercise to well below the oxidative capacity.

The basis of this limitation to sustained ATP supply probably lies in the effects of both muscle contraction and glycolysis on the signal for oxidative phosphorylation [ADP]. At the onset of exercise, an imbalance between ATP supply and demand causes [PCr] to decrease (Fig. 3) and [ADP] to increase according to the creatine kinase (CK) reaction, as shown in Fig. 4:

$$[ADP] = [ATP][Cr]/([PCr][H^+]K_{eq}), \quad (2)$$

where K_{eq} is the CK equilibrium constant. The key signaling molecule ADP is at too low a concentration (micromolar levels) to be routinely measured by ^{31}P MRS. However, the value calculated from the CK reaction has been confirmed in

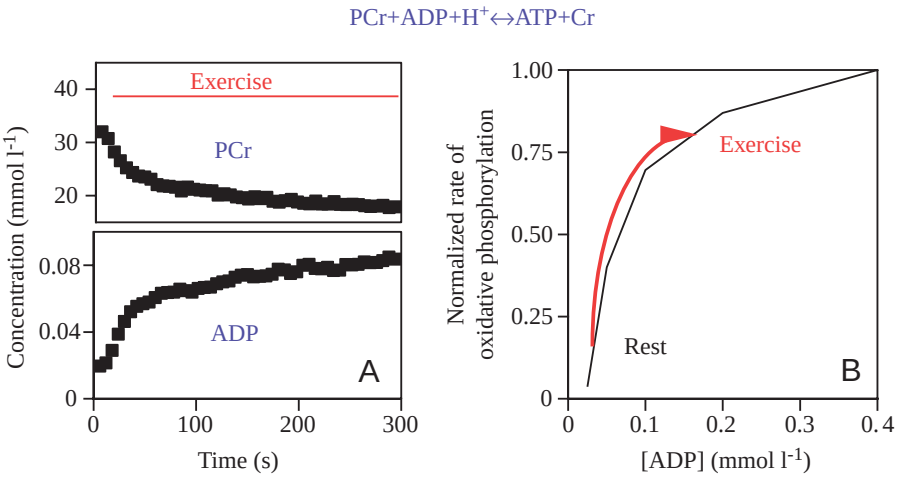

Fig. 4. Diagram of the reciprocal changes in phosphocreatine level ([PCr]) and [ADP] that occur *via* the creatine kinase reaction during exercise (A) and the effect of the rise in [ADP] with exercise (red curve and arrowhead) on mitochondrial oxidative phosphorylation (B). Data in A are unpublished results from human ankle dorsiflexor muscles, and data in B are from isolated mammalian heart mitochondria (Mootha et al., 1997).

intact muscle in which [ADP] was raised to high levels for a long enough period to be directly measured by ^{31}P MRS (Fisher and Dillon, 1988).

The elevation in [ADP] activates oxidative phosphorylation in isolated mammalian heart mitochondria (Fig. 4B), and Fig. 6B shows that the same relationship holds for human muscle *in vivo*. The drop in [PCr] continues with exercise until [ADP] increases enough to activate sufficient mitochondrial oxidative phosphorylation to balance ATP supply to ATP demand. Once an ATP balance has been attained, [PCr] breakdown is no longer needed to meet ATP demands and [PCr] reaches a steady-state level.

Not only is [ADP] central to the ATP balance in the cell, but it is also affected by the H^+ balance, as depicted in Fig. 5. Both balances affect the ADP level because the position of the CK equilibrium is a function of [PCr] and [H^+] (and therefore pH). The problem for cellular oxidative phosphorylation is that the effects of [PCr] and pH are opposite: a decline in [PCr] increases [ADP], while a decline in pH (an increase in [H^+]) reduces [ADP]. The result is that either an imbalance of ATP supply *versus* demand or in H^+ production *versus* efflux will affect the ADP level and, thereby, the oxidative phosphorylation rate during exercise.

What limits [ADP]?

The decline in pH that accompanies exercise inhibits the rise in [ADP] and may be a primary factor in limiting oxidative phosphorylation during exercise. This inability of [ADP] to reach levels that elicit the oxidative capacity is illustrated in Fig. 6A. The symbols are the highest [ADP] achieved *in vivo* during steady-state exercise in muscle reported in the literature (i.e. 0.06–0.11 mmol l^{-1}) (Harkema et al., 1997; Harkema and Meyer, 1997; Jeneson et al., 1996; Kushmerick et al., 1992; Paganini et al., 1997; Walter et al., 1997). The line shows the relationship between the rate of oxidative phosphorylation and [ADP] in isolated mitochondria (data normalized to the mitochondrial oxidative capacity; Mootha et al., 1997). This plot demonstrates that the steady-state [ADP] in exercising muscle is far below that required to elicit the oxidative capacity of mitochondria. These results support the contention that the maximum [ADP] never rises high enough during steady-state exercise to elicit the oxidative capacity (Gollnick, 1985; Hochachka and Matheson, 1992). Our contention is that this inability to activate the oxidative capacity is primarily an intracellular property due to the decline in intracellular pH resulting from glycolytic H^+ production during exercise.

The evidence for an effect of pH on oxidative phosphorylation comes from recent MRS studies in animals and humans (McCully et al., 1993). First, steady-state exercise studies have shown that steady-state [PCr] is depleted to only approximately half the resting level at the highest steady-state exercise level. Higher exercise levels cannot be maintained because pH drops throughout the course of the exercise (McCully et al., 1993). In addition, rodent and human studies have shown that the rate of [PCr] recovery slows at lower pH (Paganini et al., 1997; Walter et al., 1997). Some evidence

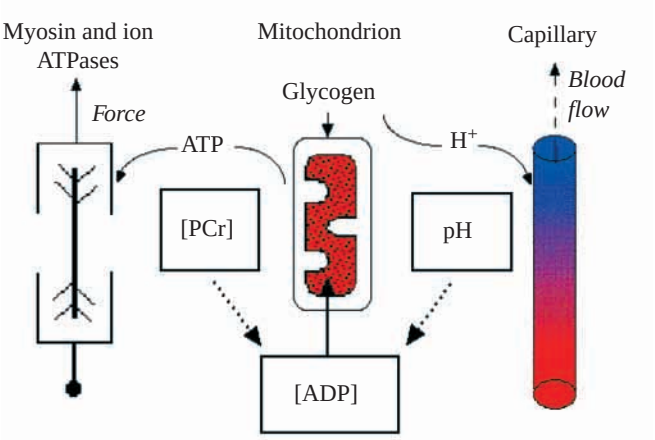

Fig. 5. The central position of ADP in muscle energetics. The ADP level is the signal for oxidative phosphorylation, which reflects both the balance of ATP supply to demand *via* phosphocreatine ([PCr]) and the balance of H^+ production *versus* efflux *via* pH in accord with the creatine kinase reaction (equation 2).

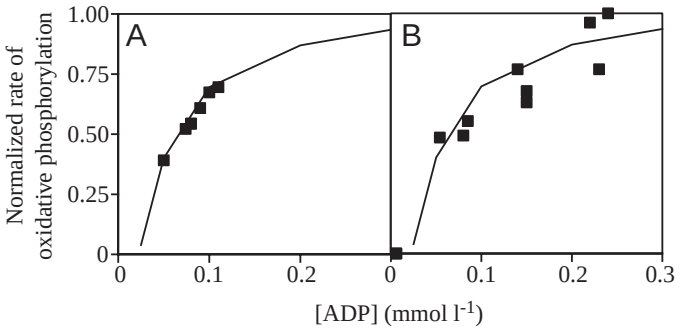

Fig. 6. ADP levels during steady-state (A) and transient (B) exercise determined for mammalian muscle. The line is the normalized rate of oxidative phosphorylation *versus* [ADP] in isolated mitochondria (Mootha et al., 1997). The symbols in A represent the highest steady-state [ADP] reported in mammalian skeletal muscle (Harkema et al., 1997; Harkema and Meyer, 1997; Jeneson et al., 1996; Kushmerick et al., 1992; Paganini et al., 1997; Walter et al., 1997). The symbols in B represent measurements from the human quadriceps at each step of an exercise stress test (Richardson et al., 1995). These data are normalized to the maximal level and superimposed on the relationship for isolated mitochondria (solid line) (Mootha et al., 1997).

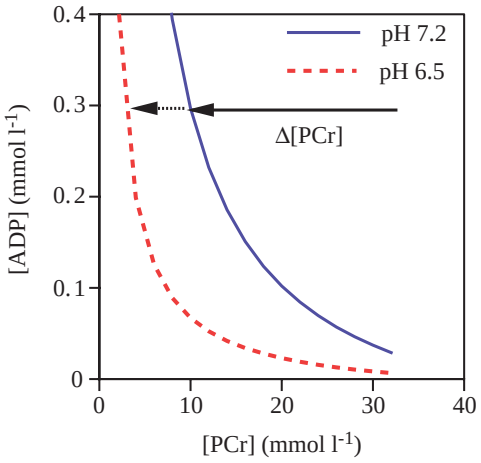

Fig. 7. [ADP] calculated from the creatine kinase reaction (equation 2) as a function of phosphocreatine concentration, [PCr], at two pH levels. The solid arrow indicates the effect of a change in [PCr] at pH 7.2 on [ADP]. The broken arrow indicates the change in [PCr] needed to maintain [ADP] with a reduction in pH from 7.2 to 6.5.

exists for a direct effect of pH on oxidative phosphorylation (Harkema et al., 1997b), but we suggest that the effect of pH is on the signal activating oxidative phosphorylation [ADP]. Thus, the elevation of [H^+] during sustained exercise does not directly inhibit force production, as occurs at higher [H^+], but rather affects sustained ATP production by limiting the rise in [ADP]. How can [ADP] ever rise high enough to elicit the mitochondrial oxidative capacity?

Keep [PCr] low and/or pH high

The key to generating a higher [ADP] than is possible during steady-state exercise is apparent in equation 2: either keep [PCr] low and/or pH high (i.e. H^+ low). Fig. 7 shows how both these strategies can be used to elevate [ADP] to levels that activate the mitochondrial oxidative capacity (approximately 0.3 mmol l^{-1}). The strategy of keeping [PCr] low to elevate [ADP] is shown by the lines calculated from the CK equilibrium at two pH values that represent the range typically found in muscle (equation 2). The horizontal line marked Δ[PCr] shows how much [PCr] must decline to raise [ADP] to 0.3 mmol l^{-1} at pH 7.2. The short dotted arrow indicates that [PCr] must drop further to keep [ADP] constant if pH declines from 7.2 to 6.5. How do muscles implement these strategies to achieve high [ADP]?

One method of achieving high [ADP] is to increase muscle ATPase activity and cause [PCr] to drop to offset a decline in pH. A large drop in [PCr] has been achieved in human muscle by a classic exercise step test that uses intense exercise above the steady-state work level to elicit $\dot{V}_{O_2max}$. An MRS study of the quadriceps exercising at $\dot{V}_{O_2max}$ (Richardson et al., 1995) showed a reduction in [PCr] to a minimal level (3 mmol l^{-1}) in the face of a pH decline to 6.5. The result is that [ADP] can reach a level (0.25 mmol l^{-1}) that is close to that eliciting maximum oxidative phosphorylation rates in isolated mitochondria, as shown in Fig. 6B. The correspondence between the normalized respiration rate *versus* [ADP] plot for human muscle and isolated mitochondria confirms that our MRS determination of [ADP] reflects the biochemically active level of this important signaling molecule. These ADP concentrations in human muscle exercising to $\dot{V}_{O_2max}$ far exceed the values observable during steady-state exercise in Fig. 6A. Thus, the decline in [PCr] resulting from intense, transient muscle exercise characteristic of a $\dot{V}_{O_2max}$ test offsets a low pH to elevate [ADP] briefly (<5 min) to levels that elicit the mitochondrial oxidative capacity.

The tailshaker muscle is able to elicit a high [ADP] and maintain it during sustained rattling by using the second strategy: keep pH high (Kemper et al., 1999). Two properties of the tailshaker muscle achieve this goal by ensuring a rapid H^+ and lactate efflux rate: small fiber size and extremely high muscle blood flow rates. The tailshaker muscle fiber diameter is approximately half (33 μm; Clark and Schultz, 1980) the diameter of human quadriceps fibers (67 μm; Hoppeler et al., 1985), resulting in a small diffusion distance to the fiber periphery. Removal of protons from the fiber is facilitated by muscle blood flow rates (468 ml 100 g^{-1} min^{-1}; Kemper et al., 2001) exceeding those found in human athletes (350 ml 100 g^{-1} min^{-1}; Richardson et al., 1995) or racehorses (<300 ml 100 g^{-1} min^{-1}; Armstrong et al., 1992). The end result is an intracellular pH in tailshaker muscle of 7.2 (Kemper et al., 1999) compared with 6.5 in the human quadriceps at $\dot{V}_{O_2max}$ (Richardson et al., 1995). This high pH combined with a low sustained [PCr] (10 mmol l^{-1}) in tailshaker muscle result in an [ADP] of 0.15 mmol l^{-1}. This [ADP] is well above any other reported sustained level and should elicit more than 80 % of the oxidative capacity of mitochondria according to Fig. 6 (Kemper

et al., 1999). The high aerobic flux and accompanying blood flow permit a high H^+ and lactate efflux and keep pH high. These results suggest that muscles can generate an [ADP] that comes close to the level that elicits the oxidative capacity in mitochondria. Thus, the limit to sustained oxidative ATP synthesis reflects the ability to activate mitochondria fully, which depends upon the interactions between glycolytic flux, H^+ efflux and oxidative phosphorylation (Fig. 2).

Concluding remarks

This review contends that sustainable performance is determined at the muscle level. We describe three phases of muscle power output (Meyer and Foley, 1996) to illustrate the role of intracellular factors in determining sustained performance. The first phase is a high-power-output fatiguing range in which the by-products of rapid ATP supply cause a rapid loss of muscle force production. The second phase has lower by-product accumulation but no immediate fatigue, so power output can be maintained for periods from seconds to minutes. We argue that high simultaneous fluxes of glycolysis and of oxidative phosphorylation provide parallel supplies of ATP, but also result in accumulation of H^+ that depresses [ADP] and prevents oxidative ATP supply from meeting demand. Thus, power output cannot be maintained beyond minutes in this phase because of a failure to match ATP supply to demand. The final phase is the lowest but sustainable level of work. The combination of glycolysis and oxidative phosphorylation can generate sufficient ATP to meet contractile ATP demand and sustain power output for periods beyond a few minutes. The factors limiting this final phase of muscle power output are consistent with our third contention that the constraint on [ADP] by pH in steady-state exercise is fundamental to cellular energetics and reflects the interaction between glycolysis and oxidative phosphorylation in the muscle cell.

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