An enzymatic approach to lactate production in human skeletal muscle during exercise

LAWRENCE L. SPRIET, RICHARD A. HOWLETT, and GEORGE J. F. HEIGENHAUSER

Department of Human Biology and Nutritional Sciences, University of Guelph, Guelph, Ontario N1G 2W1 CANADA; and Department of Medicine, McMaster University, Hamilton, Ontario L8N 35 CANADA

ABSTRACT

SPRIET, L. L., R. A. HOWLETT, and G. J. F. HEIGENHAUSER. An enzymatic approach to lactate production in human skeletal muscle during exercise. *Med. Sci. Sports Exerc.,* Vol. 32, No. 4, pp. 756–763, 2000. **Purpose:** This paper examines the production of lactate in human skeletal muscle over a range of power outputs (35–250% $\dot{V}O_{2max}$) from an enzymatic flux point of view. The conversion of pyruvate and NADH to lactate and NAD in the cytoplasm of muscle cells is catalyzed by the near-equilibrium enzyme lactate dehydrogenase (LDH). As flux through LDH is increased by its substrates, pyruvate and NADH, the factors governing the production of these substrates will largely dictate how much lactate is produced at any exercise power output. In an attempt to understand lactate production, flux rates through the enzymes that regulate glycogenolysis/glycolysis, the transfer of cytoplasmic reducing equivalents into the mitochondria, and the various fates of pyruvate have been measured or estimated. **Results:** At low power outputs, the rates of pyruvate and NADH production in the cytoplasm are low, and pyruvate dehydrogenase (PDH) and the shuttle system enzymes (SS) metabolize the majority of these substrates, resulting in little or no lactate production. At higher power outputs (65, 90, and 250% $\dot{V}O_{2max}$), the mismatch between the ATP demand and aerobic ATP provision at the onset of exercise increases as a function of intensity, resulting in increasing accumulations of the glycogenolytic/glycolytic activators (free ADP, AMP, and P_i). The resulting glycolytic flux, and NADH and pyruvate production, is progressively greater than can be handled by the SS and PDH, and lactate is produced at increasing rates. Lactate production during the onset of exercise and 10 min of sustained aerobic exercise may be a function of adjustments in the delivery of O_2 to the muscles, adjustments in the activation of the aerobic ATP producing metabolic pathways and/or substantial glycogenolytic/glycolytic flux through a mass action effect. **Key Words:** OXIDATIVE PHOSPHORYLATION, MUSCLE ENERGY STATE, EXERCISE INTENSITY, GLYCOGENOLYSIS/GLYCOLYSIS, PYRUVATE, LACTATE DEHYDROGENASE, PYRUVATE DEHYDROGENASE, NADH

During exercise, carbohydrate (CHO) is metabolized in the cytoplasm of skeletal muscle cells to produce pyruvate in the glycolytic pathway (Fig. 1). The CHO fuel is provided by the uptake of glucose from the blood and from glycogen stored inside the muscle. Once produced, the pyruvate can be further metabolized in the cytoplasm or transported across the inner mitochondrial membrane and metabolized inside the mitochondria. The most important mitochondrial pathway of pyruvate metabolism is conversion to acetyl-coenzyme A (acetyl-CoA) with the reduction of NAD to NADH in a reaction catalyzed by the pyruvate dehydrogenase (PDH) complex. The acetyl-CoA is then available to enter the tricarboxylic acid (TCA) cycle where reducing equivalents are produced for use in the electron transport chain to generate ATP in the process of oxidative phosphorylation. The oxidative use of 1 mmol glucose or glucosyl unit results in the production of ~39 mmol ATP. Conversely, the most important cytoplasmic pathway of pyruvate metabolism is conversion to lactate with the oxidation of NADH to NAD in the lactate dehydrogenase (LDH) reaction. When 1 mmol glucosyl unit is metabolized to lactate, it provides either 2 mmol (exogenous glucose) or 3 mmol (muscle glycogen) ATP.

Pyruvate can also combine with glutamate to form 2-oxoglutarate and alanine in the alanine aminotransferase (AAT) reaction in the cytoplasm, and to a minor extent in the mitochondria (Fig. 1). This reaction appears to be important in the early stages of exercise to increase the content of the TCA cycle intermediates but only accounts for ~2–5% of pyruvate disposal (12,13). Other reactions catalyzed by pyruvate carboxylase and malic enzyme also compete for pyruvate in the cytoplasm but do not appear to be quantitatively important (25), although they have not been studied in human muscle. NADH, the other substrate for LDH, can also be reconverted to NAD in the cytoplasm via the near-equilibrium malate-aspartate and alpha-glycerophosphate shuttle systems (SS), which transfer reducing equivalents to the mitochondria (Fig. 1). The malate-aspartate shuttle appears to be the quantitatively important system (26,32,33).

0195-9131/00/3204-0756/0
MEDICINE & SCIENCE IN SPORTS & EXERCISE®

Submitted for publication December 1998.
Accepted for publication December 1998.

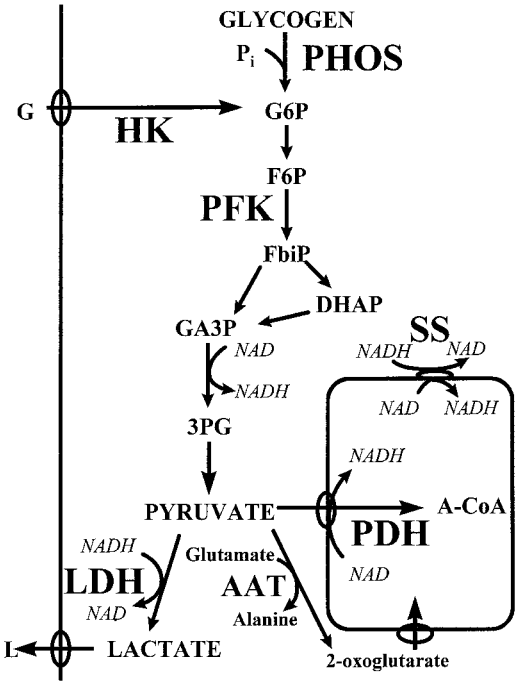

Figure 1—Schematic of the glycogenolytic/glycolytic pathways highlighting key enzymes and major fates of pyruvate. The *vertical line* indicates the muscle membrane separating blood and muscle cytoplasm, the *rectangle* depicts the mitochondrion, the *ellipses* indicate a transport process, and the *arrows* indicate the direction of net flux. G, glucose; L, lactate; A-CoA, acetyl-coenzyme A; PHOS, glycogen phosphorylase; HK, hexokinase; PFK, phosphofructokinase; SS, malate-aspartate shuttle system; LDH, lactate dehydrogenase; AAT, alanine amino transferase; PDH, pyruvate dehydrogenase. Flux through PHOS, HK, and PFK involves a 6-carbon moiety and through SS, PDH, and LDH involves a 3-carbon moiety. Therefore, the metabolism of a 6-carbon glucosyl unit may result in up to a 2-fold higher flux through SS, PDH, and LDH.

Determining the exact mechanisms of lactate production in skeletal muscle during all exercise conditions has been difficult and will not be resolved in this paper. Readers are urged to consult the numerous research papers and reviews that have been published on this topic (5–7,14,22). The purpose of this paper is to use an enzymatic approach to examine lactate production over a wide range of exercise power outputs in human skeletal muscle. This approach examines the flux through the key enzymes of glycogenolysis/glycolysis, the SS, and pyruvate metabolism at 35, 65, 90, and 250% $\dot{V}O_{2max}$. The first 10 min of cycle exercise at each of the aerobic power outputs and 30 s of cycle sprinting at ~250% $\dot{V}O_{2max}$ are examined. Because the measurements and estimates of enzyme and pathway fluxes are derived from needle muscle biopsy samples, this approach only provides average responses for the fiber type populations that exist in the sampled vastus lateralis muscles. Also, the subjects who volunteered for these studies were active, but not well-trained aerobically.

REGULATION OF GLYCOGENOLYTIC/GLYCOLYTIC ENZYMES

The rate of lactate production in skeletal muscle at any power output will depend mainly on the flux of substrate through the glycogenolytic/glycolytic pathway and the relative activities of the SS and the PDH and LDH pathways of pyruvate metabolism. Generally, the higher the demand for ATP, the greater the activation of the enzymes that regulate the glycogenolytic/glycolytic flux. These enzymes include glycogen phosphorylase (PHOS) and phosphofructokinase (PFK), and to a much smaller extent, hexokinase (HK) (Fig. 1). They are nonequilibrium enzymes that are covalently and allosterically regulated by factors related to the intensity of the muscle contraction (hormones and Ca^{2+}) and the severity of the demand for ATP. Therefore, the regulators associated with the energy status of the cell, or the muscle contents of ATP, and free ADP, AMP, inorganic phosphate (P_i), and H^+, are important in signaling the intensity of the contraction and demand for ATP. In sprint exercise, ammonia and inosine monophosphate may also be important.

Evidence suggests that skeletal muscle glycogen PHOS activity is regulated by a two-stage process at the onset of exercise (2,10,31). The first stage is transformation from the less active form of PHOS *b* to the more active *a* form, mediated mainly by Ca^{2+} and to a lesser extent by epinephrine. This stage may be regarded as a gross control mechanism that determines the potential upper limit for glycogenolytic flux. The second stage is posttransformational control of PHOS *a* (and to a lesser extent, PHOS *b*) by substrate availability (free P_i) and allosteric modulation (free AMP). This level of control fine tunes glycogenolytic flux to the ATP demand via the regulators linked to the energy state of the cell.

The nonequilibrium enzyme PFK is also controlled by regulators linked to the energy status of the cell, but in an entirely different manner than PHOS (for review, see 37). The major regulator of PFK activity is ATP, which not only binds to its active site (as a reaction substrate) but also to an allosteric or regulatory site. At rest, when ATP levels are high and positive modulators are low, ATP inhibits PFK. Citrate and H^+ potentiate the binding of ATP to the allosteric site and inhibition of the enzyme. However, during exercise, free ADP, AMP, and P_i accumulate and decrease the binding of ATP at the regulatory site, thereby releasing PFK inhibition. These positive modulators are dominant during exercise as PFK activity increases despite accumulations of citrate and H^+.

Skeletal muscle LDH is a tetrameric enzyme that exists in five isoforms due to the existence of two types of subunits, a muscle and a heart form, both of which favor the production of lactate. As a near-equilibrium enzyme, it is not under covalent or allosteric control but is sensitive to the concentrations of its substrates and products. Therefore, increases in pyruvate and NADH increase the flux through LDH in human skeletal muscle fiber types.

POTENTIAL MECHANISMS FOR LACTATE PRODUCTION

During exercise at low power outputs, the demand for ATP, the glycolytic flux, and the rate of pyruvate production are all low, and most of the pyruvate is converted to acetyl-

CoA in the mitochondria through activation of PDH. The NADH produced in the glycolytic pathway is also transferred across the mitochondrial membrane via the SS. Therefore, with both substrates of the LDH reaction low, the production of lactate is minimal.

At higher power outputs, with a higher demand for ATP, activation of the glycolytic pathway increases. If the rates of pyruvate and NADH production exceed the ability of PDH to metabolize pyruvate and/or the SS to transfer reducing equivalents into the mitochondria, lactate will be produced. However, whereas lactate production is ultimately related to the activities of the various enzymes in the glycolytic pathway, the SS, and the PDH complex, events in the mitochondria determine how active the glycolytic pathway needs to be, to assist in meeting the demand for energy.

According to one prevalent hypothesis, the electron transfer and oxidative phosphorylation processes in the mitochondria are near-equilibrium and are therefore controlled by the level of their substrates, oxygen (O_2), NADH, free ADP, and free P_i (39). The availability of these substrates will determine how well the demand for ATP is matched by aerobic ATP synthesis. When exercise begins, free ADP and P_i increase and processes that increase O_2 delivery and NADH provision are activated. However, at moderate aerobic exercise intensities and above, aerobic ATP provision cannot initially meet the demand for ATP and anaerobic pathways (substrate phosphorylation) contribute to ATP provision. In this case, ATP breakdown is greater than ATP production and ATP levels decrease slightly, producing significant increases in free ADP and AMP, decreases in phosphocreatine (PCr) and increases in P_i, leading to activation of the glycolytic pathway.

It has been argued that lactate production occurs only in response to situations where the muscle cell lacks sufficient O_2 to metabolize pyruvate and fat in the mitochondria (7,22). Others have argued that lactate production at the onset of intense aerobic exercise (65–100% $\dot{V}O_{2max}$) is not related to the availability of O_2 but slow activation of the metabolic pathways (e.g., PDH activation, TCA cycle, beta-oxidation) that provide substrate for aerobic ATP production (11,18,38). An additional interpretation is that lactate production is unrelated to O_2 availability and is simply a required by-product of glycolytic flux (5,7). A portion of the produced pyruvate would always be converted to lactate via LDH regardless of the rate of pyruvate production in a mass action manner. At low intensities and glycolytic flux, lactate production would be hard to detect. At higher power outputs with higher glycolytic flux, the lactate production would be very significant.

The most common situations where these possibilities may lead to lactate production include the transition from rest to exercise, the transition from one power output to a higher power output and exercise at power outputs that are higher than can be sustained with only oxidative phosphorylation. These possibilities may also contribute to the much lower but continued rates of lactate production during sustained exercise at moderate and high aerobic power outputs. In these situations, it is important to remember that lactate production may result from more than one of these causes, especially when muscle fiber type differences are considered.

There is evidence that following aerobic training (with or without the proliferation of mitochondria), lactate production at a given submaximal power output is reduced (3,16,17,35). In other words, the increase in glycolytic flux and resulting lactate production at the onset of exercise and during sustained aerobic exercise at a given submaximal power output is reduced. PCr degradation, glycogen utilization, and the accumulations of the by-products of ATP hydrolysis are reduced consequent to an increased ability to oxidize fat and/or provide O_2 to the mitochondria after short-term and prolonged aerobic training with proliferation of mitochondria (3,17,35). These same metabolic changes are present during intense aerobic exercise after acute perturbations (short-term training, increased fat availability, caffeine ingestion) that appear to increase fat metabolism and/or NADH provision, when no changes in mitochondrial potential have occurred (4,9,10,16).

GLYCOGENOLYTIC/GLYCOLYTIC FLUX AND EXERCISE INTENSITY

It is clear that the potential for lactate production in any exercise situation will be dependent on the flux in the glycogenolytic/glycolytic pathway. It is therefore important to remember that the flux in this pathway varies drastically depending on the exercise intensity. At low power outputs, the demand for ATP is low and can be provided at a high enough rate through aerobic combustion of CHO and fat. Consequently, the flux through the glycolytic pathway is low, providing small amounts of ATP, reducing equivalents and pyruvate.

The situation during power outputs above 100% $\dot{V}O_{2max}$ is very different. These power outputs cannot be sustained solely by aerobic ATP production. The ATP that cannot be provided aerobically must be generated anaerobically via substrate phosphorylation from the degradation of PCr in the creatine kinase reaction and in the glycolytic pathway with the conversion of glycogen to lactate. Therefore, the demand on the glycogenolytic/glycolytic pathway is not only to produce pyruvate for aerobic ATP production but also for anaerobic ATP provision. Although the amount of ATP generated by the metabolism of each mole of glucose from stored glycogen to lactate is low, the rate of ATP production that can be achieved is much higher than from CHO-derived aerobic ATP production. For this to be true, the flux through the glycogenolytic/glycolytic pathway must be substantially higher than during aerobic exercise. This results in pyruvate and NADH production rates that are much higher and can be handled in the PDH and SS reactions, respectively, and lactate and NAD must be produced by LDH. Because the sum of cytosolic [NAD] and [NADH] is fixed, the regeneration of NAD is important for sustained high glycolytic flux as the NAD is reutilized higher up in the pathway (glyceraldehyde phosphate dehydrogenase reaction). The produced lactate accumulates in the muscle or is transported out of the cell.

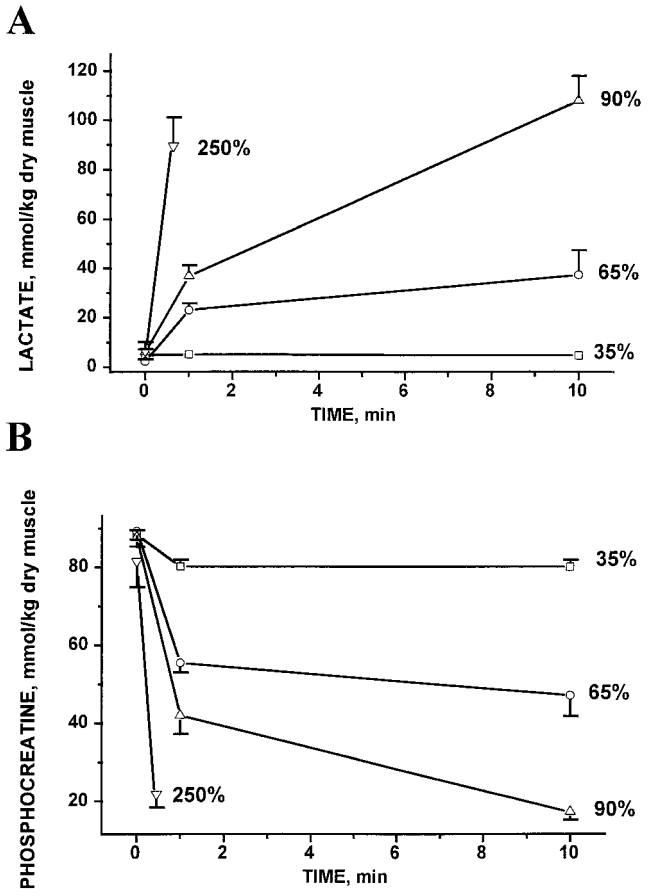

Figure 2—Human muscle lactate (A) and phosphocreatine (B) contents during cycling for 10 min at varying power outputs (35–90% $\dot{V}O_{2max}$) and 30 s at 250% $\dot{V}O_{2max}$. Data are means ± SE and were obtained from references 18, 24, 30, and 36.

Therefore, the glycogenolytic/glycolytic pathway must be sensitive to extremes in demand, the low flux of aerobic exercise and the very high flux of sprint exercise. The maximal activities of PHOS and PFK measured *in vitro* are very high, and flux through these enzymes approaches these activities during maximal sprint exercise. Conversely, because the maximal activities *in vitro* of the enzymes involved only in aerobic metabolism (SS and PDH) are much lower, flux rates approach these activities during exercise at high aerobic power outputs.

EXERCISE INTENSITY AND SKELETAL MUSCLE LACTATE PRODUCTION

Lactate accumulates in human vastus lateralis skeletal muscle as a function of increasing exercise power output (Fig. 2). However, muscle lactate contents underestimate the production of lactate during exercise as varying amounts of lactate will be transported out of the muscle. Therefore, measured rates of lactate release from other studies (20,27) were added to the muscle measurements to estimate average LDH flux rates (Table 1). Similarly, flux rates through additional enzymes were estimated from skeletal muscle biopsy measurements of fuels, metabolites and enzymes in the relevant pathways (Table 1).

35% $\dot{V}O_{2max}$. The transition from rest to 1 min of exercise at 35% $\dot{V}O_{2max}$ (~60 W) resulted in little disturbance to the energy status of the muscle cells. The ATP content was unchanged, PCr decreased by less than 10 mmol·kg^{-1} dry muscle (dm), and free P_i, ADP, and AMP increased only slightly (Figs. 2–4). The muscle was able to meet the increased requirements for ATP through aerobic means. Consequently, the flux through PHOS, PFK, and the glycolytic pathway was low (Table 1) and essentially matched the aerobic use of pyruvate as no muscle lactate accumulation could be detected after 1 and 10 min of exercise (Fig. 2). A significant amount of PDH was transformed to its active form (PDH *a*) to convert pyruvate to acetyl-CoA (Fig. 5). Previous studies have demonstrated that transformation to PDH *a* is equivalent to PDH flux in normal exercise situations (18,29). Additional acetyl-CoA may also be derived from the metabolism of fat. It is unlikely that the AAT reaction was very active and resulted in significant increases in TCA cycle intermediates, although this has not been measured during cycling at this power output. After 8 min of cycling at 40% $\dot{V}O_{2max}$, arterial blood lactate did increase slightly from 0.45 to 1.08 mM, and a small but measurable lactate efflux (0.75 mmol·min^{-1}·exercising leg·$^{-1}$) was present (27).

65% $\dot{V}O_{2max}$. The energy status of the muscle cells in the initial minute of exercise at 65%$\dot{V}O_{2max}$ (~165 W) was significantly altered, indicating a temporary mismatch between the demand for ATP and aerobic resynthesis of ATP. Consequently, anaerobic pathways provided the balance of the ATP, until aerobic metabolism could match the required rate of ATP provision. Although the content of muscle ATP was well defended, PCr decreased by ~34 mmol·kg^{-1} dm in the 1st minute of exercise and free P_i, ADP, and AMP increased noticeably (Figs. 3 and 4). Higher levels of these metabolites increased PHOS and PFK activities such that glycolytic flux was ~5-fold higher than at 35% $\dot{V}O_{2max}$ (Table 1, Fig. 6). Muscle glycogen was quantitatively the most important CHO source for ATP resynthesis during the transition to moderate aerobic exercise. Estimated PDH flux (activity of PDH *a*) increased rapidly in the 1st minute, reaching ~2.7 mmol pyruvate·kg^{-1} wet muscle (wm)·min^{-1} (Table 1, Fig. 5). Assuming there was sufficient O_2 to support the aerobic combustion of the resulting acetyl-CoA, this rate was not high enough to metabolize all of the produced pyruvate. The same may have been true for the ability of the SS to handle the production of NADH. A small portion (2–5%) of the produced pyruvate may have been metabolized in the AAT reaction, resulting in an increase in TCA cycle intermediates (12,13). The remaining pyruvate and NADH would increase lactate production via the LDH reaction. The estimated flux through LDH in the 1st minute at 65% $\dot{V}O_{2max}$ was 2-fold higher than the flux through PDH (Table 1, Fig. 5). In the final 9 min of exercise at this power output, muscle lactate content was unchanged, but arterial lactate increased from ~1 to 3 mM, and lactate release was ~4 mmol·min^{-1}·exercising^{-1} leg after 8 min of cycling (27).

TABLE 1. Estimated flux through the key enzymes linked to lactate production in human skeletal muscle during cycle exercise at various power outputs.

		Power Output, % $\dot{V}O_{2max}$			
	MAX *in Vitro*	35% (58 W)	65% (164 W)	90% (229 W)	250% (625 W)
PHOS	40–45	0.55	3.6	7.0	35.5
HK	~3	0.2	0.35	0.5	0.5
PFK	40–60	0.75	3.95	6.5	32.25
SS	~27	1.5	2.7	3.4	4.5
PDH	4.5	1.5	2.7	3.4	4.5
LDH	~350	0	5.2	9.6	60

Flux, mmol substrate · kg^{-1} vastus lateralis (wet) muscle · min^{-1}. $\dot{V}O_{2max}$, maximal oxygen consumption; MAX, maximal enzyme activity measured *in vitro*; W, watts; PHOS, glycogen phosphorylase; HK, hexokinase; PFK, phosphofructokinase; SS, malate-aspartate shuttle system; PDH, pyruvate dehydrogenase; LDH, lactate dehydrogenase. Flux through PHOS, HK, and PFK involves a 6-carbon moiety and through SS, PDH, and LDH involves two, 3-carbon moieties. Therefore, the metabolism of a 6-carbon glucosyl unit may result in up to a 2-fold higher flux through SS, PDH, and LDH.

Estimated maximal *in vitro* activities (37°C) were taken from the following references; PHOS (2,3); HK (1,15,34); PFK (1,19); SS (32,33); PDH (18,30); LDH (15,34). LDH flux was estimated at each power output from pre and post 30 s or 1 min muscle lactate content measurements plus estimated lactate transport from muscles. It was assumed that no lactate left the muscle at 35% $\dot{V}O_{2max}$. At 65, 90, and 250% $\dot{V}O_{2max}$, it was assumed that lactate efflux was 20%, 25%, and 10% of the muscle lactate accumulations, respectively. PDH flux was assumed to be equivalent to PDH *a* measurements at each power output (18,28,30). The flux through the SS could be no higher than PDH flux. PFK flux was estimated as that required to account for pyruvate metabolism by LDH and PDH. HK flux was estimated from glucose uptake measurements of Katz and coworkers (21,23). Lastly, PHOS flux was the CHO required to match the PFK flux that was not accounted for by HK. At the two highest power outputs, it also included the build up of glycolytic intermediates between the PHOS and PFK steps (18,30).

The increase in ATP demand during the transition from rest to exercise at 65% $\dot{V}O_{2max}$ was too great for the aerobic processes in the muscle to immediately handle. The exact limitation(s) that prevented a more rapid increase in aerobic metabolism at this power output are unknown. It could be a lack of available O_2 that limits ATP production rate and ultimately determines the rates of acetyl-CoA use from both CHO and fat. It may also be the rate at which the key enzymes regulating CHO and fat metabolism (e.g., PDH transformation, TCA cycle, and beta-oxidation) can be activated that limits the production of reducing equivalents and ultimately ATP provision. This energy mismatch leads to increased PCr use and free ADP, AMP, and P_i accumulations. These signals activate the glycogenolytic/glycolytic enzymes, and the increased glycolytic flux increases pyruvate production. Increases in pyruvate will increase flux through the near-

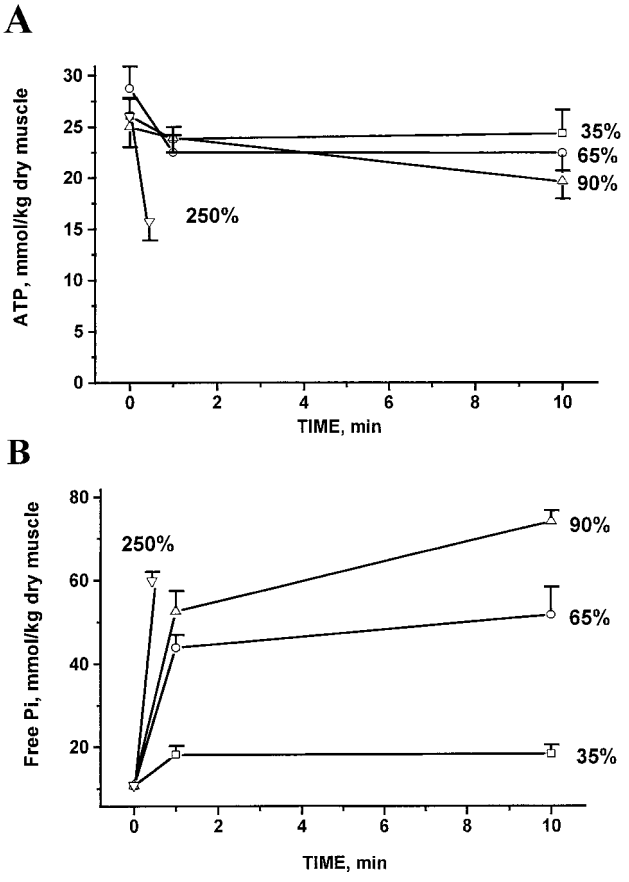

Figure 3—Human muscle ATP (A) and free inorganic phosphate (B) contents during cycling for 10 min at varying power outputs (35–90% $\dot{V}O_{2max}$) and 30 s at 250% $\dot{V}O_{2max}$. P_i, inorganic phosphate. Data are means ± SE and were obtained from references 18, 24, 30, and 36.

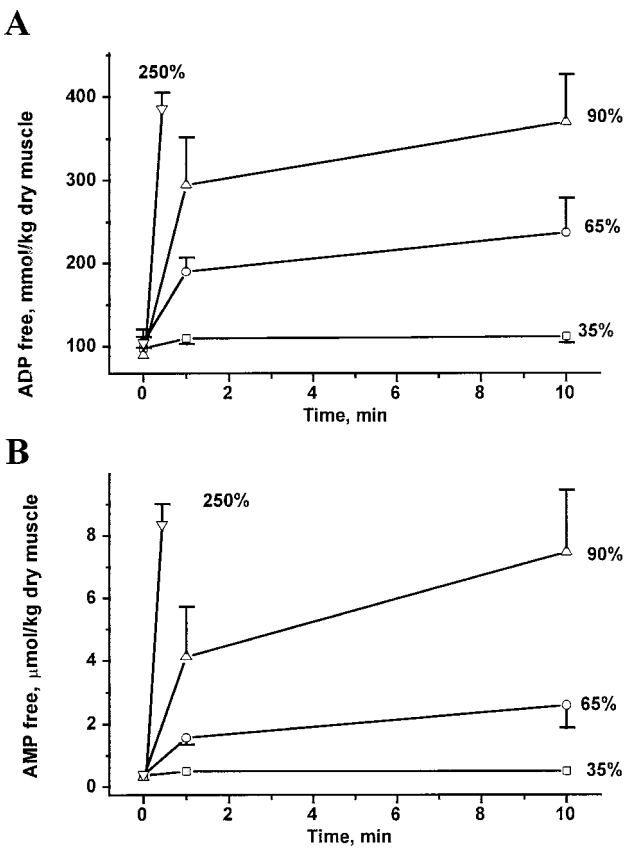

Figure 4—Human muscle free ADP (A) and free AMP (B) contents during cycling for 10 min at varying power outputs (35–90% $\dot{V}O_{2max}$) and 30 s at 250% $\dot{V}O_{2max}$. Data are means ± SE and were obtained from references 18, 24, 30, and 36. Free ADP and AMP calculated as described by Dudley et al. (8).

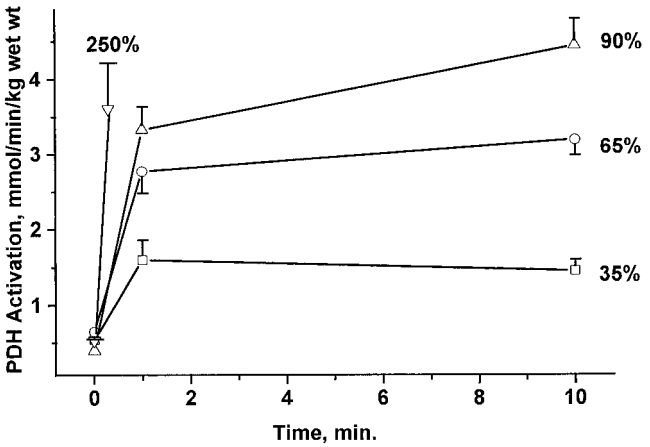

Figure 5—Human muscle pyruvate dehydrogenase activation (transformation) to the active (*a*) form during cycling for 10 min at varying power outputs (35–90% $\dot{V}O_{2max}$) and 30 s at 250% $\dot{V}O_{2max}$. Data are means ± SE and were obtained from references 18, 28, and 30.

equilibrium reactions catalyzed by LDH and AAT, help to transform the nonequilibrium enzyme PDH to its active form, and provide substrate for PDH *a*.

The same explanation may account for the ongoing lactate production in the exercise period from minute 1 to 10. However, it is not clear why lactate production would continue when O_2 delivery to the working mitochondria and metabolic activation have had time to adjust to the demands of the power output. It may be that the adjustments to the power output are not perfect, although the low rate of lactate production during continued exercise represents a very small mismatch between pyruvate production and pyruvate disposal through PDH. It is also unclear how the lower rates of glycogenolytic/glycolytic flux are regulated, as the signals that control the transformation of PHOS to its more active *a* form and the allosteric activators of PHOS *a* and *b* remain constant after the initial minute of exercise at 65% $\dot{V}O_{2max}$. It may involve changes in the amount of PHOS in the *a* form and/or the sensitivity of both the *a* and *b* forms to allosteric regulators.

90% $\dot{V}O_{2max}$. The mismatch between the demand for ATP and the ability to produce ATP aerobically in the working muscles in the transition from rest to 1 min of exercise at 90% $\dot{V}O_{2max}$ (~230 W) was greater than at 65% $\dot{V}O_{2max}$. Lactate increased from ~6 to 37 mmol·kg^{-1} dm and PCr decreased by 44 mmol·kg^{-1} dm in the 1st minute of exercise (Fig. 2). Accumulations of free ADP, AMP, and P_i were greater, and glycolytic flux was 2-fold greater than exercise at 65% $\dot{V}O_{2max}$ (Figs. 3 and 4, Table 1). Muscle glycogen was clearly the dominant CHO fuel for ATP production during intense aerobic exercise (Table 1). PDH flux increased rapidly to ~3.4 mmol pyruvate·kg^{-1} wm·min^{-1}, but LDH activity was 3-fold higher in the 1st minute of exercise (Fig. 6, Table 1). Again, the AAT reaction metabolized ~2–5% of the produced pyruvate at this power output (12,13).

It is also clear that a metabolic steady state was never reached during 10 min of sustained cycling at 90% $\dot{V}O_{2max}$ as muscle lactate accumulation continued beyond 1 min (Fig. 2). Arterial and venous blood lactate concentrations increased from ~1 mM at rest to 4–6 and 6–8 mM at 5 and 10 min of exercise (9,20). Free P_i, ADP, and AMP contents also continued to accumulate, PCr stores continued to decrease, and PDH flux was maximal at 10 min. At this power output, it seems possible that lactate production continued because some muscle fibers were experiencing an inadequate delivery of O_2, thereby maintaining the signals for high glycolytic activity. This is supported by the continuing decrease in the PCr store, suggesting that aerobic metabolism was not able to match the demand for ATP in all fibers. Also, although the average whole body power output was 90% $\dot{V}O_{2max}$, the demand on some fibers may have exceeded their aerobic potential, thereby requiring continued anaerobic ATP provision (PCr degradation and lactate production). These suggestions are strong possibilities because the subjects in the cited studies were not aerobically trained and had not adapted to optimize O_2 delivery to the muscle and the use of O_2 by the muscle.

250% $\dot{V}O_{2max}$. At the most intense power output studied, subjects sprinted maximally on an isokinetic cycle ergometer for 30 s. The power output decreased from ~800–900 W in the initial seconds of exercise to ~500 W after 30 s and averaged ~625 W (24,36). The average power output represented ~250% of the power output required to elicit $\dot{V}O_{2max}$ in these subjects. The demand for ATP in this situation was so severe that it required both the aerobic and anaerobic systems to reach maximal rates of ATP resynthesis as quickly as possible. Breath-by-breath measurements suggest that $\dot{V}O_2$ reached ~75–80% of $\dot{V}O_{2max}$ in 30 s (30)

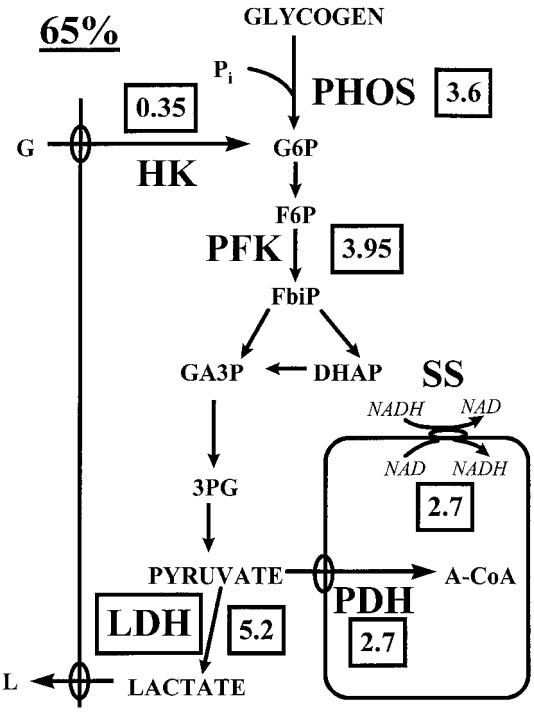

Figure 6—Estimations of flux through the key enzymes linked to lactate production during the 1st minute of cycling at 65% $\dot{V}O_{2max}$. Units for flux, mmol substrate·kg^{-1} vastus lateralis muscle (wet)·min^{-1}. Abbreviations and symbols as in Figure 1, and see Table 1 for calculations.

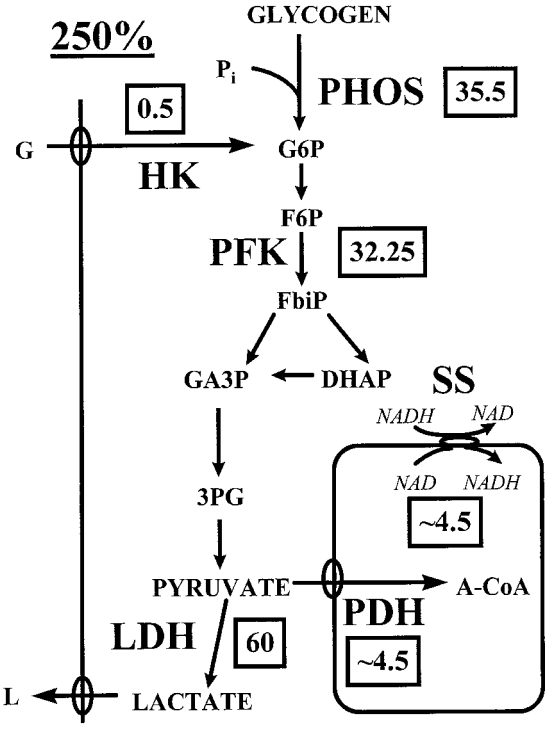

Figure 7—Estimations of flux through the key enzymes linked to lactate production during 30 s of sprint cycling at 250% $\dot{V}O_{2max}$. Units for flux, mmol substrate·kg^{-1} vastus lateralis muscle (wet)·min^{-1}. Abbreviations and symbols as in Figure 1 and see Table 1 for calculations.

and contributed only ~20–30% of the total required ATP during the 30-s bout (24,30). Anaerobic pathways provided the remainder of the ATP with glycolysis contributing ~65–70% (muscle lactate increased to ~90 mmol·kg^{-1} dm) and PCr degradation contributing 30–35% (PCr decreased to ~20 mmol·kg^{-1} dm) of the total anaerobic contribution. Therefore, the overall energy contribution for 30 s of sprint cycling was 20–30% aerobic, 46–53% anaerobic glycolysis and 24–27% from PCr degradation.

At this power output, the calculated fluxes through muscle PHOS and PFK approached the maximal values measured *in vitro* and were 50- to 75-fold higher than at 35% $\dot{V}O_{2max}$ (Table 1, Fig. 7). At these high glycolytic rates, pyruvate was metabolized mainly through LDH (flux averaged 60 mmol pyruvate·kg^{-1} wm·min^{-1}), even though activation of PDH was already maximal (~3.5–4 mmol pyruvate·kg^{-1} wm·min^{-1}) after only 15 s of sprinting (28). The accumulations of free ADP, AMP and P_i were extremely rapid and higher after only 30 s of exercise than after 1 min of aerobic exercise (Figs. 3 and 4), accounting for the large increase in glycolytic flux.

The extreme ATP demand at this power output required that maximal activation of aerobic and anaerobic ATP producing pathways were reached as rapidly as possible. The aerobic systems were 75–80% activated at the end of 30 s, but PDH was fully activated after 15 s of sprinting. Because there was an abundance of pyruvate available, it suggests that the delivery of O_2 to the mitochondria is the factor that limits how quickly aerobic metabolism is turned on during this type of exercise. In spite of this limitation, aerobic metabolism contributed 20–30% of the required ATP. It should also be noted, that even if the aerobic systems could turn on in a square wave fashion at the onset of sprinting, the aerobic ATP contribution would only rise to 40% of the total required energy. This makes it very clear how important the anaerobic systems are for providing the balance of the energy required during maximal sprint situations. This includes the production of lactate and regeneration of NAD in the LDH reaction.

SUMMARY

This paper examined the production of lactate in human skeletal muscle over a range of power outputs (35, 65, 90, and 250% $\dot{V}O_{2max}$) from an enzymatic point of view. Flux through the near-equilibrium enzyme LDH is controlled by increases in the reaction substrates, NADH and pyruvate. The factors governing the production of these substrates will largely dictate how much lactate is produced at any exercise power output.

At low power outputs, the signals that turn on glycogenolysis/glycolysis accumulated to a minor extent, resulting in low rates of pyruvate and NADH production in the cytoplasm. PDH and the SS enzymes metabolized the majority of these LDH substrates, resulting in little or no lactate production. At moderate and high power outputs (65 and 90% $\dot{V}O_{2max}$), the mismatch between the ATP demand and ATP provision at the onset of exercise increased as a function of exercise intensity, resulting in accumulations of the glycogenolytic/glycolytic activators (free ADP, AMP, and P_i). The resulting glycolytic flux, and NADH and pyruvate production was greater than could be handled by the SS and PDH reactions, and lactate production was related to the exercise intensity. The delivery of O_2 to the muscle, the activation rate of the aerobic ATP producing pathways, and a mass action effect of increasing glycolytic flux may all have contributed to lactate production during the transition from rest to exercise.

At the highest power output studied (~250% $\dot{V}O_{2max}$), the signals that stimulate both the aerobic and anaerobic ATP producing pathways were maximal. Aerobic pathways produced only 20–30% of the total ATP requirement, leaving the remainder for the anaerobic glycolytic and PCr systems. Consequently glycogenolytic/glycolytic flux approached the maximal activities of PHOS and PFK measured *in vitro*, and pyruvate was converted to lactate at a rate that was at least 15- to 20-fold higher than conversion to acetyl-CoA.

In conclusion, this paper has described the production of lactate at varying power outputs from an enzymatic viewpoint. However, the mitochondrial events that ultimately result in activation of the enzymes involved in the production of lactate remain obscure.

The authors' experiments cited in this paper were supported by grants from the Natural Science and Engineering Research and Medical Research Councils of Canada. R. A. Howlett was supported by a Gatorade Sports Science Institute student research award. G. J. F. Heigenhauser is a Career Investigator of the Heart and Stroke Foundation of Ontario.

Address for correspondence: Lawrence L. Spriet, Ph.D., Dept. of Human Biology & Nutritional Sciences, University of Guelph, Guelph, Ontario, N1G 2W1 Canada. E-mail: spriet.ms@aps.uoguelph.ca.

REFERENCES

1. BLOMSTRAND, E., B. EKBLOM, and E. A NEWSHOLME. Maximal activities of key glycolytic and oxidative enzymes in human skeletal muscle from differently trained individuals. *J. Physiol.* 381: 111–118, 1986.
2. CHASIOTIS, D., K. SAHLIN, and E. HULTMAN. Regulation of glycogenolysis in human muscle at rest and during exercise. *J. Appl. Physiol.* 53:708–715, 1982.
3. CHESLEY, A., G. J. F. HEIGENHAUSER, and L. L. SPRIET. Regulation of muscle glycogen phosphorylase activity following short-term endurance training. *Am. J. Physiol.* 270(*Endocrinol. Metab.*): E328–E335, 1996.
4. CHESLEY, A., R. A. HOWLETT, G. J. F. HEIGENHAUSER, E. HULTMAN, and L. L. SPRIET. Regulation of muscle glycogenolytic flux during intense aerobic exercise after caffeine ingestion. *Am. J. Physiol.* 275(*Reg. Integr. Comp. Physiol.*):R596–R603, 1998.
5. CONNETT, R. J., T. E. GAYESKI, and C. R. HONIG. Lactate accumulation in fully aerobic, working, dog gracilis muscle. *Am. J. Physiol.* 246(*Heart Circ. Physiol.*):H120–H128, 1984.
6. CONNETT, R. J., C. R. HONIG, T. E. J. GAYESKI, and G. A. BROOKS. Defining hypoxia: a systems view of VO_2, glycolysis, energetics, and intracellular PO_2. *J. Appl. Physiol.* 68:833–842, 1990.
7. CONNETT, R. J, and K. SAHLIN. Control of glycolysis and glycogen metabolism. In: *Handbook of Physiology, Section 12, Exercise: Regulation and Integration of Multiple Systems*, L. B. Rowell and J. T. Shepherd (Eds.). New York: Oxford University Press, 1996. pp. 870–911.
8. DUDLEY, G. A., P. C. TULLSON, and R. L. TERJUNG. Influence of mitochondrial content on the sensitivity of respiratory control. *J. Biol. Chem.* 262:9109–9114, 1987.
9. DYCK, D. J., C. T. PUTMAN, G. J. F. HEIGENHAUSER, E. HULTMAN, and L. L. SPRIET. Regulation of fat-carbohydrate interaction in skeletal muscle during intense aerobic cycling. *Am. J. Physiol.* 265:E852–E859, 1993.
10. DYCK, D. J., S. J. PETERS, P. S. WENDLING, A. CHESLEY, E. HULTMAN, and L. L. SPRIET. Regulation of muscle glycogen phosphorylase activity during intense aerobic cycling with elevated FFA. *Am. J. Physiol.* 270(*Endocrinol. Metab.*):E116–E125, 1996.
11. FROM, A. H. L., S. D. ZIMMER, S. P. MICHURSKI, et al. Regulation of the oxidative phosphorylation rate in the intact cell. *Biochemistry* 29:3731–3743, 1990.
12. GIBALA, M. J., M. A. TARNOPOLSKY, and T. E. GRAHAM. Tricarboxylic acid cycle intermediates in human skeletal muscle at rest and during prolonged cycling. *Am. J. Physiol.* 272(*Endocrinol. Metab.*):E239–E344, 1997.
13. GIBALA, M. J., D. A. MACLEAN, T. E. GRAHAM, and B. SALTIN. Anaplerotic processes in human skeletal muscle during brief dynamic exercise. *J. Physiol.* 502.3:703–713, 1997.
14. GLADDEN, L. B. Lactate transport and exchange during exercise. In: *Handbook of Physiology, Section 12, Exercise: Regulation and Integration of Multiple Systems*. L. B. Rowell and J. T. Shepherd (Eds.). New York: Oxford University Press, 1996, pp. 614–648.
15. GREEN, H. J., I. G. FRASER, and D. A. RANNEY. Male and female differences in enzyme activities of energy metabolism in vastus lateralis muscle. *J. Neurol. Sci.* 65:323–331, 1984.
16. GREEN, H. J., R. HELYAR, M. BALL-BURNETT, B. FARRANCE, and D. RANNEY. Metabolic adaptations to training precede changes in muscle mitochondrial capacity. *J. Appl. Physiol.* 72:484–491, 1992.
17. HOLLOSZY, J. O., and E. F. COYLE. Adaptations of skeletal muscle to endurance exercise and their metabolic consequences. *J. Appl. Physiol.* 56:831–838, 1984.
18. HOWLETT, R. A., M. L. PAROLIN, D. J. DYCK, E. HULTMAN, N. L. JONES, G. J. F. HEIGENHAUSER, and L. L. SPRIET. Regulation of skeletal muscle glycogen phosphorylase and pyruvate dehydrogenase at varying exercise power outputs. *Am. J. Physiol.* 275(*Reg. Integr, Comp. Physiol.*):R418–R425, 1998.
19. JANSSON, E., and C. SYLVEN. Activities of key enzymes in the energy metabolism of human myocardium and skeletal muscle. *Clin. Physiol.* 6:465–471, 1986.
20. KATZ, A., K. SAHLIN, and A. JUHLIN-DANNFELT. Effect of beta-adrenoceptor blockade on H^+ and K^+ flux in exercising humans. *J. Appl. Physiol.* 59:336–341, 1985.
21. KATZ, A, S. BROBERG, K. SAHLIN, and J. WAHREN. Leg glucose uptake during maximal dynamic exercise in humans. *Am. J. Physiol.* 251(*Endocrinol. Metab.*):E65–E70, 1986.
22. KATZ, A., and K. SAHLIN. Regulation of lactic acid production during exercise. *J. Appl. Physiol.* 65:509–518, 1988.
23. KATZ, A., K. SAHLIN, and S. BROBERG. Regulation of glucose utilization in human skeletal muscle during moderate dynamic exercise. *Am. J. Physiol.* 260(*Endocrinol. Metab.*):E411–E415, 1991.
24. MCCARTNEY, N., L. L. SPRIET, G. J. F. HEIGENHAUSER, J. M. KOWALCHUK, J. R. SUTTON, and N. L. JONES. Muscle power and metabolism in maximal intermittent exercise. *J. Appl. Physiol.* 60:1164–1169, 1986.
25. MOLE, P. A., K. M. BALDWIN, R. L. TERJUNG, and J. O. HOLLOSZY. Enzymatic pathways of pyruvate metabolism in skeletal muscle: adaptations to exercise. *Am. J. Physiol.* 224:50–54, 1973.
26. NEWSHOLME, E. A., and A. R. LEECH. *Biochemistry for the Medical Sciences.* Toronto: Wiley, 1983, pp. 200–204.
27. ODLAND, L. M., G. J. F. HEIGENHAUSER, D. WONG, M. G. HOLLIDGE-HORVAT, and L. L. SPRIET. Effects of increased fat availability on fat-carbohydrate interaction during prolonged aerobic exercise in humans. *Am. J. Physiol.* 274:R894–R902, 1998.
28. PAROLIN, M. L., A. CHESLEY, M. P. MATSOS, L. L. SPRIET, N. L. JONES, and G. J. HEIGENHAUSER. Regulation of skeletal muscle glycogen phosphorylase and PDH during maximal intermittent exercise. *Am J. Physiol.* 277(5 Pt 1):E890–900, 1999.
29. PUTMAN, C. T., L. L. SPRIET, E. HULTMAN, et al. Pyruvate dehydrogenase activity and acetyl-group accumulation during exercise after different diets. *Am. J. Physiol.* 265(*Endocrinol. Metab.*): E752–E760, 1993.
30. PUTMAN, C. T., N. L. JONES, L. C. LANDS, T. M. BRAGG, M. G. HOLLIDGE-HORVAT, and G. J. F. HEIGENHAUSER. Skeletal muscle pyruvate dehydrogenase activity during maximal exercise in humans. *Am. J. Physiol.* 269(*Endocrinol. Metab.*):E458–E468, 1995.
31. REN, J. M., and E. HULTMAN. Regulation of phosphorylase *a* activity in human skeletal muscle. *J. Appl. Physiol.* 69:919–923, 1990.
32. SCHANTZ, P. G., B. SJOBERG, and J. SVEDENHAG. Malate-aspartate and alpha-glycerophosphate shuttle enzyme levels in human skeletal muscle: methodological considerations and effect of endurance training. *Acta Physiol. Scand.* 128:397–407, 1986.
33. SCHANTZ, P. G., and J. HENRIKSSON. Enzyme levels of the NADH shuttle systems: measurements in isolated muscle fibers from humans of differing physical activity. *Acta Physiol. Scand.* 129: 505–515, 1987.
34. SIMONEAU, J-A., and C. BOUCHARD. Human variation in skeletal muscle fiber-type proportion and enzyme activities. *Am. J. Physiol.* 257(*Endocrinol. Metab.*):E567–E572, 1989.
35. SPINA, R. J., M. M.-Y. CHI, M. G. HOPKINS, P. M. NEMETH, O. H. LOWRY, and J. O. HOLLOSZY. Mitochondrial enzymes increase in muscle in response to 7–10 days of cycle exercise. *J. Appl. Physiol.* 80:2250–2254, 1996.
36. SPRIET, L. L., M. I. LINDINGER, R. S. MCKELVIE, G. J. F. HEIGENHAUSER, and N. L. JONES. Muscle glycogenolysis and hydrogen ion concentration during maximal intermittent cycling. *J. Appl. Physiol.* 66:8–13, 1989.
37. SPRIET, L. L. Phosphofructokinase activity and acidosis during short-term tetanic contractions. *Can. J. Physiol. Pharmacol.* 69: 298–304, 1991.
38. TIMMONS, J. A., T. GUSTAFSSON, C. J. SUNDBERG, E. JANSSON, and P. L. GREENHAFF. Muscle acetyl group availability is a major determinant of oxygen deficit in humans during submaximal exercise. *Am. J. Physiol.* 274(*Endocrinol. Metab.*):E377–E380, 1998.
39. WILSON, D. F. Factors affecting the rate and energetics of mitochondrial oxidative phosphorylation. *Med. Sci. Sports Exerc.* 26: 37–43, 1994.