CHAPTER 2

LITERATURE REVIEW

2.1 BIOENERGETICS

If one could select a single term that might be considered a common denominator for every aspect of physical activity the term energy is probably the most appropriate. It is through the release of energy that a muscle is able to contract. The manner in which this energy store is depleted essentially depends on the fitness of the individual and the kind of physical activity being performed. The modification or increase of energy stores through training, significantly improves physical performance (Fox and Bowers, 1993). Other methods of altering energy stores via ergogenic substances mainly target one or more of the body's energy systems to aid the natural production and release of energy for enhanced performance.

In the body nutrients, as an indirect energy source, go through a profound series of chemical reactions or metabolic pathways leading to the formation of adenosine triphosphate (ATP), the body's direct source of energy for all biological work. Studying these pathways allows one to make valid and safe applications to physical activity programs concerning nutrition and performance, the onset and delay of muscular fatigue, body weight control, specificity of training programs and heat balance (Fox and Bowers, 1993).

Unlike the physical properties of matter, it is difficult to define energy in concrete terms of size, shape or mass. Rather, the term energy suggests a dynamic state related to a condition of change, because the presence of energy is revealed only when change has taken place. Within this context, energy relates to the ability to perform work. As work increases, the transfer of energy also increases so that change occurs (McArdle et al.,1991). Any physical or chemical process that results in the release of energy to its surroundings is termed exergonic. Exergonic reactions can be viewed as "downhill" processes and result in a release of free energy. This energy is

useful for work and is typically utilized to fuel or drive "uphill" endergonic reactions which require and absorb energy.

The above is exemplified in the energy liberated during the breakdown of food being employed to manufacture ATP, which is stored in all muscle cells. In turn, the energy released by the breakdown of this compound is used by the cell to perform its specialized work. The structure of ATP consists of one complex component, adenosine and three less complex but "energetic" phosphate groups. For the purpose of exercise physiology, ATP's chemical value lies in these phosphate groups. The bonds between the two terminal phosphate groups represent so-called high-energy bonds. When 1 mole of these phosphate bonds is broken down, 7 to 12 kilocalories of energy are liberated, with adenosine diphosphate (ADP) and inorganic phosphate (Pi) being formed as byproducts.

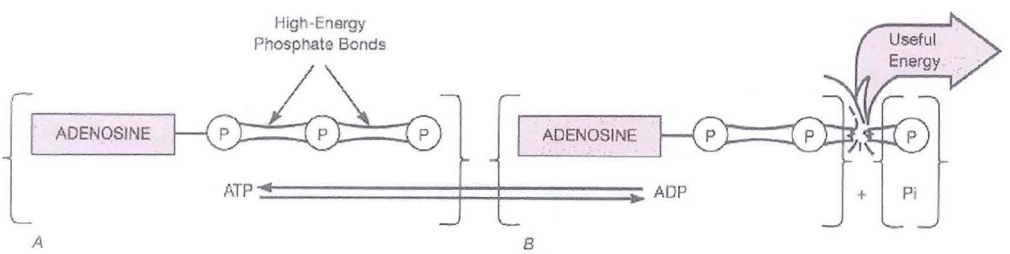

Figure 2.1: A. simplified structure of ATP, showing high-energy phosphate bonds. B. Breakdown of 1 mole of ATP to ADP and inorganic phosphate (Pi), with the release of useful energy. (Fox and Bowers, 1993).

2.2 ENERGY FOR METABOLIC WORK

Contraction of skeletal muscle, like all biological work, is powered by the energy released through hydrolysis of the high-energy ATP. This reaction is catalyzed by the enzyme myosin ATPase. One must keep in mind that at any given time there are limited amounts of ATP in the muscle cell and that ATP is constantly being used and regenerated. The regeneration of ATP is endergonic and requires energy. There are three common exergonic processes

for the production of ATP. Muscle fibres contain the metabolic machinery to produce ATP by these common energy-yielding pathways, viz.:

1. creatine phosphate (CP) system;
2. glycolysis; and
3. aerobic oxidation of nutrients.

The CP or phosphagen system involves the immediate transfer of high-energy phosphate from CP to rephosphorylate ADP to ATP. This reaction is rapid because it involves only one enzymatic step. Although this reaction is not dependent on oxygen in the cell, CP exists in finite quantities and thus the total amount of ATP that can be produced through this mechanism is limited.

The second metabolic pathway capable of producing ATP without the involvement of oxygen exists in the cytoplasm of the muscle cell and is termed glycolysis or the lactate system. Glycolysis entails the the partial degradation of carbohydrate to pyruvate or lactate and involves a series of enzymatically catalyzed steps to provide energy for the rapid, short-term resynthesis of ATP.

The final aerobic metabolic pathway active in cells for the long-term production of ATP is a combination of two complex metabolic processes and functions within the mitochondria. The process involves the initial breakdown of carbohydrate (glycolysis), fat (lypolysis) and protein (proteolysis) followed by further decarboxylation and dehydrogenation of these precursors in the Krebs cycle. Subsequently, during the electron transport mechanism the oxygen acts as the final hydrogen acceptor to form water and large amounts of ATP via oxidative phosphorylation (Durstine et al., 1993; McArdle et al., 1991; Fox and Bowers, 1993; Meyer and Meij, 1996; Martini, 1995).

2.3 FACTORS RELATED TO PERFORMANCE

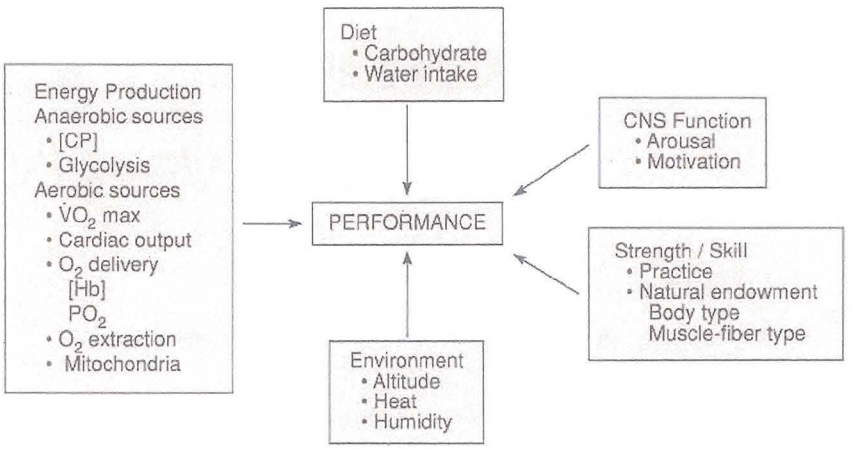

Figure 2.2 Factors affecting performance (Powers and Howley, 1994)

As indicated in Figure 2.2, various variables exists that influence the performance of an athlete. Training techniques as well as physiological, dietary and environmental factors contribute to the level of success or failure that an athlete experiences. Some of these variables could be considered to be controllable factors, i.e. diet and training, while others like physiological and environmental factors cannot be altered by the individual. Studying these variables and their influence on performance is important in understanding the human body and its capabilities and limitations.

2.3.1 Maximal Oxygen Uptake ($V0_2max$)

$V0_2max$ functionally represents the maximal amount of oxygen that can be removed from circulating blood and used by the working tissues during a specified period. Whether the relationship between increasing oxygen consumption and running speed is linear or curvilinear has been a subject of great interest and varying opinion ever since the early experiments of Hill and Lupton in 1923 (Martin and Coe, 1997). Maximal oxygen consumption can be defined as the maximal rate at which oxygen can be consumed per minute during large-muscle-group activity of continuously progressively increasing intensity until exhaustion. The region where oxygen uptake plateaus and shows no further increase, or increases only slightly with an additional

workload, is called maximal oxygen uptake, maximal oxygen consumption, maximal aerobic power or simply $V0_2max$ (McArdle et al., 1996).

The above concept has been the topic of heavy debate during the past few years. Recent literature has questioned the very existence of $V0_2max$. It is necessary to clarify the physiological basis of maximal oxygen utilization. Noakes recently considered $V0_2max$, defined as reaching a plateau of $V0_2$ at high power outputs, a myth (Noakes, 1998). Further questions arose regarding the traditional view that maximal muscle effort is constrained by cardiopulmonary system limitation. Lets assume that the phenomenon of a $V0_2$ plateau does exist, since it can be seen in well conditioned athletes who are accustomed to high intensity exercise. Attainment of a plateau is also typical of many animal species. It is true that the phenomenon is not seen universally, but that is not a valid argument against its existence (Wagner, 2000).

What happens when the metabolic processes in the body reach maximum ATP generation and a $V0_2$ plateau is reached? What could be described as the mechanistic basis of $V0_2max$? The basic equation describing ATP generation by this process is useful to consider:

$$\mathbf{3\ ADP + 3\ P_i + \tfrac{1}{2}\ 0_2 + NADH + H^+ \rightarrow 3\ ATP + NAD^+ + H_20}$$

What determines the maximal velocity of this reaction? Although a simplified equation of the more complex pathways, it is still useful because it can be reduced to the issue of whether or not 0_2, as one of the reactants in the equation, reaches concentrations low enough to be the rate-limiting component during exercise. If not, one would conclude that basic metabolic capacity, and not 0_2 availability is, at some important pathway step rate-limiting (Wagner, 2000). The following points need to be considered here:

1. Depending on conditions, maximal velocity of oxidative phosphorylation may be limited by oxygen availability, or by oxygen-independant metabolic capacity.

2. Manipulations that alter either the supply of oxygen or the concentrations of any of the non-oxygen related biochemical species independently have the capacity to alter the maximal rate of oxidative phosphorylation and thus $V0_2max$. Thus, $V0_2max$ is not an absolute concept it is acutely changeable by altering parts of the metabolic pathway.
3. The stated equation also indicates that the term "anaerobic" is a total misnomer in the context of oxidative phosphorylation. The term means absence of oxygen, and if mitochondrial partial oxygen pressure ($P0_2$) were indeed zero, both the velocity of this reaction and $V0_2$ itself would have to fall to zero and exercise would stop, according to the law of mass action. A more appropriate use of the term "anaerobic" would be to describe processes that do not depend on 0_2 irrespective of its availability (Wagner, 2000).

When considering conditions were oxygen supply limits $V0_2max$, there is clearly no single factor limiting oxygen transport. Research individually altering the fraction of inspired oxygen (F_{I02}), hemoglobin (Hb), cardiac output or muscle blood flow, or Hb-0_2 affinity have all indicated $V0_2max$ can be increased by any one of these tactics (Jones and Lindstedt, 1993; Wagner, 1996). The possible contributing factors to oxygen supply limitation to $V0_2max$ include the following: 1) arterial 0_2 saturation; 2) arterial 0_2 concentration (0_2 saturation and [Hb]); 3) muscle blood flow; and 4) muscle 0_2 extraction. The first three factors govern convective flow of 0_2 to the muscle microcirculation; the fourth reflects those factors that act together to determine 0_2 extraction. The factors that govern extraction are: a) the diffusive conductance for 0_2 between microcirculatory red cells and mitochondria; b) the perfusive conductance of the muscle microcirculation, in essence the product of [Hb] and blood flow; and c) any flow/ metabolism heterogeneity that exists in the muscle (Wagner, 2000).

Overall conclusions include the following:

- The absence of a plateau in $V0_2$ at peak exercise in some people is expected and is not evidence that $V0_2max$ is a myth;
- The ongoing debates concerning its 0_2 dependence or metabolic dependence must be viewed in the context of the experimental conditions;
- If $V0_2max$ is oxygen-dependent, one should ask which components of oxygen transport are most important in this rate-limitation;
- If $V0_2max$ is metabolically limited, one should ask, which steps of the glycolytic/ Krebs cycle/ cytochrome/ shuttle pathways are primarily responsible?; and
- Interventions manipulating either oxygen supply or metabolic components may change the basic nature of how $V0_2max$ is set from oxygen-dependent to metabolically dependent and vice versa. There is, therefore, no single universal answer to the question of what limits $V0_2max$.

Running economy and fractional utilization of $V0_2$ max also affect endurance performance. The speed at lactate threshold (LT) integrates all three of these variables and is the best physiological predictor of distance running performance (Basset and Howley, 2000).

At rest your heart rate is regulated by signals from the brain that travel to your heart via the parasympathetic nervous system. Cardiovascular training increases the sensitivity of the heart to these nerves, which lowers the heart's resting rate. Although resting heart rate is generally lower in endurance athletes, it is not always a reliable indicator of aerobic fitness. For example, resting heart rate decreases with age and with some medications (beta-blockers) and tends to increase with such factors as emotion, anticipation before exercise or a race, and chemical stimulants like caffeine and nicotine. However, monitored on a regular basis, a slower resting heart rate (early morning heart rate) indicates increasing fitness. Conversely, a consistent

increase in resting heart rate reflects over-training or possible dehydration, emotional stress, poor sleeping habits, illness, poor nutritional status, or a combination of two or more of these. Heart rate comprises one of the two important factors influencing cardiac output, the other being stroke volume.

Q (Cardiac Output, litres per minute) = SV (Stroke Volume, litres per beat) x HR (beats per minute)

Cardiac output (Q) is defined as the amount of blood ejected per minute by the heart, specifically the left ventricle. At rest there is little difference in cardiac output between trained and untrained subjects, with average values ranging between 5 and 6 litre per minute. Maximal cardiac output in trained male subjects can reach values in excess of 30 litres per minute, twice as high as in untrained individuals. This variable is the one that differs the most when elite athletes are compared with untrained individuals (Wagner, 2000). This has led many to proclaim that cardiac output is the key factor limiting $V0_2max$. Cardiac output is undeniably important, but without parallel upward adjustment in both pulmonary and muscle 0_2 diffusive transport conductance, a very high cardiac output would cause substantial arterial desaturation and also impair muscle 0_2 extraction. Both conditions are caused by shortened transit times (Dempsey and Fregosi, 1985). Cardiac function has been postulated to play another, more critical role than described in limiting maximal oxygen consumption. It is suggested that maximal exercise may be regulated primarily to prevent hypoxic cardiac damage. Thus, it may have nothing to do with 0_2 supply limitation to exercising skeletal muscle. This theory has some teleological appeal but lacks experimental support. Most healthy exercising subjects do not experience myocardial ischemia by any criteria at maximal exercise; it is possible to exercise at extreme altitude yet still have no objective evidence of ischemia or myocardial dysfunction (Wagner, 2000).

Women tend to have a slightly higher cardiac output when performing at the same level of oxygen consumption. This difference amounts to between 1.5 and 1.75 litre per minute. This means that, the cardiac output will be 1.5 to

1.75 litres per minute higher on the average in woman than in men for a given oxygen consumption. The reason for this is probably compensatory due to the woman's lower oxygen-carrying capacity of blood, resulting from lower levels of haemoglobin. Also, the maximal cardiac output of both trained and untrained woman is generally lower than that of their male counterparts (Fox and Bowers, 1993). The increase in cardiac output and redistribution of blood flow that occur during exercise can best be summarized by developing the concept of the oxygen transport system. The components of the system and their interrelationship are as follows:

$V0_2$ (oxygen transported) = SV x HR x a-$v0_2$ diff (arteriovenous oxygen difference)

From this equation we can derive that to maintain a certain $V0_2$ during exercise with lower comparative heart rates, the subjects must either experience an increase in stroke volume or a increase in arteriovenous oxygen difference. Athletes who excel in endurance sports generally have a large capacity for aerobic energy transfer. The maximal oxygen consumption recorded for competitors in distance running are at most double those of sedentary men and woman. This is not to say that the VO_2 max is the only determinant of endurance performance. Other factors, principally those at local tissue level such as capillary density, enzymes, mitochondrial size and number, and muscle fibre type, exert a strong influence on a muscle's capacity to sustain a high level of aerobic exercise (McArdle et al., 1996). The VO_2 max does, however, provide important information on the capacity of the long term energy system. In addition, this measure has significant physiological meaning in that attaining a high VO_2 max requires the integration of a high level of ventilatory, cardiovascular and neuromuscular functions (McArdle et al., 1996). Martin and Coe (1997) find a high statistical correlation between aerobic power and competitive performance.

2.3.2 Blood Lactate Accumulation

Lactate is one of the products of glycolysis. It is both produced and used by the muscles. It's rate of production increases as the exercise rate increases and as more carbohydrate is used to fuel exercise (Noakes, 1992). Glycolysis refers to the process where carbohydrates are broken down to pyruvic acid or lactic acid (Meyer and Meij, 1996). Lactic acid does not necessarily accumulate at all levels of exercise. During light and moderate exercise the energy demands are adequately met by reactions that use oxygen. In biochemical terms, the ATP for muscular contraction is made available predominantly through energy generated by the oxidation of hydrogen. Any lactic acid formed during light exercise is rapidly oxidized. As such, the blood lactic acid levels remains fairly stable even though oxygen consumption increases. Lactic acid begins to accumulate and rise in an exponential fashion at about 55% of the healthy, untrained subject's maximal capacity for aerobic metabolism. The usual explanation for the increase in lactic acid is based on the assumption of a relative tissue hypoxia in heavy exercise (McArdle et al.,1991). For this reason it would be beneficial to the athlete if either ergogenic aid could help the oxygen supply to the muscle and surrounding tissue, preventing or delaying the onset of hypoxia due to increased exercise intensity. Although the energy released during glycolysis is rapid and does not require oxygen, relatively little ATP is resynthezised in this manner. Consequently, aerobic (absence of hypoxia) reactions provide the important final stage for energy transfer, especially if vigorous exercise proceeds beyond several minutes. An untrained individual who fasted overnight and who has a sample of blood collected in the morning from an arm vein before any exercise, has a lactate level ranging from 0.44 to 1.7 mmol/L. Martin and Coe (1997) also found the equivalent of 0.3 to 0.6 mmol/L to be true for trained individuals, providing that they are not over-trained. Within an hour after an intensive training session during which blood lactate levels reach the highest achievable values (15mmol/L), muscle lactate levels will return to normal (Noakes, 1992). Most of the lactic acid produced during vigorous exercise is removed by direct oxidation (55-70%) while the balance amount is converted to glycogen (<20%), protein constituents (5-10%) and other compounds

(<10%) (Gupta et al., 1996). Lactic acid produced in working muscles is almost completely dissociated into H^+ and lactate within the range of physiological pH, which contributes to the metabolic acidosis (Hirokoba et al., 1992).

2.3.3 The Aerobic and Anaerobic Systems during Rest and Exercise

There are at least three important features of the aerobic and anaerobic systems under conditions of rest and exercise that need some consideration:

- The types of foodstuffs being metabolised,
- The relative roles played by each system, and
- The presence and accumulation of lactic acid in the blood

Rest

During rest, two thirds of the food fuel is contributed by fats and the other third by carbohydrates (glycogen and glucose). The aerobic system is the principle system in operation. The oxygen transport system (heart and lungs) is capable of supplying each cell with sufficient oxygen, therefore there is adequate ATP to satisfy all the energy requirements of the resting state. Protein is not mentioned due to the fact (as mentioned earlier) that the contribution of proteins as food fuel is negligible (Fox and Bowers, 1993).

The molecules of ATP shown coming from the anaerobic system are considered as part of the aerobic yield, because, as indicated they are likewise formed in the presence of oxygen. Although the aerobic system is the primary one in operation, one can note that there is a small but constant amount of lactic acid present in the blood (about 10mg for every 100ml of blood). The reason for this relates to the abundance and effectiveness of LDH (lactic dehydrogenase), the enzyme that catalyses the reaction of pyruvic acid to lactic acid. LDH is always converting some pyruvate to lactate. The fact that the lactic acid level remains constant and does not accumulate tells us that anaerobic glycolysis is not operating at any significant level. One can

summarize that during rest, the foodstuffs utilized are fats and carbohydrates, and the necessary ATP is produced primarily by the aerobic system (Fox and Bowers, 1993).

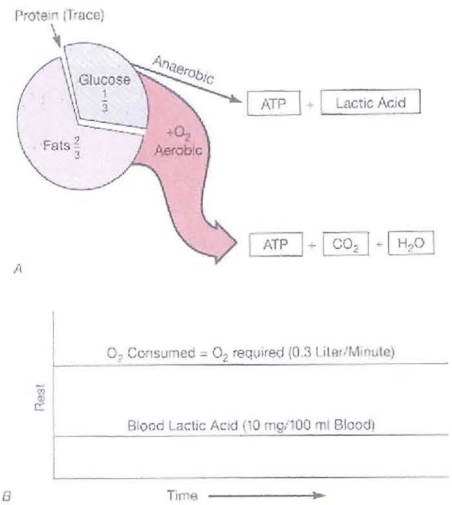

Figure 2.3: The aerobic system supplies all the ATP required in the resting state (Fox and Bowers, 1993).

Rest to Exercise Transition

During the transition from rest to light or moderate exercise, oxygen consumption increases rapidly and reaches a steady state within one to four minutes (balance between the energy required by the working muscles and ATP production via aerobic metabolism). The fact that $V0_2$ does not increase instantaneously to a steady state value suggests that anaerobic energy sources contribute to the overall production of ATP at the beginning of exercise. There is much evidence to suggest that at the onset of exercise, the ATP-PC system is the first active bioenergetic pathway, followed by glycolysis and finally aerobic energy production. Theoretically once steady state has been attained, exercise could continue indefinitely. However, other limiting factors such as the following do play a role:

- Fluid loss;
- Electrolyte depletion; and
- Maintenance of adequate fuel reserves (Fox and Bowers, 1993).

Of considerable importance during prolonged exercise is maintaining adequate fuel reserves, particularly liver and blood glucose for the functioning of the central nervous system, and muscle glycogen to power exercise. Work capacity dramatically decreases when muscle glycogen stores are depleted. It must however, be kept in mind that the maximal level at which steady state can be maintained, differs for each individual. For some the steady state level may be running a marathon at 4 minutes per km while for others it may be during walking. Steady state levels are dependant on how well circulation can deliver oxygen to working muscles and how well active tissues can utilize this oxygen. Once steady state oxygen consumption has been reached, lactate production equals removal and small amounts of lactate accumulated prior to this time, remain relatively constant until the end of exercise (McArdle et al., 1991).

The difference between the total oxygen actually consumed during exercise and the total that would have been consumed had a steady rate of aerobic metabolism been reached at the start of exercise, is referred to as one's oxygen deficit. This transitional phase represents the anaerobic energy being used. Trained athletes reach this steady state more rapidly and therefore have a smaller oxygen deficit. This is due to factors such as cardiovascular or muscular adaptations, resulting in the total aerobic energy provision being greater for a trained person and therefore the anaerobic energy transfer smaller (Fox and Bowers, 1993).

The readjustment of oxidative phosporylation to meet the new ATP demand after a step increase in work rate is delayed and follows an approximately exponential time course. Investigations into muscle 0_2 uptake ($Q0_2$) kinetics have sought to confirm one of two hypotheses, namely, whether the rate of increase in oxidative phosphorylation is limited by the adaptation of oxygen utilization or oxygen transport mechanisms. An oxygen utilization limitation reflects a metabolic inertia. This means that the rate of oxidative phosphorylation at any point during the adaptation to steady state is determined solely by levels of cellular metabolic controllers (Barstow et al., 1994) and/or mitochondrial enzyme activation (Hochacka and Matheson,

1992; Timmons et al., 1996). It implies that mitochondrial $P0_2$ ($P_{mito}\ 0_2$) in all active muscle fibres at all time points during the adaptation, is adequate to support the highest rate of oxidative phosphorylation possible for the current level of metabolic potential.

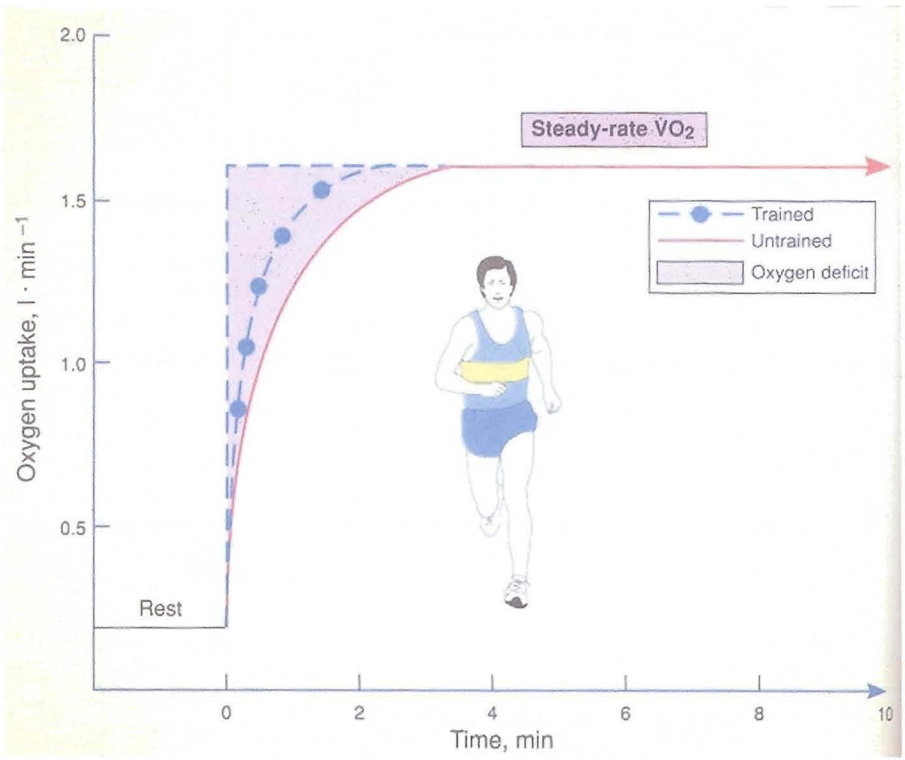

Figure 2.4: Time course of oxygen uptake during a continuous jog at a relatively slow pace for 10 minutes. The shaded area indicates the oxygen deficit or the extra quantity of oxygen that would have been consumed had the oxygen uptake reached a steady rate immediately (McArdle et al., 1991)

An oxygen transport limitation reflects the inertia of oxygen delivery to the mitochondria. In this case, at least some of the oxidative metabolic machinery is capable of increasing its utilization of oxygen more rapidly if more oxygen is made available (Hughson, 1990). This infers that mitochondrial $P0_2$ is not saturating in all active muscle fibres at all time points during the adaptation to a steady state (Tschakovsky and Hughson, 1999).

Evidence for utilization and transport limitations to $Q0_2$ kinetics

Early measurements of oxygen uptake indicated an initial rapid adaptation, followed by a continued increase over the next 2-3 min. Among the first to provide quantitative descriptions of the response (Berg, 1947; Henry, 1951; Henry and De Moor, 1956), observed that the time course of change was generally similar between the adaptation to and the recovery from exercise. Cerretelli (1966) and colleagues introduced the term "early blood lactate" to denote the obligatory lactate production associated with a slow $V0_2$ response. This concept supports the notion that there is a primary inadequacy of aerobic metabolism to meet the ATP demand at the onset of exercise. Either an oxygen transport inertia or a metabolic inertia could account for these findings.

Altered 0_2 transport

In humans, alterations in the content and $P0_2$ of the arterial blood or the blood flow adaptations have been used to test the hypothesis of an oxygen transport limitation to $Q0_2$ kinetics. Obviously, to support an oxygen transport limitation it would be necessary to show that increased oxygen delivery accelerated $Q0_2$ kinetics, compared with the "normal" condition. However, it must be recognised that what constitutes the control or normal condition is open to debate. Whether $Q0_2$ kinetics is accelerated with increases in oxygen transport depends on the exercise condition chosen as the control condition. When sea level (~ 21% inspired 0_2), upright cycling exercise is defined as the normal exercising condition, then hypoxia (10-14% inspired oxygen is commonly used) slows the $V0_2$ kinetic response (an estimate of $Q0_2$) during cycling exercise (Linnarsson et al., 1974), whereas hyperoxia (> 60% inspired 0_2) accelerates the $V0_2$ kinetic response during cycling exercises only at work rates above the ventilatory threshold but not below (MacDonald et al., 1997). Similarly, impairment of cardiac output adaptation via β-blockade and light-to-moderate exercise transition vs. rest-to-light or rest-to-moderate exercise transition, or reduction of the local arterial perfusion pressure via supine exercise have all resulted in slower $V0_2$ kinetics. In contrast, attempted impairment of muscle blood flow with lower body positive pressure during

semi-upright cycling failed to slow $V0_2$ kinetics, whereas slightly faster cardiac output kinetics in heart-transplant patients obtained by a preceding exercise bout did not speed up $V0_2$ kinetics (Grassi et al., 1996).

Although this evidence suggests that the adjustment of $Q0_2$ can often be impaired with reductions in oxygen transport, there is little evidence to suggest that $Q0_2$ kinetics can be accelerated, except perhaps at work rates above the ventilatory threshold. This evidence might lead to the conclusion that 0_2 transport is not limiting under normal exercise conditions. However, this does not preclude a role for an 0_2 transport limitation under a number of common exercise conditions such as exercise at altitude, athletic activities in which the exercising muscles are not well-below heart level, and activities in which the duration of contractions significantly reduced the time allowed for the muscle perfusion to occur (e.g. rowing, downhill skiing). If a different exercise mode is used as the control condition, then acceleration of $Q0_2$ kinetics relative to the normal condition can be achieved by improving 0_2 delivery (Tschakovsky and Hughson, 1999).

Most studies have inherent limitations in their measurement of actual $Q0_2$ uptake kinetics. Typically, cycling exercise $Q0_2$ kinetics is estimated from $V0_2$ (i.e. alveolar oxygen uptake). The $V0_2$ response is biphasic, with early increase influenced predominantly by increased pulmonary blood flow and second phase to steady-state levels additionally influenced by 0_2-depleted venous blood from the exercising muscle. Modelling of $V0_2$ and $Q0_2$ kinetics during exercise transients and observations of equivalence between phosphocreatine (PCr) kinetics and the time-constant of the phase-two $V0_2$ response, suggests that this second phase closely represents the dynamics of $Q0_2$ across a variety of exercise intensities (Barstow and Mole, 1991). Because the second phase is thought to represent the arrival of the oxygen-depleted blood from the exercising muscle (Whipp and Ward, 1990), these lines of evidence support the use of the phase-two time-constant when evaluating effects on $Q0_2$ kinetics under different oxygen delivery conditions via measurements of $V0_2$. However, Essfeld et al. (1991) have shown that the relationship between $V0_2$ and $Q0_2$ kinetics is sensitive to differences between

muscle blood flow and $Q0_2$ kinetics. Their modelling suggests that $V0_2$ and $Q0_2$ kinetics are similar when there is a small difference between the time constants of muscle perfusion and $Q0_2$ kinetics, but $V0_2$ estimates of $Q0_2$ should be viewed with caution when the adaptation of muscle perfusion differs from $Q0_2$.

When $Q0_2$ is estimated by using the Fick principle across the vascular bed of a muscle during voluntary exercise, certain limitations must also be recognized. The Fick principle refers to the basis of some direct methods of measuring the output of the rate and of blood flow to some of the organs, e.g., the kidneys. It can be used when arterial and venous concentrations of a substance can be measured and the amount of uptake or removal of the substance can be determined. Oxygen consumption is equal to the product of the pulmonary blood flow and the increase in oxygen content of the blood passing through the lungs, usually rearranged so that flow equals oxygen consumption divided by the arteriovenous difference in blood oxygen concentration (Stedman, 1982). With in-situ animal preparations it is possible to isolate the vascular supply and return of the exercising muscle and to ensure activation of all muscle fibres by electrical stimulation. In contrast, for studies of voluntary sub-maximal exercise in humans, the venous effluent at in-vivo venous sampling sites will invariably be a mixture of blood from both exercising and nonexercising tissues because of the heterogeneous nature of motor unit recruitment and vascular supply (Berg, 1947). Therefore, any differences in the relative contribution of nonworking and working muscle venous effluent during different phases of the dynamic adaptation to exercise, could introduce error in the estimate of exercising muscle fibre arteriovenous 0_2 difference.

In their study of blood flow and leg oxygen uptake kinetics with upright cycling exercises, Grassi et al. (1996) interpreted the transient increase in venous 0_2 content in the first 0-15 seconds and the subsequent minimal change in calculated leg $V0_2$, to indicate that 0_2 delivery was in excess of 0_2 demand in the initial 15 seconds of exercise. However, they also acknowledged the potential effect of blood flow heterogeneity in determining the mixed venous 0_2 content. One contributor to such an effect might be the potentially

disproportionate effect of the muscle pump on initial blood flow distribution vs. later in exercise, which may have been a factor in their results. Activation of the muscle pump at exercise onset would serve to increase flow through capillaries adjacent to both active and inactive fibres. Depending on the amount of active muscle mass and the effect on intramuscular pressure of the contractions, early venous effluent may, to a large degree, come from elevated flow past nonactive fibres and, therefore, result in the observation of a transient reduction or lack of increase in (a-v) $D0_2$ measured at a site draining the exercising limb. As exercise progresses, an increase in metabolic vasodilatation occurs, effectively "stealing" flow from adjacent capillary modules that are not dilated as part of a feedback regulation (Berg, 1947). The contribution of venous effluent from venules draining active fibres would be expected to predominate as exercise continues and the measured components of the Fick principle (total limb blood flow and venous effluent 0_2 content) would be more accurately related to muscle 0_2 consumption. Transit delay should therefore only account for a minor portion of the observed delay in 0_2 consumption (Tschakovsky and Hughson, 1999).

Cardiac Output Kinetics and Muscle Blood Flow Kinetics vs. Estimates of $Q0_2$ Kinetics

The first combined measurements of cardiac output and $V0_2$ kinetics indicated that cardiac output adapted more rapidly to exercise (Chance and Williams, 1956). Subsequent estimates of cardiac output kinetics in similar exercise conditions confirmed this. The kinetics of bulk blood flow is often faster (MacDonald et al., 1998). However, it has been observed by some that blood flow during the second phase of adjustment closely matches the metabolic adaptation (Grassi et al., 1996). These results have been interpreted to indicate that bulk 0_2 delivery to the exercising muscle is adequate at the onset of exercise to meet the 0_2 demands of the muscle, since 0_2 transport appears to reach steady state before 0_2 consumption.

Phosphocreatine and $V0_2$ Kinetics

Since the initial experiments of Mahler (1985) in frog sartorius muscle, similarities between PCr kinetics and $V0_2$ or $Q0_2$ kinetics have been

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demonstrated in both animal and human models across a range of work rates. This reflects what is believed to be the first-order nature of respiratory control. These data have been interpreted as evidence that metabolic controllers determine the rate of adaptation of 0_2 consumption. In addition $Q0_2$ during the transition from rest to exercise in electrically stimulated dog muscle can be adequately described as the result of changes in phosphorylation potential and redox potential (Connett et al., 1990). Interpreting these observations to mean that the kinetic responses of these metabolites control the increase in oxidative phosphorylation may be valid under conditions where 0_2 is present in saturating amounts, but they do not confirm that oxygen supply is adequate.

Analysis has shown that, at moderate exercise levels where little glycolytic contribution to ATP production occurs during the exercise onset, the kinetics of PCr will mirror those of $Q0_2$. This is because the net ATP demand is met by aerobic and PCr sources, and as aerobic supply of ATP increases exponentially the net breakdown of PCr decreases proportionally. It can be shown mathematically that, under conditions of no glycolytic contribution to ATP production, the time constant of PCr breakdown will always be equivalent to that of aerobic metabolism, regardless of whether PCr is acting as a primary controller of mitochondrial respiration or a buffer of ATP levels. This means that if the adaptation of aerobic metabolism was being limited by inadequate oxygen availability, the similarity in PCr and $Q0_2$ kinetics could simply be caused by the need for PCr depletion to compensate for the greater 0_2 deficit in the face of minimal contribution by anaerobic glycolysis (Binzoni et al., 1990).

Since the first relationship between PCr depletion and steady-state levels of $Q0_2$ in normoxia was first observed, subsequent studies have confirmed this relationship (Meyer and Foley, 1994). It implicates phosphorylation potential in the determination of cellular respiration rate. However, observation of different levels of PCr for the same $Q0_2$ or $V0_2$ under different arterial oxygenation conditions, indicates that 0_2 can exert a modulatory effect on the level of metabolic controllers required to achieve, or be associated with, a given rate of mitochondrial respiration. If this is the case for steady-state

exercise, then it is likely to also apply during the non-steady state, since the cytochrome-c oxidase reaction is a function of the combined drive of the phosphorylation potential, the redox potential, H^+ concentration, and the $P0_2$. An important implication of this is that the level of proposed metabolic controllers does not have to change as much to achieve the same rate of ATP production by oxidative phosphorylation when more oxygen is made available (Tschakovsky and Hughson, 1999).

Determinants of $Q0_2$ Kinetics: Cellular Metabolic State, Enzyme Activation, and $P_{mito}0_2$

Limitations of experimental and theoretical approaches have contributed to the scope of conflicting evidence in this field. If $Q0_2$ kinetics truly were limited only by intrinsic metabolic inertia or extrinsic oxygen transport inertia in a mutually exclusive manner, it is unlikely that such a degree of conflict would exist. Therefore one must try to understand how metabolic controllers and mitochondrial 0_2 supply interact, to determine mitochondrial respiration at any given instant during exercise.

The overall reaction of oxidative phosphorylation is:

$$\mathbf{NADH + ½\ 0_2 + H^+ + 3ADP + 3P_i \Rightarrow 3ATP + NAD^+ + H_20}$$

Oxidation of fuels in the Krebs cycle provides reducing equivalents (NADH, FADH: electron donors) for the electron transport chain (ETC). The ETC is composed of four coenzyme complexes, with the terminal one being cytochrome-c. Oxygen acts as the terminal electron receptor from cytochrome-c, in an irreversible reaction catalyzed by cytochrome-c oxidase, resulting in formation of H_20 and allowing for continued ETC flux. ATP synthesis from ADP and Pi is not directly involved in the terminal reaction of the ETC but coupled to it, such that changes in concentrations of ATP to ADP and Pi can considerably alter the rate of electron transfer. The release of free energy in the transfer of electrons down the ETC is used to "pump" H^+ from the matrix side to the outside of the inner mitochondrial membrane, creating an

electrochemical gradient. The H^+ ions then flow back along this gradient into the mitochondrial matrix through protein channels with associated ATP synthase complexes. The energy from the flow of H+ is used to rephosphorylate ADP, forming ATP (Wilson and Rumsey, 1988).

For sustained ATP turnover to occur during skeletal muscle contractions, ATP demand needs to be matched by aerobic ATP supply. For this to occur, regulation of mitochondrial oxygen consumption must be achieved by a precise communication of ATP demand to the mitochondrial ATP-producing machinery. Some mechanisms have been proposed as regulators of mitochondrial respiration, the following are likely the most important in determining its rate of adaptation:

- Cellular metabolic state
- Latent mitochondrial enzyme activation
- $P_{mito}0_2$
- Factors determining $P_{mito}0_2$
- Interaction of cellular metabolic state, enzyme activation, and $P0_2$

Whether the evidence from a given experiment supported an intrinsic metabolic inertia or an extrinsic oxygen transport inertia depended on the nature of the experimental control condition. This is likely because different exercise conditions can impact 0_2-delivery kinetics in dramatically different ways, whereas the mechanisms determining metabolic adjustments are likely more uniform across a wide variety of exercise conditions (e.g. supine vs. upright leg exercise). An exception to this may be prior exercise, which might alter the rate of adjustment of metabolic controllers (Timmons et al., 1996). Tschakovsky and Hughson (1999) propose that metabolic inertia and 0_2-transport inertia likely interact to determine the adaptation of muscle aerobic metabolism at exercise onset, under a number of common exercise conditions.

2.3.4 Prolonged Exercise

The energy to perform long-term exercise comes primarily from aerobic metabolism. A steady state oxygen uptake can generally be maintained during sub-maximal exercise of moderate duration (McArdle et al., 1991).

Major foodstuffs for prolonged exercise include carbohydrates and fats. For activities lasting up to 20 minutes, carbohydrates are the dominant fuel. As time proceeds beyond an hour, glycogen stores decrease and fats become more important as an energy source. The mix of glycogen and fat utilization will vary with different athletes for a variety of reasons:

- Initial glycogen stores;
- Proportions of fast and slow twitch muscle fibres; and
- State of training.

In prolonged (>20minutes) exercise the major energy pathway for ATP is supplied by the aerobic system. The lactic acid and ATP-PC system also contribute, but only at the beginning of the exercise, before oxygen consumption reaches a new steady-state level. Once oxygen consumption reaches a new steady state level the aerobic system is sufficient to supply all of the ATP energy required for exercise. For this reason lactic acid does not accumulate to very high levels during exercise lasting for more than an hour. Understandably the anaerobic systems might be re-engaged during "kick" efforts to win a long distance race, thereby raising blood lactate levels at the end. In other runners an event might be ended with an aerobic steady-state effort. An example of this would be marathon running. Some athletes run 42.2km in about 2.5 hours, but at the end of the race their blood lactic acid levels are only about two to three times that found at rest. The fatigue experienced by these runners at the end of a race, is therefore due to factors other than high blood lactic acid levels. Some of the more important factors leading to this type of fatigue are:

- Low blood glucose levels due to depletion of liver glycogen stores;
- Local muscular fatigue due to depletion of muscular glycogen stores;

- Loss of water (dehydration) and electrolytes, which leads to high body temperature; and
- Boredom and the physical stress in general that the body has sustained.

2.4 FACTORS LIMITING PERFORMANCE

There is no single cause of fatigue. Fatigue is task-specific, its causes are multifocal and may vary from occasion to occasion. Fatigue is often due to impairment within the active muscles themselves, in which case the fatigue is peripheral to the central nervous system (CNS) and is due to muscle fatigue. Muscle fatigue can also be due to more diffuse, or more central factors (Brooks et al., 1996).

Fatigue can be defined as the inability to maintain a power output or force during repeated muscle contractions. Causes for fatigue vary and are usually related to the type of activity performed (Powers and Howley, 1994).

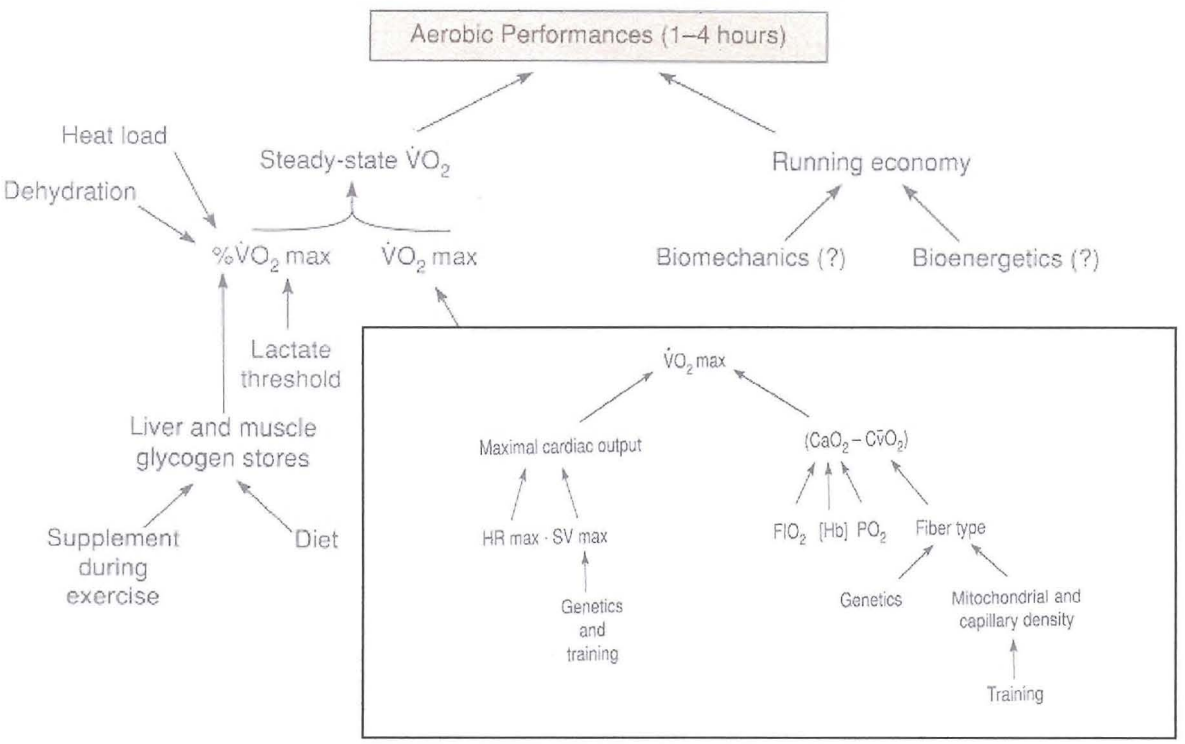

Figure 2.5: Factors affecting fatigue in aerobic performance lasting one to four hours, adapted from Powers and Howley, 1996.

2.4.1 Metabolite Depletion

The Phosphagens (ATP and CP)

Fatigue can be viewed as the result of a simple imbalance between the ATP requirements of a muscle, and it's ATP-generating capacity. When exercise begins and the need for ATP accelerates, a series of ATP-generating reactions occur to replenish the ATP. As the cross-bridges use the ATP and generate the ADP, creatine phosphate provides for the immediate resynthesis of the ATP (CP+ADP→ATP+Cr). As the creatine phosphate becomes depleted, ADP begins to accumulate and the myokinase reaction occurs to generate ATP (ADP+ADP→ATP+AMP). The accumulation of all these products stimulates glycolysis to generate additional ATP, which may result in H^+ accumulation. However, as ATP demand continues to exceed supply, a variety of reactions occur in the cell that limit work and may protect the cell from damage. ATP is needed to pump ions and maintain cell structure, so in this sense fatigue serves as a protective function. When the ATP generating mechanisms can't keep up, AMP begins to accumulate and may be metabolized to inosine monophosphate and ammonia ($AMP+H_20 \rightarrow IMP+NH_3$). IMP and NH_3 increase with exercise intensity, and the depletion of muscle glycogen. Further, if NH_3 accumulates in plasma, central nervous system function may also be affected (Powers and Howley, 1994).

The different ways of ATP production can be linked to the different muscle fibre types that are recruited during various types of activities. Up to an exercise intensity of about 40% of $V0_2$ max, the Type I slow-twitch oxidative muscle fibre is recruited to provide tension development. This fibre type is dependent on a continuous supply of blood to provide the oxygen needed for the generation of ATP from carbohydrates and fats. Any factor that may limit the amount of oxygen that is delivered to this muscle fibre type would cause a reduction in tension development in these fibres and necessitate the recruitment of Type IIa fibres to generate the needed tension. Various factors, including altitude, dehydration, blood loss or anemia could influence the oxygen supply to these fibres. Type IIa fast-twitch, fatigue-resistant muscle

fibres are recruited at an intensity of between 40%-75% of $V0_2$ max. These fibres are rich in mitochondria making them dependant on oxygen delivery for tension development. They also have a great capacity to produce ATP via anaerobic glycolysis. The mitochondrial content of Type IIa fibres is sensitive to endurance training, so that with detraining more of the ATP supply would be provided by glycolysis, leading to lactate production. If oxygen delivery to this fibre type is decreased, tension development will fall, requiring Type IIb fibres to come into play to maintain tension (Powers and Howley, 1994). Type IIb fibres are fast-twitch muscle fibres with a low mitochondrial content. This fibre can generate great tension via anaerobic sources of energy, but fatigues quickly. It comes into play at an intensity of about 75% of maximal oxygen consumption (Powers and Howley, 1994).

Muscle Glycogen

Glycogen depletion in skeletal muscle is associated with fatigue during prolonged submaximal exercise to exhaustion. It is possible for an athlete to exercise to exhaustion and fatigue because of glycogen depletion from specific muscle fibres, while glycogen remains in adjacent fibres within the tissue. These glycogen reserves can be mobilized if epinephrine levels rise, stimulating glycogenolysis, glycolysis, lactate production and release, and energy (lactate) exchange via the lactate shuttle (Brooks et al., 1996).

Blood Glucose

During prolonged exercise, glucose production may be reliant on gluconeogenesis because of hepatic glycogen depletion. This may cause the fall of glucose production below that required by the working muscle and other essential tissue such as the brain. Also, in prolonged exercise leading to dehydration and hyperthermia, shunting of blood flow away from the liver and kidneys occurs. Thus, levels of gluconeogenic precursors (lactate, pyruvate, alanine) rise, and hepatic glucose production decreases. In this case of falling blood glucose, exercise becomes subjectively more difficult because of CNS starvation and difficulty in oxidizing fats in muscle due to the absence of anaplerotic substrates (Brooks et al., 1996).