AEROBIC TRAINING ATTENUATES SKELETAL MUSCLE ANAPLEROSIS DURING EXERCISE IN HUMANS.

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ABSTRACT

We hypothesized that the exercise-induced increase in muscle tricarboxylic acid (TCA) cycle intermediates (TCAI) would be lower after aerobic training (TR), due to a better match between pyruvate production and subsequent oxidation and lower flux through the alanine aminotransferase (AAT) reaction. Eight men [22 ± 1 y; maximal aerobic capacity (VO_{2max}) = 3.9 ± 0.2 L/min] cycled at 75% of their pre-TR VO_{2max} to exhaustion (Exh), before and after 7 wk of TR (1 hr/d, 5 d/wk). Muscle biopsies (v. lateralis) were obtained at rest, 5 min of exercise and Exh. The effect of TR was evidenced by an increased time to fatigue (91 ± 6 vs 42 ± 6 min), increases in resting [glycogen] and citrate synthase maximal activity, and decreases in glycogen degradation, lactate accumulation and phosphocreatine utilization during exercise. The sum of 4 measured TCAI was similar between trials at rest, but lower after 5 min of exercise post-TR (2.7 ± 0.2 vs 4.3 ± 0.2 $mmol.kg^{-1}$ dw, $P<0.05$). Importantly, the [TCAI] at Exh post-TR (2.9 ± 0.2 $mmol.kg^{-1}$ dw) was not different compared to 5 min of exercise and thus fatigue was not attributable to a decline in TCAI. The net change in glutamate (Post: 4.5 ± 0.7 vs Pre: 7.7 ± 0.6 $mmol.kg^{-1}$ dw) and alanine (Post: 3.3 ± 0.2 vs Pre: 5.6 ± 0.3 $mmol.kg^{-1}$ dw) from Rest-5 min of exercise was attenuated post-TR ($P<0.05$), which is consistent with lower flux through the AAT reaction. We conclude that changes in muscle TCAI during exercise are not causally related to aerobic energy provision.

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CHAPTER 1

REVIEW OF LITERATURE

1.1 INTRODUCTION

It is now well established that during the first few minutes of an acute bout of moderate to intense exercise, there is a net increase in the total concentration of tricarboxylic acid (TCA) cycle intermediates (TCAI) in skeletal muscle (Gibala *et al.*, 1998; Gibala *et al.*, 1997a; Gibala *et al.*, 1997b; Graham & Gibala, 1998; Sahlin *et al.*, 1990). This increase in the TCAI pool size is commonly referred to as "anaplerosis," a term originally coined by Kornberg (1966) to describe metabolic pathways that replenish TCAI. Many of the metabolic pathways that interact with the TCA cycle involve amino acids. Notably, the reaction that is believed to be the major contributor to anaplerosis at the start of exercise is catalyzed by alanine aminotransferase (ATT), which reversibly forms alanine and 2-oxoglutarate from glutamate and pyruvate (Gibala *et al.*, 1997a; Sahlin *et al.*, 1995; Sahlin *et al.*, 1990; Spencer *et al.*, 1991).

The physiological significance of the net increase in TCAI at the onset of exercise is controversial. Some investigators have hypothesized that an increase in [TCAI] is necessary to augment TCA cycle flux or that changes in the [TCAI] influence the capacity for aerobic energy provision (Sahlin *et al.*, 1995; Sahlin *et al.*, 1990; Wagenmakers, 1998a). It has further been suggested that a decrease in TCAI pool size during prolonged exercise might be related to the development of local muscle fatigue (Sahlin *et al.*, 1995; Sahlin *et al.*, 1990; Wagenmakers *et al.*, 1990; Wagenmakers,

1998b). Alternatively, others have hypothesized that anaplerosis could simply represent a 'mass action' effect due to the mismatch between the rate of pyruvate production from glycolysis and oxidation in the TCA cycle (Constantin-Teodosiu *et al.*, 1999; Graham & Gibala, 1998). The resultant increase in pyruvate concentration drives the near-equilibrium AAT reaction towards the formation of 2-oxoglutarate and leads to an increase in TCAI.

One of the ways to elucidate the physiological significance of anaplerosis is to manipulate the concentrations of TCAI during exercise and determine the effect on oxidative energy metabolism and TCA cycle flux. One potential manipulation is aerobic (i.e. endurance) training, which is known to induce a "tighter coupling" between pyruvate production and oxidation, as evidenced by an attenuation of glycogen breakdown, lactate formation and phosphocreatine (PCr) degradation (Phillips *et al.*, 1996a; Henriksson, 1992; Phillips *et al.*, 1996b; Holloszy & Coyle, 1984). Therefore, aerobic training represents a potential model to examine how an attenuation of pyruvate production affects TCAI pool size, and how this might effect oxidative energy provision.

The following review of literature focuses on TCAI and amino acid metabolism in human skeletal muscle, along with the anaplerotic and cataplerotic roles that amino acids play in TCA cycle metabolism. Recent information comes from studies that have attempted to manipulate TCAI during exercise in an effort to determine the effect on energy metabolism. Finally, the review summarizes the relatively small body of literature that exists regarding the effect of endurance training on TCAI and amino acid metabolism in human skeletal muscle.

1.2 OVERVIEW OF MUSCLE TCA CYCLE METABOLISM

1.2.1 Role of the TCA cycle in metabolism

In order for skeletal muscle cells to produce energy by oxidative metabolism, they must undergo cellular respiration, which involves three major stages. The first stage involves the oxidation of fuels such as glucose, fatty acids and certain amino acids into two carbon fragments in the form of acetyl-coenzyme A (acetyl-CoA). In the second stage, acetyl-CoA enters the TCA cycle by combining with oxaloacetate to form citrate. Then, through a series of eight reactions, acetyl-CoA is oxidized to form CO_2 and water. The energy released is used to form the reduced coenzymes NADH and $FADH_2$, along with the substrate level phosphorylation of GDP to form GTP. The third stage involves the electron transport chain, which oxidizes the reduced coenzymes and ultimately forms ATP through the process of oxidative phosphorylation. Since the TCA cycle can utilize all the potential sources of fuel through the oxidation of acetyl-CoA, it represents a critical pathway in the provision of oxidative energy.

1.2.2 Overview of the TCA cycle reactions

There are eight reactions that make up the TCA cycle and consequently eight TCA cycle intermediates (TCAI) (Fig. 1.1). These intermediates are neither created nor destroyed in the net operation of the cycle, as evidenced by the overall equation for the cycle:

$$\text{Acetyl CoA} + 3\ NAD^+ + FAD + GDP + P_i + 2\ H_2O \rightarrow$$
$$CoASH + 2\ CO_2 + 3\ NADH + 3\ H^+ + FADH_2 + GTP$$

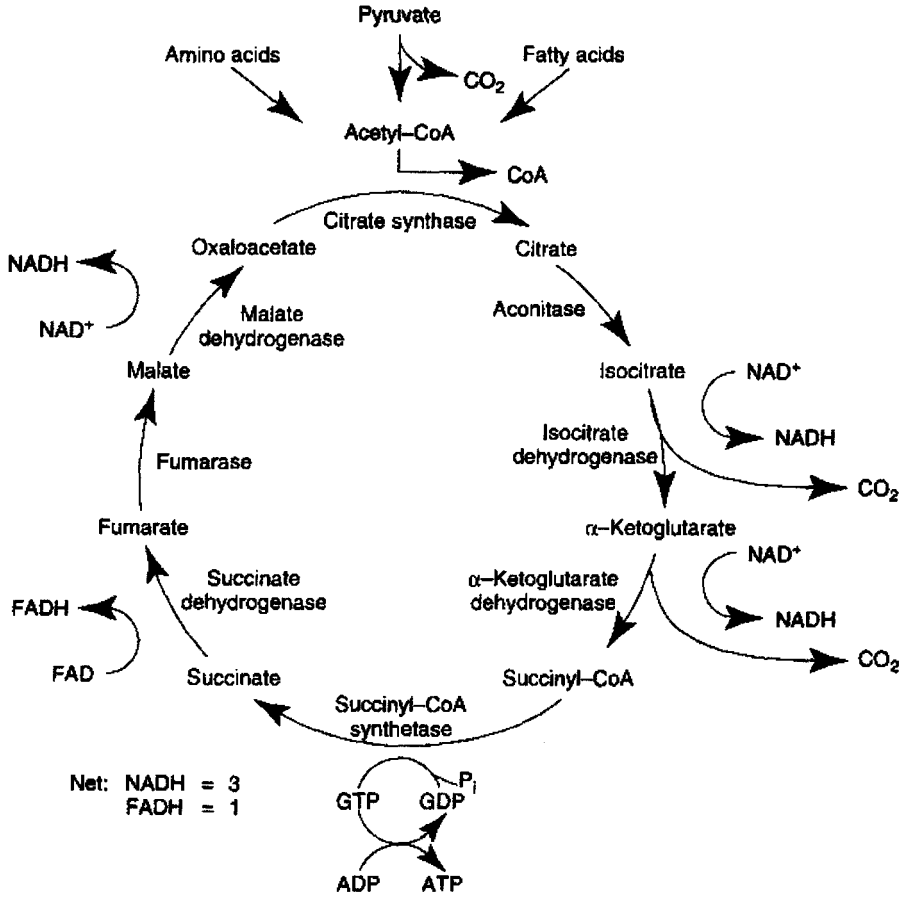

Fig. 1.1. Overview of the TCA cycle (from Powers and Howley, 1998)

The TCA cycle begins with the condensation of acetyl-CoA with oxaloacetate to form citrate, catalyzed by the enzyme citrate synthase. This reaction involves the initial formation of citroyl-CoA, which is a transient intermediate that rapidly undergoes hydrolysis to yield free CoA (that can be recycled) and citrate. This hydrolysis makes the forward reaction highly exergonic, and this large negative free-energy change is necessary for the operation of the cycle, due to the very low levels of oxaloacetate

normally present (Newsholme & Leech, 1983). The enzyme aconitase then catalyzes the reversible transformation of citrate into isocitrate.

The next reaction is the oxidative decarboxylation of isocitrate to form 2-oxoglutarate (also called α-ketoglutarate) and CO_2, catalyzed by isocitrate dehydrogenase. Isocitrate dehydrogenase has two isoforms. One uses NAD^+ as the electron acceptor and is found only in the mitochondrial matrix, and the other uses $NADP^+$ and is found in both the mitochrondrial matrix and the cytosol. The reactions of the two are otherwise identical. The $NADP^+$ form is thought to be used for reductive anabolic reactions (Lehninger *et al.*, 1993).

2-oxoglutarate subsequently undergoes oxidative decarboxylation via 2-oxoglutarate dehydrogenase (or α-ketoglutarate dehydrogenase) to form succinyl-CoA and CO_2. This is an irreversible reaction and the electron acceptor in this reaction is NAD^+. This reaction is similar to the pyruvate dehydrogenase (PDH) reaction in that both reactions involve oxidation of an oxo-acid and the formation of CO_2, along with the use of the same cofactor.

The next reaction in the TCA cycle is the reversible conversion of succinyl-CoA to succinate using succinyl-CoA synthetase. The strong negative free energy change of this reaction is used to drive the phosphorylation of GDP and represents an example of substrate level phosphorylation. Succinate is subsequently oxidized to fumarate and $FADH_2$ by succinate dehydrogenase, which is the only enzyme in the TCA cycle that is membrane bound (Lehninger *et al.*, 1993). Succinate dehydrogenase is an FAD-

containing flavoprotein that can transfer electrons directly into the electron transport chain.

Fumarate is then hydrated in a reversible reaction by fumarase to form malate. Malate is then involved in the final reaction in the TCA cycle, whereby it is oxidized to oxaloacetate via NAD-linked malate dehydrogenase (MDH). The equilibrium of this reaction lies far to the left in standard thermodynamic conditions (i.e. malate formation), but since oxaloacetate is continually removed by the exergonic citrate synthase reaction, its concentration remains low, which pulls the MDH reaction toward the formation of oxaloacetate (Lehninger *et al.*, 1993).

1.2.3 Regulation of TCA cycle flux

Much of our current knowledge regarding the regulation of TCA cycle flux is based on studies conducted using isolated heart preparations (Cooney *et al.*, 1981; Williamson & Cooper, 1980). It appears that flux through the TCA cycle is primarily controlled by the activity of three non-equilibrium enzymes, citrate synthase, isocitrate dehydrogenase and 2-oxoglutarate dehydrogenase. The three main factors that govern the rate of flux through the cycle are substrate availability, inhibition by accumulating products and allosteric feedback (Newsholme & Leech, 1983). Flux through citrate synthase is generally thought to be determined by the availability of its substrates, oxaloacetate and acetyl-CoA, although the oxaloacetate seems to be the most critical factor (Williamson & Cooper, 1980; Spriet & Howlett, 1999). It is also affected by an accumulation of its product citrate, which is known to inhibit citrate synthase (Lehninger

et al., 1993). This effect does not seem to be a factor during exercise in human skeletal muscle however, due to the observation that flux through the cycle clearly increases despite increases in the concentration of citrate (Aragon & Lowenstein, 1980; Putman *et al.*, 1995). Increases in the concentration of ATP can also act as an inhibitor to citrate synthase flux, but this can be alleviated by ADP, an allosteric activator of this enzyme (Lehninger *et al.*, 1993).

When the ratio of $NADH/NAD^+$ becomes too large, both dehydrogenase reactions are severely inhibited by mass action. Isocitrate dehydrogenase is also inhibited by increasing concentrations of ATP, while 2-oxoglutarate dehydrogenase is affected by its product, succinyl-CoA (Lehninger *et al.*, 1993). Finally, contraction mediated increases in Ca^{2+} activate both dehydrogenases, and increases in free ADP have been shown to activate isocitrate dehydrogenase (Hansford, 1985; Spriet & Howlett, 1999).

1.3 OVERVIEW OF SKELETAL MUSCLE AMINO ACID METABOLISM

1.3.1 The free amino acid pool

There are 20 different amino acids found in the human body, which are continuously exchanged between various protein compartments and the free amino acid pool as proteins are constantly being synthesized and simultaneously broken down (Wagenmakers, 1998b). The amount of free amino acids in the body is very small and is dependent upon the balance between rates of synthesis and degradation (Graham *et al.*, 1995a). For example, Wagenmakers (1998b) estimated that in a 70 kg individual with

about 12 kg of protein, 200-230 grams are in the form of free amino acids. About 130 grams of these free amino acids are present in skeletal muscle, while only about 5 grams are present in the blood (Wagenmakers, 1998b). It is the pool of free amino acids that is actually involved in intermediary metabolism. For comprehensive reviews of amino acid metabolism see Graham (1995a), Rennie (1996) and Wagenmakers (1998a).

1.3.2 Muscle amino acid exchange

The liver can oxidize most amino acids, but it has been shown in both rodents and humans that only six of the 20 amino acids are metabolized in skeletal muscle (Chang & Goldberg, 1978a; Chang & Goldberg, 1978b; Sahlin *et al.*, 1990; Eriksson *et al.*, 1985). These include the branched-chain amino acids (BCAA) leucine, isoleucine and valine, along with asparagine, aspartate and glutamate (Hood & Terjung, 1990). These amino acids provide the amino groups to produce glutamine and alanine, which can be released from the skeletal muscle (Ahlborg *et al.*, 1974; Wahren *et al.*, 1976).

One of the ways to determine which amino acids are metabolized in muscle is to examine muscle amino acid exchange after an overnight fast. In this situation, there is a net protein breakdown because synthesis is less than degradation. Amino acids that are not metabolized in the muscle are expected to be in the same relative proportion as they are in muscle protein, but if they are not, the amino acids must have undergone transamination, oxidation, or synthesis. This is in fact the case with the BCAA, glutamate, aspartate and asparagine. They are either released in amounts lower than their occurrence in protein or not released at all (Ahlborg *et al.*, 1974; Wahren *et al.*, 1976),

therefore they must be involved in metabolism in the muscle. In fact, glutamate is constantly taken up by the skeletal muscle from the circulation, so it would be expected to be in a higher concentration, which gives an indication that it is highly involved in amino acid metabolism (Graham & MacLean, 1998). Conversely, glutamine and alanine are released in a much higher proportion to their occurrence in muscle protein, with the relative occurrence being 7% for glutamine and 9% for alanine, while the release is 48% and 32% of the total amino acid release respectively (Ahlborg *et al.*, 1974; Wahren *et al.*, 1976), therefore they must be products of key reactions in metabolism in the muscle. After a mixed meal, the percentage of glutamine released from muscle increases to 71% of the total amino acids released, and since glutamine contains two N-atoms per molecule, it serves as a carrier for 82% of the N-released from muscle (Ahlborg *et al.*, 1974; Wahren *et al.*, 1976). Glutamine and alanine therefore play key roles in the safe transport of amino nitrogen to the liver for production of urea and subsequent excretion by the kidneys. The significance of glutamine as the main end product of amino acid metabolism is due to the fact that it is involved in metabolism elsewhere in the body. It is important as a fuel for the immune system and mucosal cells in the intestine, as well as a participant in the production of purines, NAD^+, and a precursor of urinary ammonia (Wagenmakers, 1998b).

Glutamine may be the main amino acid released from muscle, but the BCAAs and glutamate are the main amino acids taken up by the muscle. After a mixed meal, BCAAs and glutamate have been shown to comprise more than 90% of the total amino acid uptake by skeletal muscle (Elia *et al.*, 1989). BCAAs come from dietary protein and

reach the skeletal muscle in higher concentrations because they make it past the gut and the liver without being metabolized due to low levels of BCAA aminotransferase (Wagenmakers, 1998b). The source of glutamate is unclear, but it is thought that the splanchnic area constantly produces glutamate in fed or fasted states (Wagenmakers, 1998b).

1.3.3 The interrelationship between BCAA, glutamate, alanine and glutamine metabolism

The three branched-chain amino acids (BCAA), leucine, isoleucine and valine, appear to be the dominant amino acids oxidized within human skeletal muscle and they all undergo similar catabolic reactions. The first step in the oxidation of BCAA is the removal of the NH_3 group, which is donated to 2-oxoglutarate to form glutamate and branched-chain oxo acids (BCOA). The glutamate formed from this reaction can be involved in a number of other reactions. For example, it can become a substrate for the AAT reaction, it can be converted to glutamine and be released from the muscle, it can combine with other oxo acids to reform amino acids, or it can combine with oxaloacetate to form aspartate and 2-oxoglutarate (Graham *et al.*, 1995a).

The second step in the oxidation of BCAA is a non-equilibrium reaction catalyzed by branched-chain oxo acid dehydrogenase (BCOAD), which causes oxidative decarboxylation of the BCOA. The fate of the BCOA differs depending on the BCAA that was oxidized (Fig 1.2). The remaining carbons of leucine can form acetyl-CoA or acetoacetate, which go on to be oxidized; therefore leucine is considered purely

ketogenic. Isoleucine can go on to form acetyl-CoA and succinyl-CoA, so it is both ketogenic and glucogenic. Finally, valine carbons go on to make succinyl-CoA only, and therefore it is considered purely glucogenic (Graham *et al.*, 1995a).

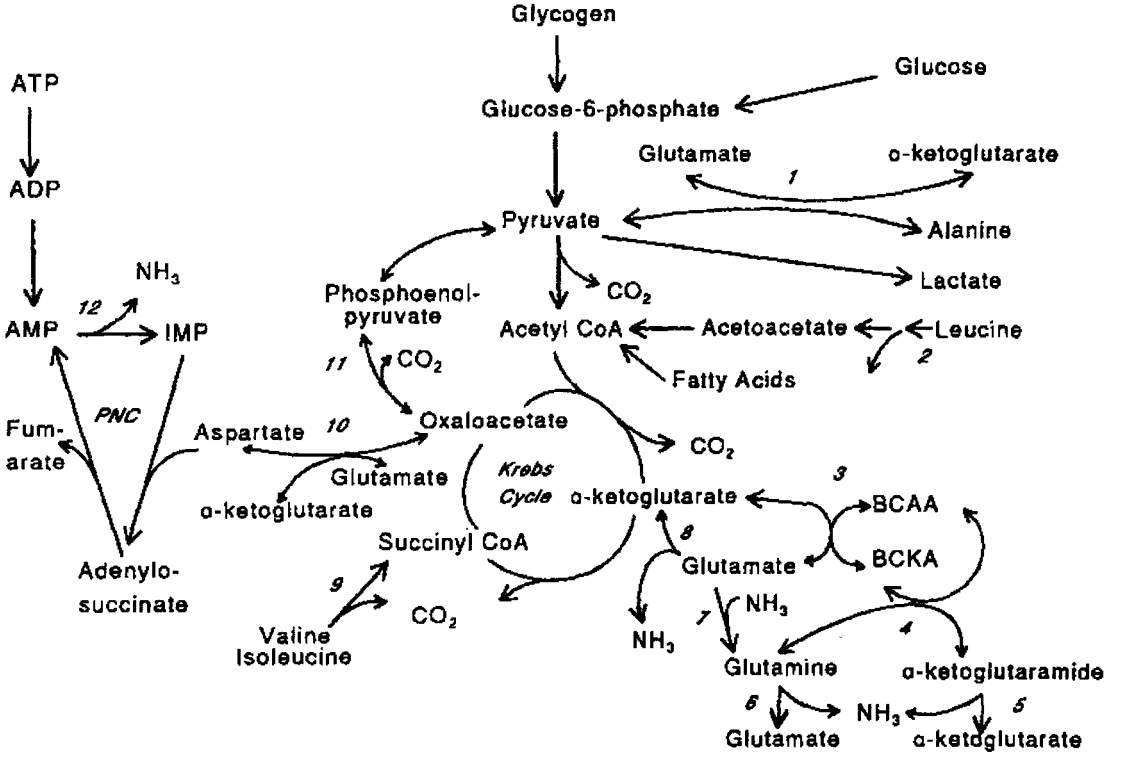

Fig. 1.2. Schematic presentation of muscle amino acid metabolism – general overview. Reaction steps referred to are as follows: 1, AAT; 2, leucine decarboxylation and catabolism to acetoacetate; 3, branched chain aminotransferase; 4, glutamine transaminase; 5, ω-amidase; 6, glutaminase; 7, glutamate dehydrogenase; 9, valine and isoleucine catabolism to succinate via BCOAD; 10, aminotransferase; 11, phosphoenolpyruvate carboxykinase; 12, AMP deaminase (Rennie, 1996).

As noted previously, glutamate is formed in the first step of BCAA oxidation. Glutamate can combine with pyruvate in a reversible reaction to form alanine and 2-oxoglutarate via alanine aminotransferase. It can also form 2-oxoglutarate directly with the enzyme glutamate dehydrogenase, which oxidatively deaminates it to release NH_3. Another fate of glutamate is to form glutamine using the enzyme glutamine synthetase. This reaction requires energy from ATP dephosphorylation, as well as the addition of an amino group. Glutamine is then carried through the blood to the liver where it is deaminated by glutaminase to reform glutamate. The amino group can then be excreted in the form of urea. Therefore, glutamate plays a central role in amino acid metabolism in the muscle (Graham & MacLean, 1992).

1.4 INTERACTION OF AMINO ACIDS WITH THE TCA CYCLE DURING EXERCISE

1.4.1 Changes in muscle amino acids during exercise

Even though it has been shown that amino acids only contribute approximately 2-6 % of the energy required during a bout of exercise (Phillips *et al.*, 1993; Tarnopolsky *et al.*, 1995; el Khoury *et al.*, 1997), they are very active in intermediary energy metabolism. Changes in the free amino acid pool concentrations during exercise give some insight into reactions that may be occurring in the skeletal muscle. One of the first studies to examine changes in muscle amino acid concentrations during an acute bout of exercise was conducted by Bergstrom and colleagues (1985). They reported that during the first ten minutes of exercise at 70% VO_{2max}, the muscle concentrations of alanine and

glutamine increased, while there was a decrease in muscle glutamate (Bergstrom *et al.*, 1985). Subsequently, numerous studies have confirmed the observation of an increase in muscle alanine and decrease in glutamate during exercise at various intensities, while changes in muscle glutamine are more variable (MacLean *et al.*, 1994; MacLean *et al.*, 1991; Sahlin *et al.*, 1990; Katz *et al.*, 1986). Glutamate has consistently been shown to decrease by 50-70% within the first 10 min of exercise, while alanine increases 50-60% by the same time point (MacLean *et al.*, 1994; Van Hall *et al.*, 1995). This has been shown using exercise intensities greater than 50% VO_{2max}. At lower exercise intensities there is about a 20% decrease in glutamate, while the concentration of alanine remains stable (Sahlin *et al.*, 1995). When exercise is prolonged (~30-90 min), [glutamate] shows no further change (Henriksson, 1991; MacLean *et al.*, 1994; Sahlin *et al.*, 1990), while the increase in alanine begins to decline until it reaches near resting levels (Van Hall *et al.*, 1995). Contrary to an early study that described an increase in muscle glutamine (Bergstrom *et al.*, 1985), most studies report that muscle [glutamine] remains fairly constant (Norman *et al.*, 1995; Jacobs *et al.*, 1992), or decreases by ~10-15% during 90 – 120 min of prolonged exercise (Sahlin *et al.*, 1990; Van Hall *et al.*, 1995).

1.4.2 Changes in muscle TCAI during exercise

During the first few minutes of an acute bout of modest-to-intense exercise, there is an increase in the pool size of the TCAI, referred to as anaplerosis (Gibala *et al.*, 1998; Gibala *et al.*, 1997a; Gibala *et al.*, 1997b; Gibala & Saltin, 1999; Graham & Gibala, 1998; Sahlin *et al.*, 1990). The increase in TCAI pool size reaches a peak within the first

few minutes of exercise (Gibala *et al.*, 1997a), and then declines with prolonged exercise to exhaustion (Gibala *et al.*, 1997b). This initial increase can be up to ~3-4 fold higher than resting values, and while there is a decrease in pool size at the time of exhaustion, it still remains ~2 fold higher than resting values (Gibala *et al.*, 1997b; Sahlin *et al.*, 1990). The increase in [TCAI] pool size is dependent upon the exercise intensity, where the pool size increases with increasing intensity (Gibala *et al.*, 1998). This is also shown by low intensity exercise, where there is little change in the TCAI pool size (Sahlin *et al.*, 1995). It is also important to note that the relative increases in total pool size are not reflective of the changes in individual intermediates (Gibala *et al.*, 2000). The increase in pool size is due mostly to the increase in the concentrations of intermediates in the second half of the cycle, namely succinate, fumarate and malate, with malate accounting for ~50% of the total change (Gibala & Saltin, 1999; Gibala *et al.*, 1999; Gibala *et al.*, 1997a; Gibala *et al.*, 1998; Gibala *et al.*, 1997b; Sahlin *et al.*, 1990).

1.4.3 Anaplerotic reactions in muscle

In order for there to be an increase in the pool size of the TCA cycle, there must be an alternative source of carbon, aside from acetyl-CoA, entering the cycle (Fig. 1.3). There are six main reactions that can potentially cause anaplerosis of the TCA cycle as noted in a review by Graham and Gibala (1998). These include the purine nucleotide cycle (PNC) (net-reaction, Eqn. 1), and the reactions catalyzed by alanine aminotransferase (Eqn. 2), glutamate dehydrogenase (Eqn. 3), pyruvate carboxylase (Eqn. 4), phosphoenolpyruvate carboxykinase (Eqn. 5) and malic enzyme (Eqn. 6).

(1) aspartate + GTP → fumarate + ammonia + GDP + P_i

(2) glutamate + pyruvate ←→ 2-oxoglutarate + alanine

(3) glutamate + NAD^+ ←→ 2-oxoglutarate + ammonia + NADH

(4) pyruvate + CO_2 +ATP → oxaloacetate + ADP + P_i

(5) phosphoenolpyruvate + CO_2 + IDP + P_i ←→ oxaloacetate + ITP

(6) pyruvate +CO_2 + NAD(P)H ←→ malate + $NAD(P)^+$

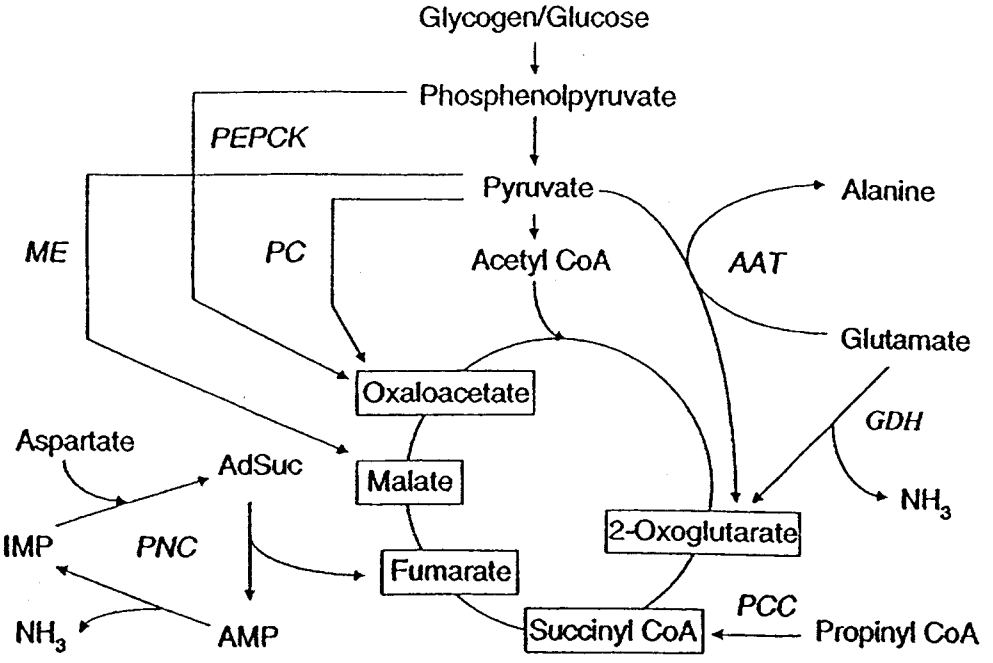

Fig 1.3. Anaplerotic pathways in cardiac and skeletal muscle. AdSuc, adenylosuccinate; AAT, alanine aminotransferase; GDH, glutamate dehydrogenase; ME, malic enzyme; PC, pyruvate carboxylase; PCC, propionyl-CoA carboxylase; PEPCK, phosphoenolpyruvate carboxykinase; PNC, purine nucleotide cycle (net reaction) (Gibala *et al.*, 2000).

1.4.3.1. *The purine nucleotide cycle*

It was originally believed that the increase in TCAI pool size in the early stage of exercise was due to the anaplerotic effects of the purine nucleotide cycle (PNC) (Aragon & Lowenstein, 1980; Flanagan *et al.*, 1986; Swain *et al.*, 1984), but this theory was largely based on research conducted using animal models. For example, a study by Aragon and Lowenstein (1980) examined the changes in TCAI induced by treatment with hadacidin, which is an inhibitor of adenylosuccinate synthetase. Their results lead them to conclude that ~72% of the expansion of the TCAI in the first 10 min of exercise was due to the purine nucleotide cycle.

This theory has not been supported by human data (Gibala *et al.*, 1997a; Sahlin *et al.*, 1990; Tarnopolsky *et al.*, 2001). For example, Gibala *et al.* (1997a) showed that during the first minute of brief dynamic exercise at ~80% of maximal leg work capacity (W_{max}) the accumulation of ammonia was too small for the increase in TCAI to be a result of the purine nucleotide cycle or the glutamate dehydrogenase reaction. A more definitive study by Tarnopolsky and colleagues (2001) studied people who were deficient in the enzyme AMP deaminase (AMPD), which is the key enzyme in the PNC to determine if complete removal of a functioning PNC pathway would affect anaplerosis. They found that the anaplerosis was similar in the AMPD patients compared to the control group, clearly demonstrating that the PNC could not be a major contributor to this phenomenon in humans (Tarnopolsky *et al.*, 2001).

1.4.3.2. *The alanine aminotransferase reaction*

The majority of research suggests that the alanine aminotransferase reaction is the main contributor to anaplerosis at the start of exercise (Graham & Gibala, 1998; Sahlin *et al.*, 1995; Sahlin *et al.*, 1990; Spencer *et al.*, 1991; Gibala *et al.*, 1997a). The reaction is believed to work by a mass action affect caused by excess pyruvate. The excess pyruvate is formed because of a mismatch between pyruvate formation in glycolysis and its oxidation via the TCA cycle. If there is an increased flux through the AAT pathway, one would expect to see a decrease in the concentration of glutamate, an increase in alanine, and anaplerosis of the TCA cycle, which is consistent with what is reported in current research (Gibala *et al.*, 1997a; Gibala *et al.*, 1997b; Sahlin *et al.*, 1990). The AAT reaction is thought to be the most important for anaplerosis of the TCA cycle because there is a stoichiometric increase in TCAI that is comparable to the increase in alanine in the first few minutes of moderate to intense exercise, along with a large decrease in muscle glutamate (Gibala *et al.*, 1997a; Sahlin *et al.*, 1990).

1.4.3.3. *Reactions involving glutamate dehydrogenase, pyruvate carboxylase, phosphoenolpyruvate carboxykinase and malic enzyme*

Glutamate dehydrogenase does not appear to be a major contributor to anaplerosis due to the relatively small increase in ammonia during the initial phase of exercise (Gibala *et al.*, 1997a; Sahlin *et al.*, 1990). The anaplerotic effect of the enzymes pyruvate carboxylase and phosphoenolpyruvate carboxykinase is thought to be small due to the low maximal activities of these enzymes in mammalian skeletal muscle (Brodal & Hjelle,

1990; Crabtree *et al.*, 1972). Finally, malic enzyme, which is found in significant amounts in human skeletal muscle, is not believed to contribute to anaplerosis. In order for it to have an anaplerotic effect, pyruvate would have to undergo reductive carboxylation and this seems unlikely for kinetic reasons (Graham & Gibala, 1998).

Although the previous sections have focused on anaplerosis, it must be noted that changes in [TCAI] are a result of both anaplerotic and cataplerotic reactions, and that there can also be a loss of TCAI through the cataplerotic reactions. Thus, the [TCAI] at any given point in time represents the net balance between carbon flux and removal from the TCA cycle.

1.4.4 Significance of changes in muscle TCAI during exercise

It remains debatable whether expansion of the TCAI pool contributes to the large increase in TCA cycle flux at the onset of exercise (Sahlin *et al.*, 1990; Wagenmakers, 1998a), or is simply a mass action phenomenon. Gibala et al. (1998) noted that there is a very large increase in TCA cycle flux (up to ~100-fold higher than rest) that is associated with a very small relative increase in TCAI pool size (i.e. 3-4 fold increase). In order to resolve this debate, it is necessary to conduct experiments that manipulate the concentrations of TCAI and determine what effect, if any, this has on muscle energy metabolism and performance. In this regard, several studies have attempted to manipulate the [TCAI] during exercise and determine the effect on aerobic energy provision, as summarized below.

1.4.4.1. *Carbohydrate Availability*

Spencer et al. (1991) showed that CHO ingestion during exercise attenuates the decline in TCAI pool during prolonged exercise, and reduced the accumulation of IMP. The levels of both alanine and lactate were higher in the CHO ingested trial as compared to the control trial. They speculated that the attenuation of the TCAI was due to a greater increase in hexose monophosphates for glycolysis, which would allow a greater and longer increase in pyruvate to allow the mass action reactions to continue. The additional CHO would have also provided substrate for the TCA cycle in the form of acetyl CoA, and thus this study could not directly assess the effect of altered [TCAI] on metabolism. Moreover, measurements were only obtained at rest and following prolonged exercise to exhaustion.

A recent study by Gibala and colleagues (2002) used reduced glycogen availability to determine the effect on [TCAI]. They hypothesized that the reduction of glycogen would lead to a reduced flux of pyruvate through the AAT pathway. Contrary to their hypothesis, they found an increase in anaplerosis, and attributed it to low amounts of glycogen causing a decreased flux through the PDH complex and/or increasing the conversion of glutamate carbon into TCAIs (Gibala *et al.*, 2002). Also, since they did not find a change in PCr degradation at the beginning of exercise, they concluded that the acceleration of the TCAI expansion had no effect on oxidative energy provision (Gibala *et al.*, 2002).

Wagenmakers and colleagues (1990) proposed the idea that a reduction in carbohydrate availability would lead to a greater oxidation of BCAAs. This could cause

a 'carbon drain' on the TCA cycle, which leads to fatigue. The carbon drain occurs if glutamate, which is formed from 2-oxoglutarate in the first step of BCAA oxidation, is converted to glutamine and released from the muscle (Wagenmakers *et al.*, 1990). However, when this theory was directly tested by having subjects ingest BCAA in order to further stimulate the activity of branched-chain oxo acid dehydrogenase during exercise, and presumably increase the carbon drain, there was no measurable effect on [TCAI] (Gibala *et al.*, 1999).

1.4.4.2. *Glutamine Supplementation*

A recent study by Bruce and colleagues (2001) used glutamine supplementation to enhance the size of the TCAI pool during a brief, acute bout of exercise. They found that glutamine supplementation increased the TCAI pool independently of pyruvate, but there was no change in the amount of PCr consumed or lactate formed after 10 min of exercise (Bruce *et al.*, 2001). They concluded that either the TCAI were not limiting for oxidative energy provision or that the severity of the exercise was not sufficient for the limitation to be operational (Bruce *et al.*, 2001; Rennie *et al.*, 2001).

1.4.4.3. *Manipulation of PDH*

Gibala and Saltin (1999) used dichloroacetate (DCA) to activate PDH with the expectation of causing a more efficient coupling between the formation of pyruvate and its oxidation by the TCA cycle. This would lead to a decrease in the amount of pyruvate available for the two mass action reactions for which it is a substrate; the AAT reaction

and lactate the dehydrogenase (LDH) reaction. The measured TCAI pool size was lower at rest but not different during exercise and thus the authors were unable to examine the effect of altered [TCAI] on metabolism. The larger net increase in TCAI during the rest-work transition was attributed to a transient inhibition of PDH induced by a higher acetyl-CoA/CoASH ratio.

1.4.4.4. *McArdle's Disease*

Sahlin et al. (1995), examined differences between TCAI in healthy subjects and those with a phosphorylase deficiency (McArdle's disease). They found greatly reduced TCAI in the McArdle's group as compared to the control group during exercise at the same relative work intensities, but no differences at the same absolute workloads. They suggested that the lower [TCAI] in the patient group may have limited aerobic energy transduction and could explain the lower VO_{2max} and peak workload. They also found the expected decrease in glutamate and increase in alanine in the healthy subjects, while the McArdle's subjects had no change in muscle [alanine] and the content of muscle glutamine and glutamate was markedly reduced. This led them to believe that the purine nucleotide cycle may be the source of limited anaplerosis in this group. These results seem logical because the subjects with McArdle's disease cannot breakdown glycogen, which means a reduction in glycolysis, and in turn leads to reduced levels of pyruvate. However, it is not definitive regarding the effect [TCAI] on metabolism since the condition also limits the availability of substrate for the TCA cycle and this could cause the reduction in maximal aerobic energy provision.

Even though research has been done to try to augment the TCAI pool size and determine the affect on oxidative energy provision, no study to date has successfully reduced the pool size to see how it effects metabolism. Aerobic endurance training could be used to try to achieve this. It is hypothesized that aerobic endurance training would lead to more efficient glycogen breakdown, and this would lead to decreases in excess pyruvate. This in turn would reduce flux through the alanine aminotransferase pathway resulting in attenuated anaplerosis of the TCAI pool size.

1.5 THE EFFECT OF AEROBIC TRAINING ON MUSCLE AMINO ACID AND TCAI METABOLISM

1.5.1. Effect of training on enzymes involved in muscle AA and TCAI metabolism

Few investigations have examined the effect of training on changes in the maximal activity of AAT and most of the available evidence comes from animal models. It has been consistently reported that the maximal activity of AAT increases following training (Graham *et al.*, 1997; Cadefau *et al.*, 1990; Guy & Snow, 1977; Green *et al.*, 1983). Molé *et al.* (1972) observed an 85% increase in AAT activity in trained rats compared to sedentary controls, whereas Henriksson et al. (1986) reported an increase in enzyme activity in electrically stimulated rabbit muscle. Finally, Guy and Snow (1977) observed increases in alanine aminotransferase, aspartate aminotransferase and citrate synthase in trained horses. Two human studies, which have measured AAT following

training, have shown similar results. Cadefau and colleagues (1990) reported an increase in AAT following sprint training, whereas Amigo and associates (1998) looked at the effect of detraining and found that it caused a decrease in AAT, leading to the belief that training caused the levels to be higher in the first place.

One area of related research has examined changes in the enzyme branch-chain 2-oxoacid dehydrogenase (BCOAD), which is the non-equilibrium enzyme used in the second stage of BCAA oxidation. McKenzie *et al.* (2000) looked at the effects of endurance exercise and endurance training on the activity of the BCOAD enzyme. They found that endurance training significantly attenuated the exercise induced increase in BCOAD activation as well as the oxidation of leucine. A study by Wagenmakers *et al.* (1989) examined the effects of an acute bout of exercise using trained subjects on the activity of BCOAD. They concluded that trained individuals only achieve significant increases in BCOAD activity after a prolonged bout of intense exercise.

1.5.2. Amino acid changes in humans with training

It is difficult to find data on amino acid changes with training in humans because most of the research was done using animal models and most of these studies have focused on leucine metabolism. In a review by Graham and associates (1996) they point out that the differences between species make interpreting human data from animal data very difficult. As well, the data from animal studies is inconsistent due to differing species and exercise protocols. There is a cross-sectional study by Graham and colleagues (1995b) that examined muscle amino acid concentrations in trained vs.

untrained humans. They found that glutamate decreased and aspartate increased in both groups with exercise, and that the trained group had greater concentrations of alanine and glutamate both at rest and during exercise, but the net change of alanine and glutamate where not different between groups (Graham *et al.*, 1995b). Conversely, Milward et al. (1994) refer to unpublished data, which demonstrated a decrease in alanine in trained athletes.

Other studies have looked at blood amino acid changes with training. One study by Green *et al.* (1991) looked at changes in blood metabolites with short-term training and found no change in blood alanine concentrations. Graham and associates (1995b) looked at the concentration of amino acids in arterial blood plasma in trained and untrained subjects during a bout of exercise. They found a decrease in glutamate at rest in trained subjects, no change in glutamine, and alanine changed over time in the trained subjects but not in the untrained subjects (Graham *et al.*, 1995b).

1.6 SUMMARY

The significance of changes in the metabolism of TCAI and amino acids during an acute bout of exercise, and the influence of these changes on aerobic energy provision remains an active area of research. To effectively study this question, it is sometimes necessary to perturb the system and examine the changes that occur. The significance of changes in pyruvate flux, and the subsequent anaplerosis of the TCA cycle that occurs within the first minutes of an acute bout of exercise, has recently been examined using several nutritional interventions. However, no study has successfully attenuated

anaplerosis during exercise and determined the effect on aerobic energy provision. Aerobic endurance training represents a potential tool to manipulate the TCAI at the start of exercise, since this creates a more efficient match between pyruvate production and oxidation. If the mass action hypothesis is correct, this should result in a reduction in flux through the AAT pathway and an attenuation of the anaplerosis of the TCAI pool size. If there is a decrease in the total pool size, along with a reduction in PCr utilization and prolonged time to exhaustion in an acute bout of exercise after endurance training, it would provide evidence for the mass action hypothesis and show that there is no relation between TCA pool size and aerobic energy provision. There is also very little known about changes in amino acid metabolism in human skeletal muscle with training, and since amino acid metabolism interacts with the TCA cycle through various pathways, it is of interest to examine the changes in amino acids following training in order to gain a deeper understanding of intermediary metabolism in skeletal muscle

CHAPTER 2

EFFECT OF ENDURANCE TRAINING ON TCA CYCLE AND AMINO ACID METABOLISM

2.1 INTRODUCTION

During the initial minutes of an acute bout of moderate to intense exercise there is a net increase in the total concentration of TCAI in skeletal muscle (Gibala *et al.*, 1998; Gibala *et al.*, 1997a; Gibala *et al.*, 1997b; Graham & Gibala, 1998; Sahlin *et al.*, 1990). Although there are numerous pathways that influence carbon flux into and out of the TCA cycle, the near-equilibrium reaction catalyzed by alanine aminotransferase (pyruvate + glutamate ↔ 2-oxoglutarate + alanine) appears quantitatively most important for the rapid increase in TCAI at the start of exercise (Gibala *et al.*, 1997a). The physiological significance of this increase, called "anaplerosis," remains controversial. Some authors have suggested that an increase in [TCAI] is necessary to optimize aerobic energy provision during exercise (Sahlin *et al.*, 1990; Wagenmakers, 1998a). An alternative explanation is that the increase in [TCAI] represents a mass-action phenomenon when the rate of pyruvate production from glycolysis transiently exceeds its rate of oxidation by the pyruvate dehydrogenase enzyme complex (PDH) (Constantin-Teodosiu *et al.*, 1999; Gibala *et al.*, 1998).

In order to investigate the physiological significance of anaplerosis, it is necessary to design studies that manipulate the concentrations of TCAI and examine the effect on oxidative energy metabolism and exercise performance. Two recent studies employed

dietary manipulations to alter the rate of TCAI expansion during the initial phase of exercise. Bruce and colleagues (2001) demonstrated that oral glutamine supplementation prior to exercise increased the size of the TCAI pool after 10 min of cycle exercise at ~70% VO_{2max} compared to rest, however, there were no differences between trials in muscle phosphocreatine (PCr) degradation or lactate accumulation, implying that the rate of TCAI expansion was not limiting for oxidative energy provision (Bruce *et al.*, 2001). Similarly Gibala and colleagues (2002) examined the effect of reduced glycogen availability on [TCAI] expansion during exercise. Surprisingly, they observed a higher rate of anaplerosis after 10 minutes of exercise in the low glycogen trial, and attributed this to a decreased flux through the PDH complex and/or an increased rate of conversion of glutamate carbon into TCAIs (Gibala *et al.*, 2002). Since there was no temporal relationship between PCr degradation and the extent of TCAI expansion, they concluded that the augmentation of the TCAI had no effect on oxidative energy provision (Gibala *et al.*, 2002). Thus, the results from both studies suggest the rate of TCAI expansion does not affect aerobic energy provision at the start of exercise, however no study has examined the effect of reduced TCAI on metabolism and performance during a prolonged bout of exercise. An investigation of this type is warranted in order to test the hypothesis that a decline in TCAI during prolonged exercise compromises aerobic energy provision and contributes to local muscle fatigue.

The purpose of the present study was to examine the effect of aerobic training on muscle TCAI metabolism during prolonged exercise in humans. We hypothesized that training would induce a 'better match' between the rate of pyruvate production from

glycolysis and the rate of pyruvate oxidation via PDH, as demonstrated by the marked reduction in muscle lactate accumulation which occurs following training (Holloszy & Coyle, 1984; Phillips *et al.*, 1996a; Green *et al.*, 1995). This would concomitantly result in a reduced rate of flux through the alanine aminotransferase reaction and attenuate the magnitude of TCAI expansion during exercise, however this would not compromise aerobic energy provision.

2.2 METHODS

2.2.1 Subjects

Eight healthy men with a mean age, height and weight of 21.6 ± 1.0 y, 178.9 ± 2.0 cm, and 91.6 ± 4.3 kg, respectively, and who were not previously cycle trained, volunteered for this study. Subjects were recruited via posters placed around the McMaster University campus. Two subjects were rugby players, two were water polo players, and one was a football player, while the other three had not recently participated in any form of regular physical activity. The experimental procedure and potential risk factors were fully explained to the subjects prior to beginning the study, and all subjects gave written, informed consent. The experimental protocol was approved by McMaster University and the Hamilton Health Sciences Corporation Research Ethics Boards.

2.2.2 Pre-experimental procedures

All subjects underwent a progressive exercise test on an electrically braked cycle ergometer (Lode BV, Excalibur Sport V2.0, The Netherlands) to determine their VO_{2max}.

Subjects also performed a practice ride in order to determine the workload that corresponded to 75% of their VO_{2max}. Subjects were instructed to refrain from exercise, alcohol, and caffeine consumption for 48 hrs prior to their assigned pre-testing trial day. They were asked to record their diet for the day before their testing day, so that it could be duplicated prior to the post-testing day. Subsequent dietary analysis revealed that their diet consisted of 2733 ± 175 kcal, 53% ± 4 carbohydrate, 30% ± 3 fat, and 17% ± 2 protein (Nutritionist 5 software, Version 1.7, First DataBank Inc., San Bruno, CA). Three hours prior to the testing trials each subject consumed a high carbohydrate meal, which consisted of 711 kcal, 87% carbohydrate, 3% fat, 10% protein.

2.2.3 Experimental protocol

Upon arrival at the laboratory, subjects had a catheter inserted into an anticubital vein and the lateral portion of each thigh was prepared for needle biopsy sampling as previously described by Bergström (1975). A small incision was made superficially to the vastus lateralis muscle, through the deep fascia, at three to four sites under local anaesthesia (2% lidocaine). After a resting biopsy and blood sample had been taken, subjects moved to an electronically braked cycle ergometer (Quinton, Q-plex 1; Quinton Instrument) and began pedaling to exhaustion at a workload designed to elicit 75% VO_{2max}. Blood samples were obtained at 10 min, 30 min, and every half hour until exhaustion (Figure 2.1.b). Muscle biopsies were obtained at 5 min, 15 min, and exhaustion. During the post training trial (post-TR), the procedure was duplicated with the exception of an additional biopsy sample obtained at the time point that corresponded

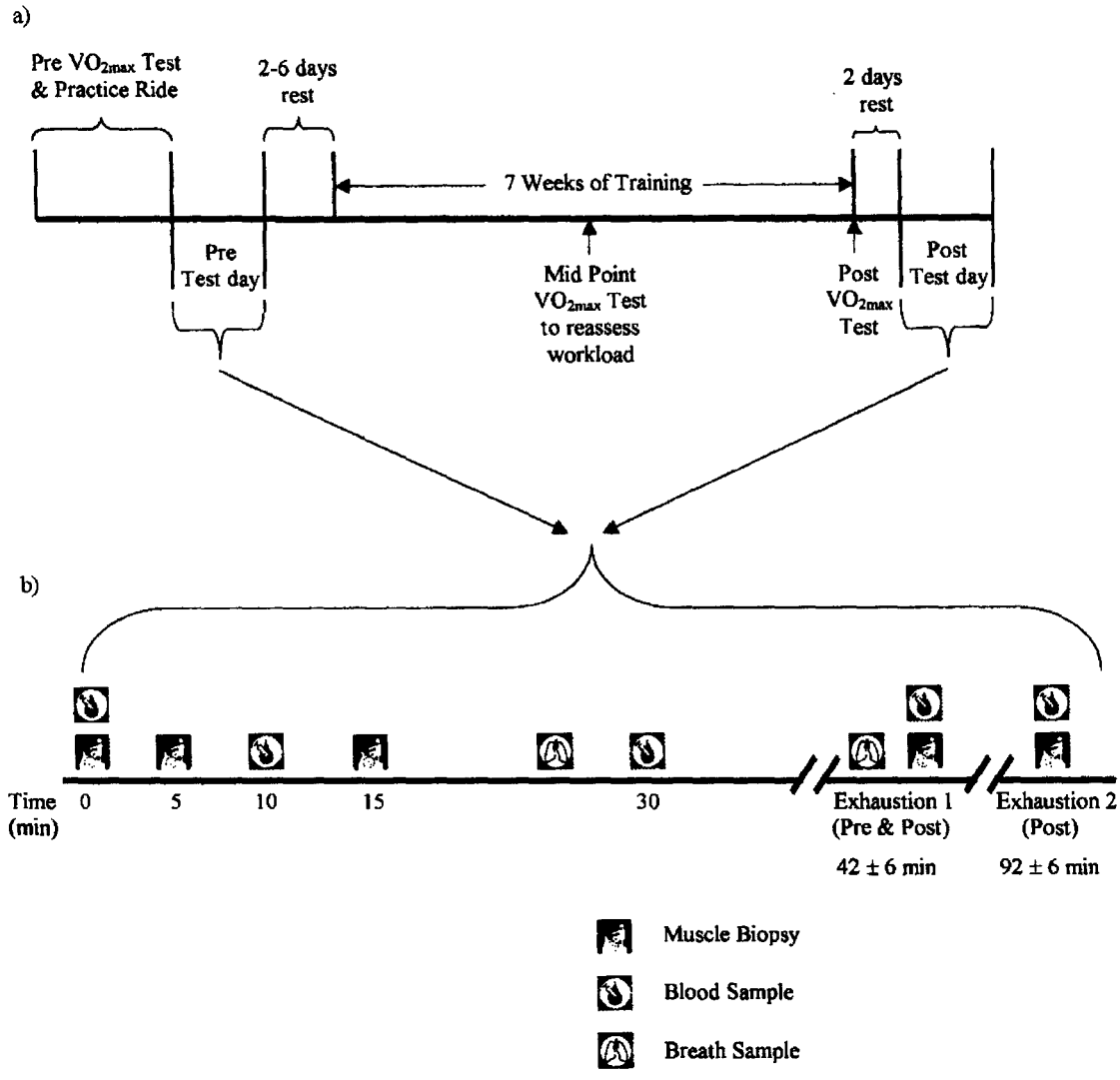

Fig 2.1. Schematic of a) training and b) experimental protocol.

to the pre training (pre-TR) exhaustion time point. This will be referred to as Exh 1, while the end of exercise time point of the post-TR trial will be referred to as End Ex. End Ex represented the point of volitional fatigue during the post-TR trial or the termination of exercise if the subjects cycled for 60 min longer than the Exh 1 time point

(Table 2.3). [The 15 min biopsy samples were included as part of an additional experiment not directly related to this project, and therefore these samples will not be further discussed in the thesis]. During exercise, expired gases were collected during the 25-30 min period of each experimental trial. Measurements of O_2 uptake (O_2) and CO_2 output (CO_2) were made using a Quinton metabolic cart (Quinton, Q-plex 1: Quinton Instrument). Respiratory exchange ratios (RER) were calculated from the O_2 and CO_2 data. Heart Rate was also determined using a telemetry monitor (Polar Electro, Woodbury, NY).

2.2.4 Training protocol

The training protocol consisted of seven weeks of cycle training (Monark Ergomedic 828E, Sweden) at a power output that elicited 75% of the subject's VO_{2max}, for one hour per day, five days per week (Figure 2.1.a). Subjects began training two to six days after their pre-TR trial day. Initially, subjects completed four 15 min rides, with 5 min breaks between rides each training day. Week 2 they completed three 20 min rides with 5 min breaks. At week 3 the breaks were reduced to 2.5 min, and for weeks 4-7, breaks were 1 min, or enough time to stretch and get a drink. VO_{2max} tests were conducted after 3 and 5 weeks to reassess each subject's workload and workloads were adjusted in order to maintain a training intensity of 75% of VO_{2max}. After the completion of seven weeks of training, subjects had 3 days rest and returned for their final VO_{2max} test. Two days later they returned to the laboratory for their post -TR trial day.

2.2.5 Muscle analyses

After muscle biopsy samples were obtained, the biopsy needle was immediately plunged into liquid nitrogen. Resting muscle samples were divided into two pieces. One piece was kept in liquid nitrogen for subsequent analysis of muscle enzyme activities, while the other piece was freeze dried, powdered and dissected free of blood and connective tissue, along with the remaining muscle samples. The freeze-dried samples were stored at -86°C until they were ready to be analyzed.

A ~10 mg portion of freeze-dried muscle was extracted on ice using 0.5 M PCA containing 1 mM EDTA (volume in µl = mg freeze dried tissue x 80) for 10 min. Samples were then centrifuged and the supernatant was collected and weighed, with 2.2 M $KHCO_3$ being added to the supernatant to neutralize the extract (volume in µl = supernatant weight (mg)/4.1). Samples were then vortexed and centrifuged and the resulting supernatant was collected and used for all muscle metabolite measurements, except glycogen. Pyruvate was analyzed using fresh extract, but otherwise the extract was stored at -86°C until each metabolite assay was performed. The extraction procedure to measure glycogen concentration was adapted from Harris and colleagues (1974) and used 500 µl of 2.0 N HCL added to ~ 2 mg freeze dried muscle. Samples were incubated at 100°C for 2 hrs, and then 500 µl of 2.0 NaOH was added. The extract was stored at -86°C until ready for analysis. Pyruvate, malate, fumarate, citrate, isocitrate, creatine (Cr), phosphocreatine (PCr), ATP, lactate (La) and glycogen (glucose assay) concentrations were determined with a Hitachi F-2500 fluorescence spectrophotometer, using fluorometric enzyme assays developed by Passoneau and Lowry (1993).

For determination of AAT and citrate synthase (CS) maximal activities, muscle samples were homogenized using methods described by Henriksson and colleagues (1986) to a 50 times dilution. CS activity was determined on an Ultrospec 3000 pro UV/Vis Spectrophotometer using a method described by Carter and colleagues (2001). An extract dilution of 50 times was used and the results were expressed in $mmol.kg^{-1}$ $ww.min^{-1}$. AAT activity was determined on a Hitachi F-2500 fluorometer using a protocol described by Passoneau and Lowry (1993) and adapted for the assay of human muscle samples. The main change was the use of 10 µl of homogenate which had been diluted to 800 times using 20 mM imidazole buffer, pH 7.0, containing 0.02% BSA (Henriksson *et al.*, 1986). All metabolite and enzyme measurements were corrected to the peak total Cr concentration for a given subject.

2.2.6 Blood analysis

Blood samples were collected into heparinized tubes. 200 µl of whole blood was combined with 1000 µl of 6 N PCA, vortex and centrifuged and the supernatant collected and stored at -86°C until ready for analysis. The PCA extract was used for the determination of glucose and La. Blood glucose and La were measured on a Hitachi F-2500 using fluorometric enzyme assays described by Passoneau and Lowry (1993).

2.2.7 Statistics

All single pre and post measurements, such as VO_{2max} and muscle enzyme activities, were analyzed using paired t-tests. All muscle metabolites were analyzed using

a one-way analysis of variance (ANOVA) owing to the uneven number of pre and post training time points and blood metabolites were analyzed using a two-way ANOVA (Pre/Post TR x time). When a significant main effect or interaction was identified, data were subsequently analysed using a Tukey HSD post hoc test. Significance for all analysis was set at $P \leq 0.05$. Due to limited muscle extract, in some instances a missing data point for a given subject was statistically generated based on the mean response of the remaining subjects. In all cases where this procedure was employed, only one data point for a given metabolite per subject had to be generated. All values are presented as means ± standard error of the mean (SEM).

2.3 RESULTS

2.3.1 Cardiorespiratory and performance results

VO_{2max} increased ($P \leq 0.05$) (3.90 ± 0.15 to 4.13 ± 0.13 $L.min^{-1}$), as did the training power output required to elicit an intensity equivalent to 75% VO_{2max} (Table 2.1). When comparing the pre and post trial rides to exhaustion, time to exhaustion increased by 116%, and there was a decrease in heart rate (10%), V_E (32%) and RER (8%) (Table 2.2). Despite the practice ride to determine 75% VO_{2max}, the measured work intensity during the pre trial was 79.6 ± 2.8 % VO_{2max}, while the same power output represented 69.0 ± 1.8% of the post-TR VO_{2max}. Of note, steady state VO_2 during the rides to exhaustion was 7% lower during the post-TR ride compared to pre-TR. Exh vs End Ex results are shown in Table 2.3 to clarify which subjects reached actual exhaustion in the post-TR trial. The subjects' height and weight did not change significantly following training (Table 2.1).

Table 2.1. ***Subject characteristics***

	Pre Training	**Post Training**
Weight (kg)	91.6 ± 4.3	91.5 ± 4.2
VO_{2max} ($L.min^{-1}$)	3.90 ± 0.15	4.13 ± 0.13*
VO_{2max} ($ml.kg^{-1}.min^{-1}$)	43.1 ± 2.3	45.8 ± 2.4*
Workload Δ's with Training (Watts)	205.6 ± 4.9	216.9 ± 4.3*

Values are means ± SEM. $n = 8$. * Significantly different from Pre Training. $P < 0.05$.

Table 2.2. ***Trial day cardiorespiratory and performance results***

	Pre Training	Post Training
Workload Trial Day (Watts)	205.6 ± 4.9	205.6 ± 4.9
% VO_{2max} worked at	79.6 ± 2.8	69.0 ± 1.8*
Time to Exhausion (min)	42.32 ± 5.98	91.41 ± 6.16*
HR (bpm)$^{+}$	175 ± 3	157 ± 1*
V_E BTPS $^{+}$	102.5 ± 11.3	69.9 ± 4.1*
VO_2 (L.min^{-1})$^{+}$	3.03 ± 0.08	2.80 ± 0.07*
VO_2 (ml.kg^{-1}.min^{-1})$^{+}$	32.7 ± 1.0	30.3 ± 1.0*
RER^{+}	1.08 ± 0.03	0.99 ± 0.01*

Values are means ± SEM. n = 8, except V_E, VO_2, & RER where n = 7. * Significantly different from Pre Training. P< 0.05. $^{+}$ Values measured at steady state (after 25-30 min of exercise) for 3-5 min and averaged.

Table 2.3. ***Individual subject exercise durations during pre-TR and post-TR trials.***

Subject	Exh Time (min)	End Ex Time (min)
S1	61.25	96.67[a]
S2	74.20	120.32[a]
S3	45.68	101.90[a]
S4	27.07	87.07[b]
S5	31.57	77.83[a]
S6	34.35	94.35[b]
S7	32.38	92.38[b]
S8	32.07	60.78[a]

[a] Exercise terminated due to volitional exhaustion. [b] Exercise terminated when subject reached pre Exh + 1 hr time limit.

2.3.2 Intramuscular metabolites

Resting glycogen was 58% higher (P≤0.05) post-TR compared to pre-TR and remained higher throughout exercise (Figure 2.2.). The net change in glycogen utilization from rest to 5 min of exercise was lower (P≤0.05) post-TR (Figure 2.5.). There was no difference in [La] at rest, however the acute exercise induced increase (P≤0.05) in [La] was attenuated by 70% after 5 min post-TR (Post 5 min: 16.7 ± 1.6 vs Pre 5 min: 56.4 ± 6.5 mmol.kg^{-1} dw) and remained 58% lower at Exh 1 as compared to

the same time point pre-TR (Figure 2.3). [Pyruvate] increased (P≤0.05) from rest during exercise pre and post-TR, but there was no difference between trials until Exh 1, when the [pyruvate] was 49% lower (P≤0.05) post-TR.

There was no difference in the concentration of PCr at rest, however, there was a 55% reduction in the utilization of PCr at 5 min (P≤0.05), and the [PCr] remained 56% higher at Exh 1 in the post-TR trial (P≤0.05) (Figure 2.4). There were no changes in [ATP] at any time point.

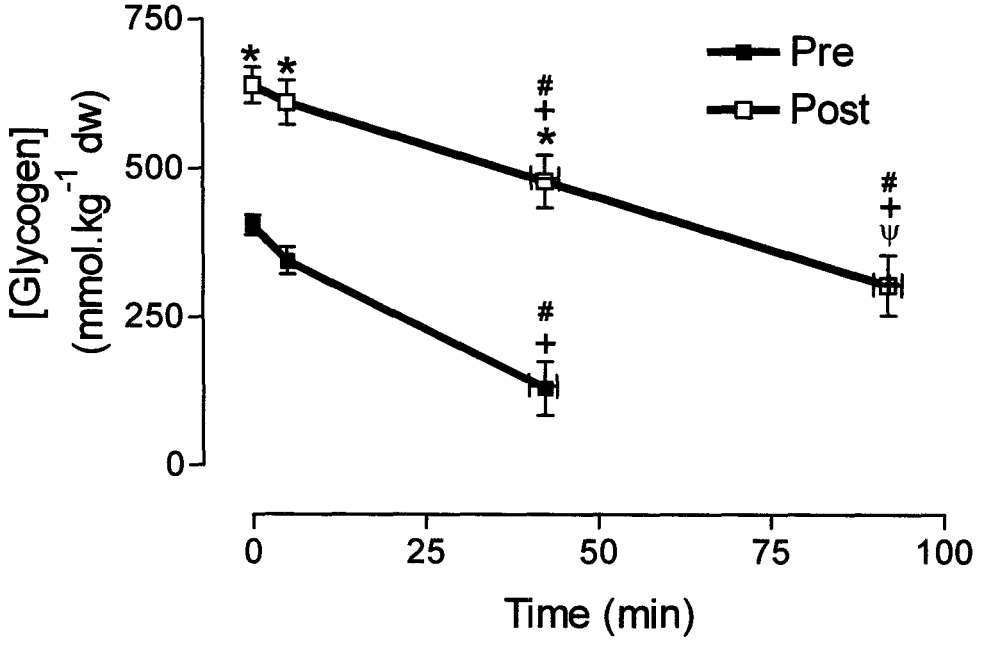

Fig 2.2. Changes in muscle [glycogen] pre vs. post training. * Significantly different from Pre and the same time point. $^{\Psi}$ Significantly different from Pre training Exh. P< 0.05.
+ Significantly different from rest in the same trial. # Significantly different from 5 min of same trial. P≤ 0.05.

Table 2.4. ***Muscle metabolite concentrations during rides to exhaustion***

	Pre Training			Post Training			
mmol.kg^{-1} d.w.	**Rest**	**5 min**	**Exh**	**Rest**	**5 min**	**Exh 1**	**End Ex**
Cr	35.5 ± 2.5	77.1 ± 4.0†	93.7 ± 2.8†$^{\#}$	37.6 ± 1.7	46.3 ± 2.5*	72.9 ± 4.8*†$^{\#}$	82.8 ± 7.4†$^{\#}$
PCr	97.7 ± 2.2	56.0 ± 5.0†	38.6 ± 3.5†$^{\#}$	95.5 ± 2.1	86.8 ± 4.9*	60.3 ± 5.0*†$^{\#}$	50.4 ± 7.1†$^{\#}$
ATP	25.3 ± 0.4	26.6 ± 0.5	26.6 ± 0.6	24.4 ± 0.6	25.5 ± 1.3	26.2 ± 0.5	26.7 ± 0.9
Lactate	4.4 ± 0.5	56.4 ± 6.5†	50.1 ± 5.4†	4.2 ± 0.4	16.7 ± 1.6*	20.9 ± 3.2*†	21.4 ± 4.1$^{\Psi}$†
Pyruvate	0.179 ± 0.030	0.627 ± 0.033†	0.764 ± 0.076†	0.142 ± 0.027	0.459 ± 0.029†	0.388 ± 0.059*†	0.439 ± 0.103$^{\Psi}$†
Glycogen	405.3 ± 16.7	345.0 ± 22.4	129.5 ± 45.3†$^{\#}$	640.0 ± 29.8*	610.7 ± 37.0*	477.8 ± 44.4*†$^{\#}$	303.4 ± 50.4$^{\Psi}$†$^{\#}$

Values are means ± SEM; $n = 8$, except pyruvate where $n = 6$, and glycogen where $n = 7$, due to small muscle samples. * Significantly different from Pre training at the same time point. $P < 0.05$. $^{\Psi}$ Significantly different from Pre training Exh. $P < 0.05$. † Significantly different from Rest during the same trial. $P < 0.05$. $^{\#}$ Significantly different from 5 min during the same trial. $P < 0.05$.

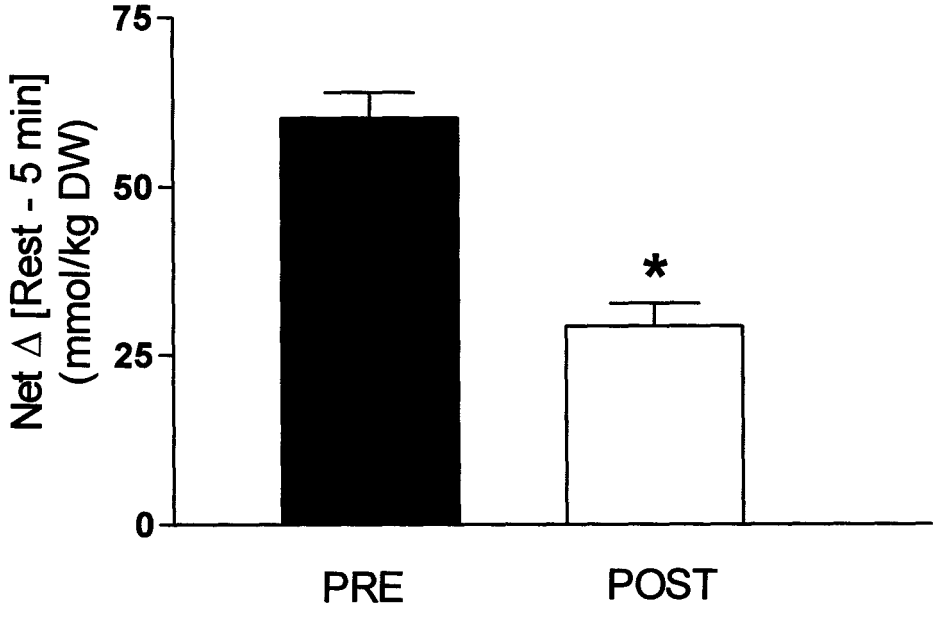

Fig 2.3. Net change in muscle glycogen from rest to 5 min of exercise, pre vs. post training. * Significantly different from Pre. P≤ 0.05.

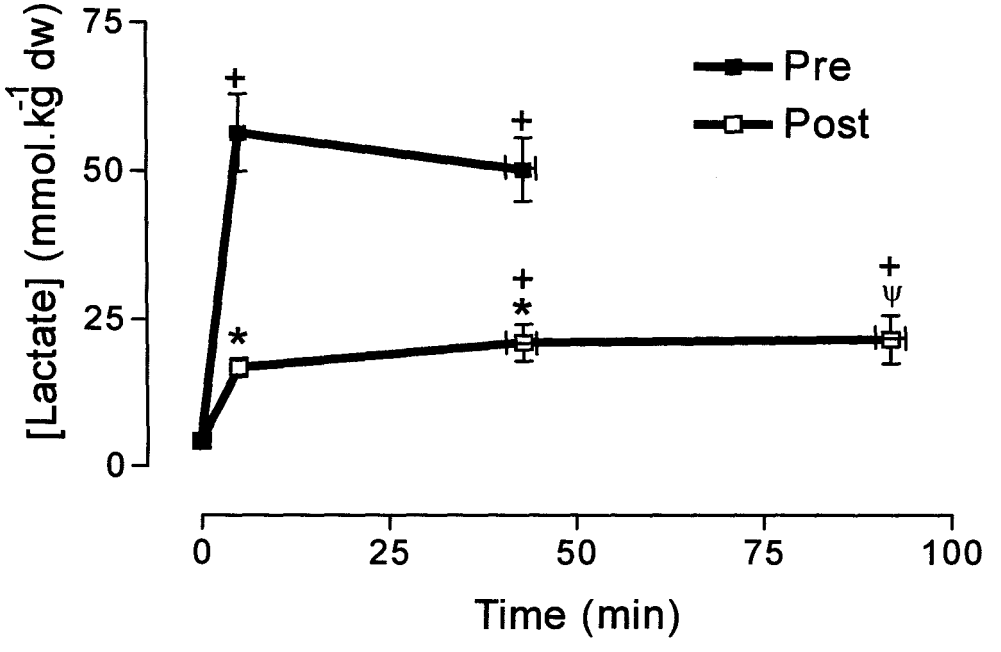

Fig 2.4. Changes in muscle [lactate] pre vs. post training. * Significantly different from Pre and the same time point. $^{\Psi}$ Significantly different from Pre training Exh. P< 0.05.
+ Significantly different from rest in the same trial. # Significantly different from 5 min of same trial. P≤ 0.05.

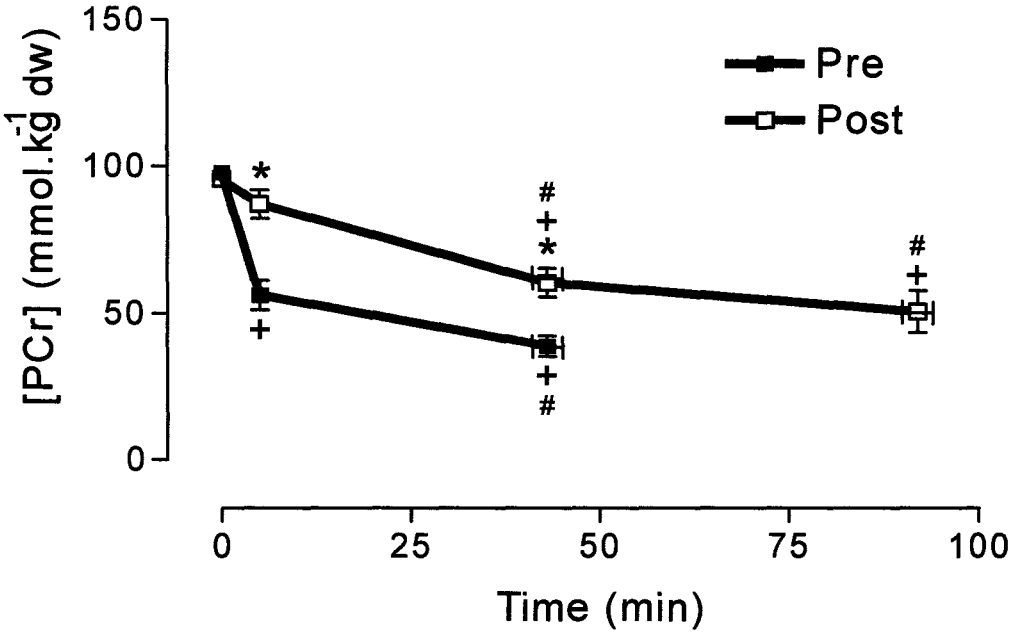

Fig 2.5. Changes in muscle [PCr] pre vs. post training. * Significantly different from Pre and the same time point. + Significantly different from rest in the same trial. # Significantly different from 5 min of same trial. $P \leq 0.05$.

2.3.3 TCAI

There was no difference in the total concentration of the 4 measured TCAI (ΣTCAI) at rest, however, the acute exercise-induced expansion of the ΣTCAI was substantially reduced following training (Figure 2.5). The ΣTCAI after 5 min of exercise was 45% lower ($P \leq 0.05$) post-TR compared to pre-TR (Post: 2.7 ± 0.2 vs Pre: 4.3 ± 0.2 $mmol.kg^{-1}$ dw). The ΣTCAI at Exh 1 and End Ex during the post-TR trial (2.9 ± 0.05 and 3.2 ± 0.02 $mmol.kg^{-1}$ dw, respectively) were not different compared to 5 min of exercise. This contrasted from the pre-TR trial, in which the ΣTCAI declined during exercise such that the value at Exh (3.5 ± 0.2 $mmol.kg^{-1}$ dw) was lower ($P \leq 0.05$) compared to 5 min of exercise. Values for the four individual TCAI are summarized in Table 2.5. The exercise-induced increase in the ΣTCAI was dominated by changes in malate, and this intermediate accounted for 50-60% of the overall pool size during both trials.

2.3.4 Muscle amino acids

There was no difference in the resting concentrations of glutamate or alanine when the pre and post-TR trials were compared (Table 2.6). However, the net change in both amino acids from rest to 5 min of exercise was smaller following training, such that the decrease in glutamate and increase in alanine were each attenuated by ~40% (Figure 2.6). Glutamate remained lower during exercise compared to rest and alanine was higher in both experimental trials ($P \leq 0.05$).

Table 2.5. ***TCAI concentrations in muscle during rides to exhaustion***

	Pre Training			Post Training			
mmol.kg^{-1} d.w.	**Rest**	**5 min**	**Exh**	**Rest**	**5 min**	**Exh 1**	**End Ex**
Malate	0.274 ± 0.033	2.848 ± 0.133†	1.935 ± 0.171†$^{\#}$	0.266 ± 0.035	1.453 ± 0.142*†	1.470 ± 0.139†	1.600 ± 0.155†
Fumarate	0.075 ± 0.013	0.864 ± 0.051†	0.601 ± 0.096†$^{\#}$	0.076 ± 0.007	0.412 ± 0.034*†	0.466 ± 0.059†	0.471 ± 0.054†
Citrate	0.248 ± 0.026	0.450 ± 0.038	0.741 ± 0.053†$^{\#}$	0.275 ± 0.029	0.763 ± 0.059*†	0.814 ± 0.072†	0.889 ± 0.060†
Isocitrate	0.062 ± 0.005	0.157 ± 0.011†	0.148 ± 0.020†	0.074 ± 0.011	0.126 ± 0.012†	0.151 ± 0.014†	0.155 ± 0.018†
Sum of TCAI	0.658 ± 0.065	4.319 ± 0.166†	3.489 ± 0.239†$^{\#}$	0.691 ± 0.076	2.754 ± 0.199*†	2.901 ± 0.234†	3.115 ± 0.239†

Values are means ± SEM; n = 8, * Significantly different from Pre training at the same time point. P< 0.05. † Significantly different from Rest during the same trial. P< 0.05. $^{\#}$ Significantly different from 5 min during the same trial. P< 0.05.

Table 2.6. ***Amino acid concentrations in muscle during rides to exhaustion***

	Pre Training			Post Training			
mmol.kg^{-1} d.w.	**Rest**	**5 min**	**Exh**	**Rest**	**5 min**	**Exh 1**	**End Ex**
Alanine	6.89 ± 0.37	12.49 ± 0.51†	12.08 ± 0.96†	7.38 ± 0.44	10.65 ± 0.47†	12.61 ± 0.76†	12.41 ± 0.69†
Glutamate	11.16 ± 0.76	3.50 ± 0.38†	3.99 ± 0.26†	11.22 ± 0.75	6.75 ± 1.09*†	5.49 ± 0.37†	5.32 ± 0.68†

Values are means ± SEM; $n = 8$, * Significantly different from Pre training at the same time point. $P < 0.05$. † Significantly different from Rest during the same trial. $P < 0.05$.

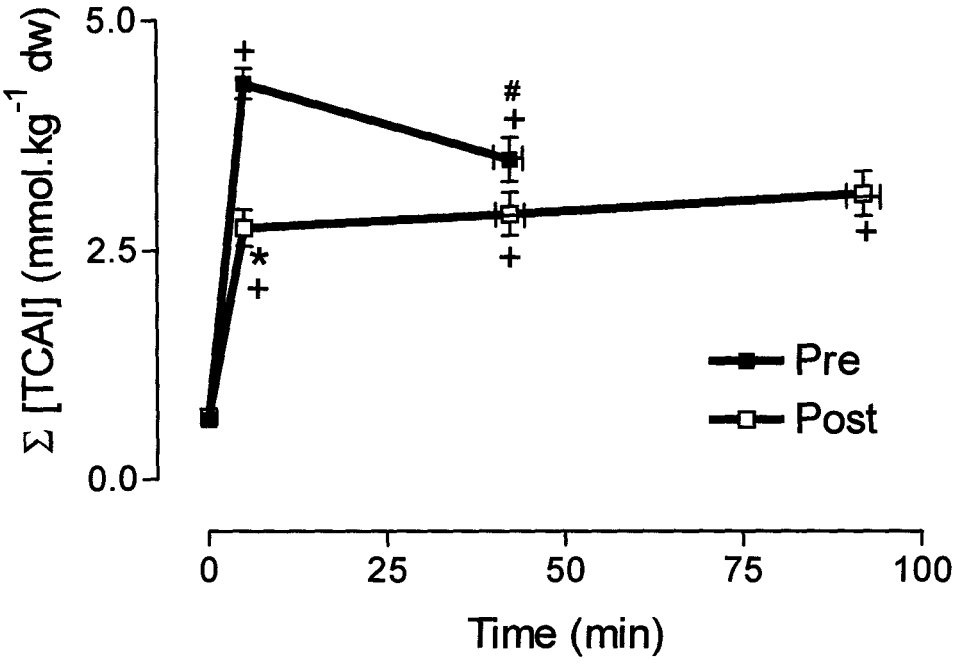

Fig. 2.6. Changes in [ΣTCAI] pre vs. post training. * Significantly different from Pre and the same time point. + Significantly different from rest in the same trial.
Significantly different from 5 min of same trial. P≤ 0.05.

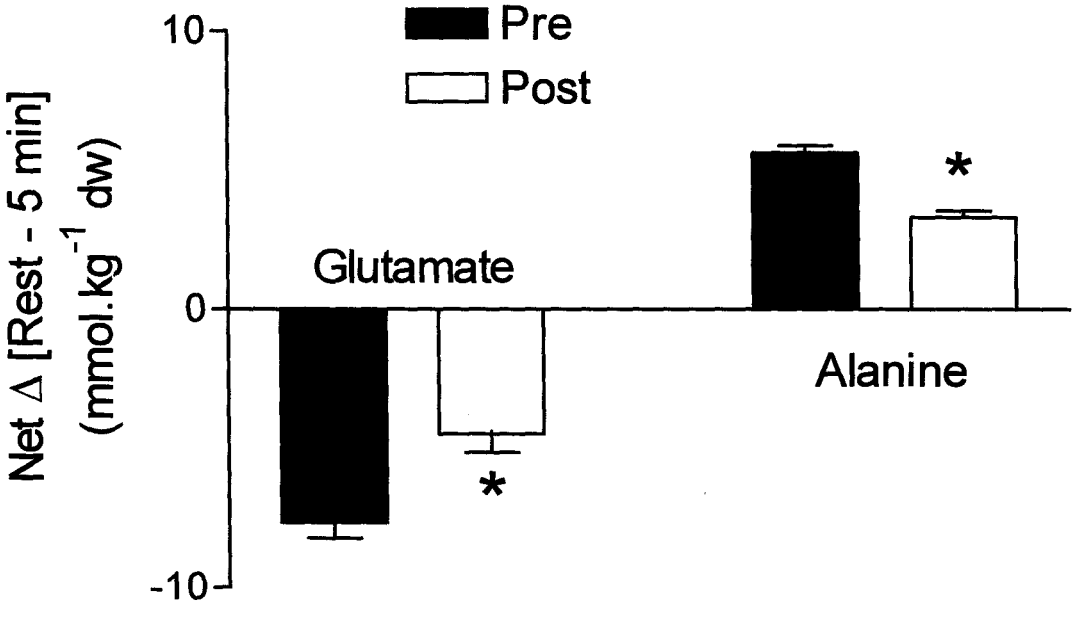

Fig. 2.7. Net change in muscle alanine and glutamate from rest to 5 min of exercise, pre vs. post training. * Significantly different from Pre. P≤ 0.05.

2.3.5 Enzyme Activities

The maximal enzyme activities of AAT and CS increased from pre-TR to post-TR (Figure 2.7). There was a 36% increase (P≤0.05) in AAT activity and a 32% increase (P≤0.05) in CS activity post-TR.

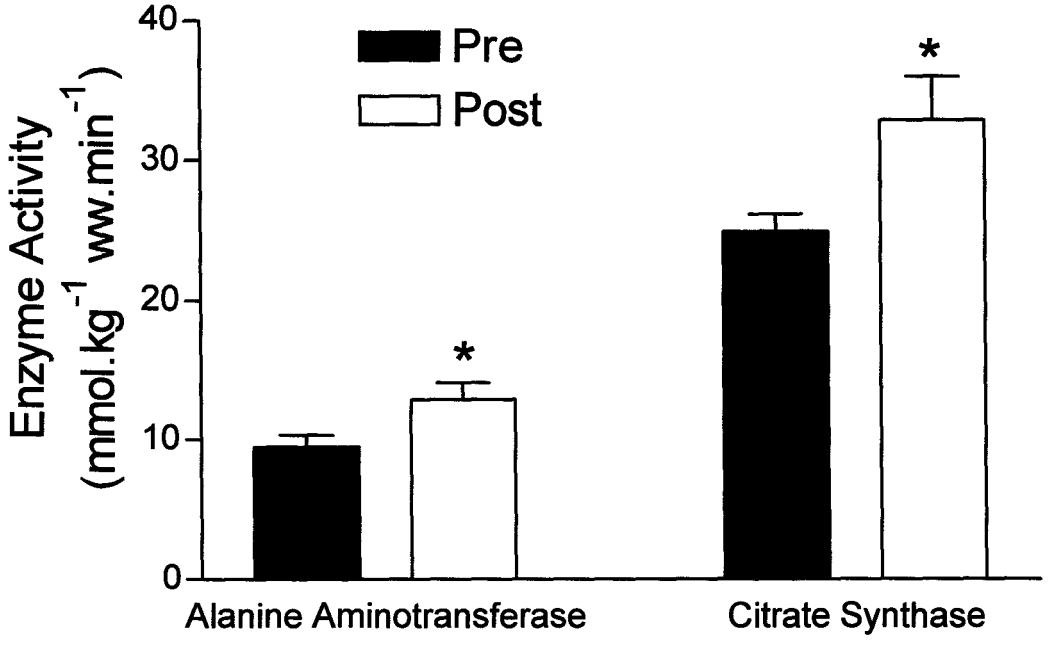

Fig. 2.8. Changes in the activity of muscle AAT and citrate synthase, pre vs. post training. * Significantly different from Pre. P≤ 0.05.

Table 2.7. ***Muscle enzyme activities***

mmol.kg^{-1} w.w./min	**Pre Training**	**Post Training**
Citrate Synthase	24.89 ± 1.23	32.77 ± 3.08*
Alanine Aminotransferase	9.53 ± 0.81	12.92 ± 1.21*

Values are means ± SEM. *n* = 8. * Significantly different from Pre Training. P< 0.05.

2.3.6 Blood glucose and blood lactate

The most significant change in metabolites measured in the blood was the decrease ($P \leq 0.05$) in [La] at 10 and 30 min of exercise in the post-TR trial compared to the pre-TR trial (Table 2.7). La was 39% lower at 5 min of exercise and became 54% lower by 30 min post-TR. Blood glucose concentrations did not change significantly at any time point.

Table 2.8. ***Blood lactate and glucose concentrations during rides to exhaustion***

	Pre Training			Post Training		
mmol.L^{-1}	**Rest**	**10 min**	**30 min**	**Rest**	**10 min**	**30 min**
Lactate	0.73 ± 0.15	5.11 ± 0.39†	6.48 ± 0.43†	0.81 ± 0.13	3.13 ± 0.25*†	2.95 ± 0.25*†
Glucose	4.39 ± 0.27	3.97 ± 0.30	3.76 ± 0.19	4.63 ± 0.33	4.01 ± 0.14	4.09 ± 0.14

Values are means ± SEM; $n = 8$, * Significantly different from Pre training at the same time point. $P < 0.05$.
† Significantly different from Rest during the same trial. $P < 0.05$.

2.4 DISCUSSION

The main finding of this study was an attenuation of the acute exercise-induced increase in the TCAI pool following 7 weeks of aerobic training. This attenuation was presumably due to a more efficient coupling between pyruvate production from glycolysis and oxidation in the PDH reaction. The subsequent reduction in pyruvate availability would have resulted in reduced flux through the AAT reaction as well as through lactate dehydrogenase (LDH). Indeed, this 'better match' between pyruvate production and oxidation was evidenced by the dramatic reduction in muscle and blood lactate accumulation after training. There also appeared to be a decrease in flux through AAT as shown by the smaller net changes in muscle glutamate and alanine from rest to 5 min of exercise post-TR. Since this pathway is considered to be the main mechanism that contributes to anaplerosis at the start of exercise (Gibala *et al.*, 1997a), it appears that decreased flux through AAT was responsible for the attenuated increase in muscle TCAI following training.

The results from this study clearly demonstrate that there was a training effect induced by the 7 weeks of aerobic training. VO_{2max} increased significantly from 3.90 ± 0.15 to 4.13 ± 0.13 L/min ($P \leq 0.05$) and during the rides to exhaustion at the same absolute workload, the subjects had reductions in steady state HR (175 ± 3 to 157 ± 1 bpm), V_E (101.2 ± 9.9 to 67.6 ± 4.3 $L.min^{-1}$) and RER (1.07 ±0.03 to 0.98 ± 0.01) ($P \leq 0.05$). Moreover, the accumulation of La in the muscle and the blood was dramatically reduced post-TR, and there was a decrease in the utilization of PCr. Finally,

there was a large increase in the resting [glycogen], and a decrease in the net utilization of glycogen from rest to 5 min of exercise.

In spite of the marked reduction in muscle [TCAI] during exercise following training, aerobic energy provision was not compromised, as evidenced by the attenuation in PCr degradation during exercise. These data therefore argue against the hypothesis that changes in muscle TCAI during exercise play a regulatory role in aerobic energy provision. For example, Wagenmakers (1998b) stated that a decrease in TCAI "may lead to a reduction of TCA-cycle activity, inadequate ATP turnover rates and muscle fatigue," but the results of this study suggest otherwise. After training, the [TCAI] at 5 min of exercise was lower than the value at Exh prior to training, and remained unchanged throughout the duration of the exercise. However, in spite of the lower [TCAI], subjects cycled for 49 ± 4 min longer compared to the pre-TR trial. Thus, there was a clear dissociation between [TCAI], exercise duration, and markers of aerobic energy provision.

The general pattern of change in individual [TCAI] pre-TR in the present study was similar to those previously reported during prolonged cycling exercise (Gibala *et al.*, 1997b; Sahlin *et al.*, 1990; Gibala *et al.*, 1997b). Gibala and colleagues (1997b) showed that the relative increases in malate, fumarate, citrate and isocitrate from rest to 5 min of exercise were 518%, 550%, 40%, and 174%, respectively, while our study showed increases of 939%, 1052%, 81%, and 153%. The present findings therefore confirm that malate is the major contributor to anaplerosis in an absolute sense. The discrepancy in the relative changes in TCAI between the two studies may be due to the fact that Gibala and colleagues used subjects who regularly engaged in cycling or running exercise (Gibala *et*

al., 1997b), while our subjects were not previously cycle trained and we used a slightly higher work exercise intensity. This observation is also consistent with the notion that trained subjects display a smaller exercise-induced increase in muscle TCAI (Gibala *et al.*, 1997b).

There are very few training studies available which contain data that can be compared with the findings from the present study. We are aware of only two studies that have measured changes in muscle TCAI following aerobic training, and both of these studies only measured citrate (Coggan *et al.*, 1993; Phillips *et al.*, 1996a). It was reported that [citrate] was higher after 90 min (Phillips *et al.*, 1996a) and 120 min (Coggan *et al.*, 1993) of exercise following training as compared to pre-TR, whereas the present study demonstrated higher [citrate] at 5 min and Exh 1 following training. This is the first study to examine the effect of aerobic training on muscle glutamate and alanine. We observed no effect of training on the resting concentrations of these amino acids, which contrasts with the findings of a cross-sectional study conducted by Graham et al. (1995). Possible reasons for this discrepancy include the training background of the subjects and possible genetic differences present in a between subject design. Subjects in the Graham et al. (1995) study had participated in endurance training for $\geq$ 6 months prior to the experiment, whereas our subjects trained for only 7 weeks, which may not have been long enough to elicit changes in the resting amino acid profile. Also, the trained subjects in the Graham et al. study may differ in some genetic qualities, such as fibre type differences, which are beneficial to endurance training and may have led them to become involved in endurance training in the first place. Another contrasting finding from our

study was the increased [alanine] at Exh pre and post-TR, while previous investigators have shown that the increased [alanine] seen in the first minutes of exercise slowly declines until exhaustion (Sahlin *et al.*, 1995; Sahlin *et al.*, 1990). Sahlin and colleagues (1990) also showed an increase in the release of alanine into the circulation in the first 30 min of exercise, therefore it would be advantageous to measure blood [alanine] in the present study to determine if that accounts for the differences between the studies.

There are few reports of AAT activity in human skeletal muscle, however our results are in the range of previously published values. Wroblewski & LaDue (1956) reported an AAT activity in sedentary human skeletal muscle of 4.8 units/ml/min whereas Cadefau and colleagues (1990) reported increases in AAT activity following sprint training, with a range from 7.1 to 16.7 units/g tissue (Pre vs. Post training respectively). Similarly, Amigo and colleagues (1998) found AAT activities in the range of 6.8 to 27.5 units/g tissue (Control vs Trained, respectively). Our study observed increases in ATT from 9.5 ± 0.8 to 12.9 ± 1.2 mmol/kg ww/min following training, however the significance of this increase is difficult to explain. An increase in CS following aerobic training, as seen in this study, is usually indicative of an increase in mitochondrial volume, but since AAT is present in both the cytosol and the mitochondria, it is more difficult to determine where the increase occurred. One factor which implies the increase primarily occurred in the mitochondria is the similar relative increases in AAT and CS, which were 36% and 32% respectively. One way to resolve this compartmentalization issue would be to measure the activity of AAT in separated mitochondrial and cytosolic fractions. Of note, a study by Ji and colleagues (1987)

separated the cytosolic and mitochondrial fractions of skeletal muscle from trained and sedentary rats and examined the activity of AAT. They found that only the mitochondrial fraction had a significant increase in AAT activity in the trained group compared to the sedentary one (Ji *et al.*, 1987).

One unexpected finding was the lower steady state VO_2 observed during the post-TR trial at the same absolute workload. It was expected that the steady state VO_2 would remain the same in the post-TR trial because the subjects cycled at the same absolute workload, but instead we observed a 7% reduction in oxygen uptake. This decrease may be potentially explained by an increased efficiency of cycling post-TR. In a review by Coyle (1995), he describes the relationship between VO_2 and cycling gross mechanical efficiency as well as the influence of muscle fibre type proportion on cycling efficiency. He proposes that with more oxidative muscle fibres (i.e. type I and IIa), there is better cycling efficiency, and with better cycling efficiency, there is a reduction in the level of VO_2 required to do the same amount of work. While our study did not assess potential muscle fibre type shifts following training, it is plausible that the improved cycling efficiency observed in our untrained group of subjects may have been related to fibre type shifts or improved neuromuscular coordination.

Some of the limitations of this study could provide areas for further research in this field. One problem in deciphering changes in TCAI metabolism is due to the presence of the TCAI and other metabolites in both the cytosol and the mitochondria, as described earlier for AAT. In order to correctly determine what is occurring in the mitochondria, measurements of TCA cycle metabolism must be replicated with isolated

mitochondria. Another limitation of the present study was the measurement of only four of the TCAI. Due to the size of the muscle samples collected, not all of the TCAI could be measured. Since 2-oxoglutarate and oxaloacetate are found in such low concentrations in the muscle (Gibala *et al.*, 1997b), the amount of muscle extract required to accurately measure their concentrations is large, and was not available for the present study. However, these intermediates are important for the understanding of TCA cycle metabolism and the anaplerosis in the initial minutes of exercise because 2-oxoglutarate is the entry point of carbons into the TCA cycle through the AAT reaction and oxaloacetate is the substrate for the entry of carbons from acetyl-CoA. Therefore, future research measuring changes in these crucial TCAI after aerobic training is warranted. Another limitation to the study of TCA cycle metabolism is the measurement of flux through the cycle. The present study only measured static changes in the TCAI during exercise after training, but the flux through the cycle remains unknown. Investigators have tried to relate changes in the [TCAI] to flux through the cycle, but this study has shown a dissociation between the two. Measurement of flux through the TCA cycle is difficult because the methods usually employed to measure flux, such as carbon labelling, follow the path of the labelled molecule and determine its rate of appearance in the final products, but since there are so many reactions that interact with the TCA cycle, the label becomes lost in these side reactions. The measurement of flux through the cycle is important for the understanding of changes during exercise, therefore should be an area for further research. One problem with the use of muscle biopsies is the relative occurrence of the different types of muscle fibres present in each of the separate muscle

biopsies. It is assumed that the muscle samples are fairly similar, but the fibres of one biopsy could be more oxidative than another, which may mean different [glycogen] or number of mitochondria. It could also have an impact on recruitment and usage of the different fibre types. Therefore a study that also measures the relative occurrence of the different fibre types could be done, and the metabolites and enzymes measured could be normalized for fibre type, in a similar manner to the correction for peak total creatine concentration.

A potential future study to help clarify the changes in the [TCAI] during exercise would be through the use of an AAT blocker, such as cycloserine. This would allow for the examination of changes in TCAI in the absence of flux through the AAT reaction. If there is attenuation in the anaplerosis in the initial minutes of exercise, as seen in the present study, without changes in aerobic energy utilization, then the hypothesis of the mass action effect of pyruvate through the AAT reaction could be further validated.

2.5. CONCLUSIONS

The main finding from this study was an attenuation of the acute exercise-induced expansion of the muscle TCAI pool following 7 weeks of aerobic training. The reduced [TCAI] during exercise is consistent with decreased flux through the AAT pathway, as evidenced by the smaller net changes in muscle glutamate and alanine. The reduced flux through AAT is attributable to a tighter coupling between pyruvate production from glycolysis and its oxidation by PDH. However, in spite of the marked reduction in [TCAI] during exercise post-TR, aerobic energy provision was not compromised and the point of volitional fatigue was unrelated to a decline in [TCAI] pool size. We therefore conclude that changes in muscle [TCAI] during exercise in humans are not causally related to aerobic energy provision.

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APPENDIX I

SUBJECT INFORMATION AND CONSENT FORMS

McMaster University

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EXERCISE METABOLISM RESEARCH GROUP (EMRG)
DEPARTMENT OF KINESIOLOGY, MCMASTER UNIVERSITY

CONSENT TO PARTICIPATE IN RESEARCH

You are asked to participate in a research study being conducted by the investigators listed below at McMaster University, Hamilton, Ontario. Prior to your participation, you are asked to read and complete this form and the two accompanying forms which outline the purpose, procedures, and risks associated with the study, and also provide other essential information regarding your rights and responsibilities as a subject. The two accompanying forms are entitled "Description of Medical Procedures" and "Subject Screening Questionnaire." All experimental procedures will be conducted in the Metabolism Research Laboratory, Room A103, Ivor Wynne Centre or the Cardio-Respiratory Research Laboratory, Health Sciences Centre, Room 3U28.

LIST OF INVESTIGATORS

Name	Campus Address	Daytime Phone Number
Martin Gibala, Ph.D.	Kinesiology, AB122	905-525-9140 ext. 23591
George Heigenhauser, Ph.D.	Medicine, HSC 3U27	905-525-9140 ext. 22679
Krista Howarth, B.Sc.	Kinesiology, A103	905-525-9140 ext. 27037
Paul Leblanc, M.Sc.	Medicine, HSC 3U27	905-525-9140 ext. 22346

PROJECT TITLE

"Amino Acid and TCA Cycle Metabolism in Human Skeletal Muscle: Effect of Endurance Training."

PURPOSE OF THE STUDY

Our laboratory is interested in the regulation of energy metabolism in human skeletal muscle. The purpose of the proposed study is to examine changes which occur in the metabolism of amino acids and TCA cycle intermediates following aerobic exercise training.

DESCRIPTION OF TESTING AND EXPERIMENTAL PROCEDURES

Following routine medical screening and the completion of a health questionnaire, you will be required to make 40 visits to the laboratory over a period of approximately 8 weeks. Specifically, the study will consist of: 2 visits in order to determine your maximal oxygen uptake (peak aerobic fitness level, or "VO_2max"), 1 practice trial ride, 2 experimental trials (which will include muscle biopsy and venous blood sampling), and 35 exercise training rides.

On your first visit to the laboratory, you will perform an exercise test on a cycle ergometer (stationary bicycle) in order to determine your VO_2max. This test will last ~15 minutes, and will involve cycling at increasingly higher workloads (exercise intensities) while the amount of

oxygen taken up by your body is determined from a mouthpiece connected to a gas analyzer. There is no discomfort associated with this procedure, except for the physical exertion of the exercise test. Approximately 2-3 days following the VO_2max test, you will report back to the laboratory and perform a 15 min "practice ride" in order to verify that the power output setting on the cycle elicits 75% of your VO_2max.

Approximately 3-4 days following the practice ride, you will report back to the laboratory in order to perform the first of two experimental exercise trials. Upon arrival, a catheter will be inserted into a forearm vein for blood sampling and your leg will be prepared for the extraction of muscle biopsy samples. The nature of these two medical procedures, and the inherent risks associated with them, are thoroughly described on the attached sheet entitled "Description of Medical Procedures." A blood sample and muscle biopsy sample will be obtained at rest. You will then perform cycle exercise until volitional fatigue (i.e., until you cannot maintain the required power output) at a workload designed to elicit ~75% of your VO_2max. Your heart rate will be monitored throughout the exercise test, and periodically gas measurements will be made from a mouthpiece attached to a gas analyzer. Venous blood samples will be drawn every ~20 minutes during exercise, and needle biopsy samples will be obtained after 5 min, 15 min and at exhaustion. During the second experimental trial a biopsy will also be taken at the point of exhaustion from the first exercise trial. Upon completion of the exercise test, you will be permitted to leave the laboratory following a half-hour of routine, post-exercise monitoring. Shower and change facilities are available in the laboratory should you require them.

Approximately 3 days following the first experimental exercise trial, you will begin a 7-week program of aerobic exercise training. Each training week will consist of 5 exercise sessions, each lasting 60 minutes, at an average work intensity of 70-75% of VO_2max. No invasive measurements will be made during the training rides, however measurements of heart rate and expired gases may be made periodically. Approximately 2-3 days following the final training ride, you will report back to the laboratory and perform the second experimental exercise trial. This trial, including muscle biopsies and blood sampling, will be identical in all respects to the first experimental exercise trial described above. Finally, on a separate occasion 2-3 days following the second experimental trial, you will be required to perform a final VO_2max test in order to determine the effectiveness of the endurance training program on maximal aerobic power.

DESCRIPTION OF POTENTIAL RISKS AND DISCOMFORTS

Please refer to the attached form entitled "Description of Medical Procedures" for a complete description of the invasive medical procedures to be performed during the study and the potential risks and discomforts associated with these procedures.

REMUNERATION

You will receive an honorarium of $600.00 in order to compensate for your effort and time commitment. Remuneration is normally provided upon completion of the study.

PROVISION OF CONFIDENTIALITY

Any information that is obtained in connection with this study will remain confidential, and appropriate measures will be taken by all investigators to ensure privacy. The results from this study will be used for educational purposes and may be published in scientific journals, presented at scientific meetings or disseminated using other appropriate methods. Regardless of presentation format, subjects will not be identified by name and your personal data will be identified by a code number only. Upon completion of the study, you will have access to your own data and the group data for your own interest.

PARTICIPATION AND WITHDRAWAL

You can choose whether to be in this study or not. If you volunteer to be in this study, you may withdraw at any time without consequences of any kind. You may exercise the option of removing your data from the study. You may also refuse to answer any questions which you do not want to and still remain in the study. The investigators also reserve the right to withdraw you from this research project if circumstances arise which warrant doing so. Should you withdraw from the study prior to its completion, a partial honorarium payment will be made based on the relative proportion of the study which was completed.

RIGHTS OF RESEARCH PARTICIPANTS

You may withdraw your consent at any time and discontinue participation without penalty. You are not waiving any legal claims, rights or remedies because of your participation in this research study. This study has been reviewed and received ethics clearance through the Hamilton Health Sciences Corporation / Faculty of Health Sciences Research Ethics Board (Project Number 00-92). If you have questions regarding your rights as a research participant, contact:

MREB Secretariat
McMaster University
1280 Main Street W., CNH-111
Hamilton, ON L8S 4L9

Telephone: 905-525-9140, ext. 24765
Fax: 905-540-8019
E-mail: grntoff@mcmaster.ca

SIGNATURE OF RESEARCH PARTICIPANT/LEGAL REPRESENTATIVE

I have read and understand the information provided for the study as described herein and in the accompanying forms entitled "Description of Medical Procedures" and "Subject Screening Questionnaire." My questions have been answered to my satisfaction, and I agree to participate in this study. I have been given a copy of this form.

_____
Name of Participant

_____
Name of Legal Representative (if applicable)

_____ _____
Signature of Participant or Legal Representative Date

SIGNATURE OF INVESTIGATOR

In my judgement, the participant is voluntarily and knowingly giving informed consent and possesses the legal capacity to give informed consent to participate in this research study.

_____ _____
Signature of Investigator Date

McMaster University

Department of Kinesiology | 1280 Main Street West, Hamilton, Ontario, Canada L8S 4K1 | Phone 905.525.9140, Fax 905.523.6011, http://kinlabserver.mcmaster.ca

EXERCISE METABOLISM RESEARCH GROUP (EMRG)
DEPARTMENT OF KINESIOLOGY, MCMASTER UNIVERSITY

DESCRIPTION OF MEDICAL PROCEDURES

The study in which you are invited to participate involves two procedures which require medical involvement: muscle biopsy sampling and venous blood sampling. Prior to any involvement, you are asked to read this form which outlines the potential medical risks inherent to these procedures. In addition, you must also complete the "Subject Screening Questionnaire" which is designed to identify any medical reason which might preclude your participation as a subject.

Muscle Biopsy Procedure

This procedure involves the removal of a small piece of muscle tissue using a sterile hollow needle. The area over your quadriceps muscle (vastus lateralis) will be cleaned and a small amount of local anesthetic ("freezing") will be injected into and under the skin. A small incision (~4 mm) in the skin will then be made in order to create an opening through which to put the biopsy needle into your thigh. There is a small amount of bleeding from the incision, but this is minimal. The incision will be covered with sterile gauze and surgical tape. At those times during the experiment when a biopsy is required, the bandage will be removed and the biopsy needle will be inserted into your thigh through the incision. A small piece of muscle (~50-100 mg; about the size of the eraser on the end of a pencil) will quickly be obtained and then the needle will be removed from your leg. During the time that the sample is being taken (~5 sec), you may feel the sensation of deep pressure in your thigh and on some occasions this is moderately painful. However, the discomfort very quickly passes and you are quite capable of performing exercise. If a biopsy sample is required during an exercise trial, the exercise bout is briefly interrupted in order to obtain the sample, and the muscle may feel a little "tight" during the first few seconds as you begin to exercise again.

Following the exercise bout, the biopsy sites will be closed with sterile bandage strips or a suture, and wrapped with a tensor bandage. You should refrain from excessive muscle use for the remainder of the day. Once the anesthetic wears off, your leg may feel tight and often there is the sensation of a deep bruise or "Charlie Horse". You should not take any aspirin-based medicine for 24 hours following the experiment as this can promote bleeding in the muscle. However, other analgesics such as Ibuprofen or Tylenol are acceptable alternatives. It is also beneficial to keep your leg elevated when you are sitting, and the periodic application of an ice pack will help to reduce any swelling and residual soreness. The following day your leg will probably feel uncomfortable going down stairs. The tightness in the muscle usually disappears within 1-2 days, and subjects routinely begin exercising normally within 2-3 days. In order to allow the incisions to heal properly and minimize any risk of infection, you should avoid prolonged submersion in water for 2-3 days. Daily showers are acceptable, but baths, swimming, saunas, etc. should be avoided.

Potential Risks. The biopsy technique is routinely used in physiological research, and complications are rare provided that proper precautions are taken. However, there is a risk of internal bleeding at the site of the biopsy, which can result in bruising and temporary discolouration of the skin. On occasion a small lump of fibrous tissue may form under the site of the incision, but this normally disappears within 2-3 months. As with any incision there is also a slight risk of infection, however this risk is virtually eliminated through proper cleansing of the area and daily changing of wound coverings. If the incision does not heal within a few days or you are in any way concerned about inflammation or infection, please contact us immediately. In very rare occasions there can be damage to a superficial sensory nerve which will result in temporary numbness in the area. There is also an extremely remote chance (1 in 1,000,000) that you will be allergic to the local anesthetic.

It is the collective experience of members in our laboratory that, in healthy young subjects, 1 in 2,500 have experienced a local skin infection; 1 in 1,100 have experienced a small lump at the site of the biopsy (in all cases this disappeared within approximately one week using gentle massage over the area of the lump); 1 in 1,750 have experienced a temporary loss of sensation in the skin at the site of incision (an area of numbness about the size of a quarter that lasted up to 4 months), and 1 in 100 have experienced mild bruising around the site of incision that lasted for ~4-5 days. While there is also a theoretical risk of damage to a small motor nerve branch of the medial vastus lateralis, we have never observed this in any of the research subjects who have been biopsied in our laboratory.

Venous Catheterization and Blood Sampling

A small Teflon catheter will be inserted into a forearm vein with the assistance of a small needle, which is subsequently removed. The discomfort of this procedure is transient and is very similar to having an injection by a needle, or when donating blood. Once the needle is removed there should be no sensation from the catheter. During the course of the experiment, blood will be drawn periodically from the catheter. In any one experiment the total blood loss is typically less than 100 ml, which is approximately 1/6 of the blood removed during a donation to a blood bank. It is not enough of a blood loss to affect your physical performance in any way. After each blood sample has been taken, the catheter is "flushed" with a sterile saline solution in order to prevent blood from clotting in the catheter. This is a salt solution that is very similar in composition to your own blood and it will not affect you. Following removal of the catheter, pressure will be placed on the site in order to minimize bleeding and facilitate healing.

Potential Risks. The insertion of a venous catheter for blood sampling is a common medical practice and involves minimal risk provided proper precautions are taken. The catheter is inserted under completely sterile conditions, however there is a theoretical risk of infection. There is also chance of bleeding if adequate pressure is not maintained upon removal of the catheter. This may cause some minor discomfort and could result in bruising/skin discoloration which could last up to a few weeks. There is also the remote risk that trauma to the vessel wall could result in the formation of a small blood clot, which could travel through the bloodstream and become lodged in a smaller vessel. However, we have never experienced such a complication in our laboratory after several thousand venous catheter placements.

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EXERCISE METABOLISM RESEARCH GROUP (EMRG)
DEPARTMENT OF KINESIOLOGY, MCMASTER UNIVERSITY

SUBJECT SCREENING QUESTIONNAIRE

Your responses to this questionnaire are confidential and you are asked to complete it for your own health and safety. If you answer "YES" to any of the following questions, please give additional details in the space provided and discuss the matter with one of the investigators. You may refuse to answer any of the following questions.

Name: _____ Date: _____

1. Have you ever been told that you have a heart problem?

   YES NO

2. Have you ever been told that you have a breathing problem such as asthma?

   YES NO

3. Have you ever been told that you sometimes experience seizures?

   YES NO

4. Have you ever had any major joint instability or ongoing chronic pain such as in the knee or back?

   YES NO

5. Have you ever been told that you have kidney problems?

   YES NO

6. Have you had any allergies to medication?

   YES NO

7. Have you had any allergies to food or environmental factors?

YES NO

8. Have you had any stomach problems such as ulcers?

YES NO

9. When you experience a cut do you take a long time to stop bleeding?

YES NO

10. When you receive a blow to a muscle do you develop bruises easily?

YES NO

11. Are you currently taking any medication (including aspirin) or have you taken any medication in the last two days?

YES NO

12. Is there any medical condition with which you have been diagnosed and are under the care of a physician (e.g. diabetes, high blood pressure)?

YES NO

APPENDIX II

RAW DATA

CITRATE SYNTHASE
(mmol.kg^{-1} ww.min^{-1})

	PRE REST	POST REST
S1	21.22	30.82
S2	22.12	24.39
S3	31.07	42.33
S4	22.80	23.22
S5	28.17	25.98
S6	23.37	29.35
S7	23.16	44.96
S8	27.21	41.10
MEAN	**24.89**	**32.77**
SD	3.48	8.72
SEM	1.23	3.08

ALANINE AMINOTRANSFERASE
(mmol.kg^{-1} ww.min^{-1})

	PRE REST	POST REST
S1	6.79	10.21
S2	8.22	9.57
S3	10.48	17.94
S4	8.42	9.06
S5	14.45	12.31
S6	8.36	11.75
S7	9.83	16.06
S8	9.71	16.44
MEAN	**9.53**	**12.92**
SD	2.30	3.43
SEM	0.81	1.21

SUBJECT WEIGHT (kg)

	PRE REST	POST REST
S1	87	86.5
S2	91	90.7
S3	87	86
S4	93.5	94.5
S5	78	78
S6	78	78.5
S7	109	108.5
S8	109	109
MEAN	**91.6**	**91.5**
SD	12.1	12.0
SEM	4.3	4.2

$VO_{2\,max}$ (L.min^{-1})

	PRE REST	POST REST
S1	3.07	3.54
S2	3.80	3.78
S3	4.46	4.41
S4	3.95	4.54
S5	4.04	4.03
S6	3.68	4.32
S7	3.99	3.89
S8	4.20	4.55
MEAN	**3.90**	**4.13**
SD	0.41	0.38
SEM	0.15	0.13

$VO_{2\,max}$ (mL.kg^{-1}.min^{-1})

	PRE REST	POST REST
S1	35.3	40.9
S2	41.8	41.7
S3	51.3	51.3
S4	42.3	48.1
S5	51.8	51.6
S6	47.1	55.0
S7	36.6	35.8
S8	38.6	41.7
MEAN	**43.1**	**45.8**
SD	6.4	6.7
SEM	2.3	2.4

EXERCISE WORKLOAD (W)

	PRE REST	POST REST
S1	185	200
S2	210	225
S3	200	215
S4	220	230
S5	220	225
S6	190	205
S7	200	205
S8	220	230
MEAN	**205.6**	**216.9**
SD	14.0	12.2
SEM	4.9	4.3

PERCENT VO_2 WORKED AT

	PRE REST	POST REST
S1	92.0	74.0
S2	82.0	73.0
S3	64.0	66.0
S4	81.0	67.0
S5	84.0	63.0
S6	77.0	64.0
S7	78.0	77.0
S8	79.0	68.0
MEAN	**79.6**	**69.0**
SD	7.9	5.1
SEM	2.8	1.8

TIME TO EXHAUSTION (min)

	PRE REST	POST REST
S1	61.25	96.67
S2	74.20	120.32
S3	45.68	101.90
S4	27.07	87.07
S5	31.57	77.83
S6	34.35	94.35
S7	32.38	92.38
S8	32.07	60.78
MEAN	**42.32**	**91.41**
SD	16.91	17.42
SEM	5.98	6.16

EXERCISE STEADY STATE HEART RATE (bpm)

	PRE REST	POST REST
S1	165	158
S2	169	158
S3	184	158
S4	179	157
S5	162	153
S6	185	158
S7	175	154
S8	183	156
MEAN	**175**	**157**
SD	9	2
SEM	3	1

EXERCISE V_E ($L.min^{-1}$)

	PRE REST	POST REST
S1	75.1	57.8
S2	84.3	70.7
S3	105.7	82.8
S4	158.2	71.1
S6	86.5	52.9
S7	82.2	74.2
S8	125.6	79.7
MEAN	**102.5**	**69.9**
SD	30.0	10.9
SEM	11.3	4.1

EXERCISE VO_2 ($L.min^{-1}$)

	PRE REST	POST REST
S1	2.81	2.47
S2	3.13	2.70
S3	2.85	2.81
S4	3.21	2.93
S6	2.82	2.73
S7	3.10	2.96
S8	3.32	3.02
MEAN	**3.03**	**2.80**
SD	0.21	0.19
SEM	0.08	0.07

EXERCISE VO_2 ($mL.kg^{-1}.min^{-1}$)

	PRE REST	POST REST
S1	32.3	28.6
S2	34.4	29.8
S3	32.8	32.7
S4	34.3	31.0
S6	36.2	34.8
S7	28.4	27.3
S8	30.5	27.7
MEAN	**32.7**	**30.3**
SD	2.6	2.7
SEM	1.0	1.0

EXERCISE
RER

	PRE REST	POST REST
S1	1.00	0.94
S2	1.02	1.01
S3	1.12	1.02
S4	1.24	0.98
S6	1.05	0.95
S7	1.00	1.01
S8	1.12	0.99
MEAN	**1.08**	**0.99**
SD	0.09	0.03
SEM	0.03	0.01

NET CHANGE IN ALANINE (Rest - 5 min)
(mmol.kg^{-1} dw)

	PRE REST	POST REST
S1	6.13	3.08
S2	5.08	4.04
S3	5.85	2.84
S4	5.08	3.50
S5	4.86	2.99
S6	6.51	3.47
S7	4.69	2.12
S8	6.59	4.16
MEAN	**5.60**	**3.28**
SD	0.76	0.67
SEM	0.27	0.24

NET CHANGE IN GLUTAMATE (Rest - 5 min)
(mmol.kg^{-1} dw)

	PRE REST	POST REST
S1	7.10	4.46
S2	6.23	4.20
S3	6.93	5.88
S4	8.48	6.58
S5	6.67	3.69
S6	6.32	4.48
S7	8.59	6.02
S8	10.99	0.42
MEAN	**7.66**	**4.47**
SD	1.62	1.92
SEM	0.57	0.68

NET CHANGE IN GLYCOGEN (Rest - 5 mir
(mmol.kg^{-1} dw)

	PRE REST	POST REST
S1	58.1	-1.0
S2	76.2	46.4
S3		
S4	67.1	-8.7
S5	81.4	50.0
S6	4.0	35.0
S7	78.6	47.7
S8	56.8	35.7
MEAN	**60.3**	**29.3**
SD	26.7	24.1
SEM	10.1	9.1

CREATINE (mmol.kg^{-1} dw)

Subject	PRE REST	PRE 5 min	PRE Exh	POST REST	POST 5 min	POST Exh 1	POST Exh 2
S1	41.9	81.8	86.1	41.3	47.5	59.0	71.6
S2	27.0	82.1	92.6	37.2	44.3	72.1	108.4
S3	35.8	89.5	97.2	38.9	43.0	88.4	82.9
S4	32.7	83.8	*88.8*	40.9	33.0	73.7	72.3
S5	47.4	68.3	109.5	43.6	48.9	98.1	117.7
S6	28.8	82.0	99.3	33.4	47.5	63.0	84.3
S7	39.0	53.4	88.6	37.5	48.0	64.6	70.4
S8	31.1	76.0	87.4	28.1	58.7	64.3	54.7
MEAN	**35.5**	**77.1**	**93.7**	**37.6**	**46.3**	**72.9**	**82.8**
SD	7.0	11.4	7.9	4.9	7.2	13.7	20.9
SEM	2.5	4.0	2.8	1.7	2.5	4.8	7.4

PHOSPHOCREATINE (mmol.kg^{-1} dw)

Subject	PRE REST	PRE 5 min	PRE Exh	POST REST	POST 5 min	POST Exh 1	POST Exh 2
S1	105.9	66.0	61.7	106.5	100.4	88.8	76.3
S2	98.3	43.2	32.7	88.2	81.1	53.2	16.9
S3	97.3	43.6	35.9	94.2	90.1	44.7	50.2
S4	103.7	52.6	*40.9*	95.5	103.5	62.7	64.1
S5	97.1	76.2	34.9	100.9	95.6	46.4	26.7
S6	101.5	48.4	31.1	96.9	82.8	67.4	46.0
S7	89.4	75.0	39.9	90.9	80.4	63.8	58.1
S8	88.3	43.4	32.0	91.3	60.7	55.1	64.6
MEAN	**97.7**	**56.0**	**38.6**	**95.5**	**86.8**	**60.3**	**50.4**
SD	6.3	14.2	10.0	5.9	13.7	14.1	20.1
SEM	2.2	5.0	3.5	2.1	4.9	5.0	7.1

TOTAL CREATINE (mmol.kg^{-1} dw)

Subject	PRE REST	PRE 5 min	PRE Exh	POST REST	POST 5 min	POST Exh 1	POST Exh 2
S1	147.8	147.8	147.8	147.8	147.8	147.8	147.8
S2	125.4	125.4	125.4	125.4	125.4	125.4	125.4
S3	133.1	133.1	133.1	133.1	133.1	133.1	133.1
S4	136.4	136.4	*136.4*	136.4	136.4	136.4	136.4
S5	144.5	144.5	144.5	144.5	144.5	144.5	144.5
S6	130.4	130.4	130.4	130.4	130.4	130.4	130.4
S7	128.5	128.5	128.5	128.5	128.5	128.5	128.5
S8	119.4	119.4	119.4	119.4	119.4	119.4	119.4
MEAN	**133.2**	**133.2**	**133.2**	**133.2**	**133.2**	**133.2**	**133.2**
SD	9.5	9.5	9.5	9.5	9.5	9.5	9.5
SEM	3.4	3.4	3.4	3.4	3.4	3.4	3.4

ATP (mmol.kg^{-1} dw)

Subject	PRE REST	PRE 5 min	PRE Exh	POST REST	POST 5 min	POST Exh 1	POST Exh 2
S1	27.1	27.9	29.0	26.1	29.1	27.8	29.3
S2	24.4	26.9	24.2	23.7	26.5	26.2	25.5
S3	23.8	24.9	27.8	22.1	25.6	26.0	25.7
S4	25.2	26.6	*26.6*	26.8	29.9	25.3	26.6
S5	26.3	27.9	24.3	25.0	25.0	27.2	28.8
S6	25.5	28.3	27.8	22.6	25.1	24.9	30.3
S7	25.1	24.5	26.0	23.7	25.2	24.4	23.5
S8	24.7	26.1	27.4	25.2	17.8	27.7	24.2
MEAN	**25.3**	**26.6**	**26.6**	**24.4**	**25.5**	**26.2**	**26.7**
SD	1.0	1.4	1.7	1.7	3.7	1.3	2.5
SEM	0.4	0.5	0.6	0.6	1.3	0.5	0.9

MALATE (mmol.kg^{-1} dw)

Subject	PRE REST	PRE 5 min	PRE Exh	POST REST	POST 5 min	POST Exh 1	POST Exh 2
S1	0.237	3.105	1.579	0.259	1.481	1.017	1.778
S2	0.227	2.842	1.505	0.110	1.778	1.311	1.210
S3	0.371	2.970	1.796	0.295	1.070	2.159	1.630
S4	0.169	3.131	*1.296*	0.436	2.132	1.426	1.264
S5	0.431	2.583	2.311	0.176	0.968	1.973	2.496
S6	0.303	2.820	2.221	0.343	1.688	1.163	1.836
S7	0.281	2.075	2.020	0.265	1.400	1.342	1.243
S8	0.175	3.256	2.753	0.244	1.110	1.370	1.344
MEAN	**0.274**	**2.848**	**1.935**	**0.266**	**1.453**	**1.470**	**1.600**
SD	0.092	0.377	0.484	0.099	0.401	0.393	0.439
SEM	0.033	0.133	0.171	0.035	0.142	0.139	0.155

FUMARATE (mmol.kg^{-1} dw)

Subject	PRE REST	PRE 5 min	PRE Exh	POST REST	POST 5 min	POST Exh 1	POST Exh 2
S1	0.077	0.950	0.434	0.093	0.416	0.299	0.563
S2	0.068	0.855	0.536	0.053	0.466	0.404	0.328
S3	0.102	0.908	0.633	0.094	0.297	0.667	0.480
S4	0.008	0.882	*0.073*	0.083	0.555	0.320	0.326
S5	0.129	0.820	0.688	0.072	0.337	0.614	0.787
S6	0.077	0.795	0.755	0.102	0.535	0.303	0.495
S7	0.085	0.599	0.679	0.072	0.336	0.433	0.374
S8	0.052	1.102	1.008	0.042	0.358	0.690	0.413
MEAN	**0.075**	**0.864**	**0.601**	**0.076**	**0.412**	**0.466**	**0.471**
SD	0.035	0.143	0.271	0.021	0.097	0.166	0.153
SEM	0.013	0.051	0.096	0.007	0.034	0.059	0.054

CITRATE (mmol.kg^{-1} dw)

Subject	PRE REST	PRE 5 min	PRE Exh	POST REST	POST 5 min	POST Exh 1	POST Exh 2
S1	0.243	0.377	0.933	0.262	0.895	0.629	0.793
S2	0.181	0.313	0.486	0.204	0.649	0.691	0.719
S3	0.270	0.396	0.833	0.263	1.036	1.021	0.998
S4	0.248	0.384	*0.782*	0.431	0.867	0.656	0.780
S5	0.402	0.600	0.679	0.161	0.602	0.666	0.833
S6	0.215	0.455	0.578	0.345	0.767	0.776	1.245
S7	0.260	0.620	0.874	0.254	0.754	0.880	0.801
S8	0.162	0.458	0.764	0.282	0.535	1.194	0.944
MEAN	**0.248**	**0.450**	**0.741**	**0.275**	**0.763**	**0.814**	**0.889**
SD	0.073	0.109	0.151	0.083	0.166	0.203	0.170
SEM	0.026	0.038	0.053	0.029	0.059	0.072	0.060

ISOCITRATE (mmol.kg^{-1} dw)

Subject	PRE REST	PRE 5 min	PRE Exh	POST REST	POST 5 min	POST Exh 1	POST Exh 2
S1	0.082	0.203	0.105	0.099	0.165	0.106	0.196
S2	0.050	0.134	0.050	0.047	0.105	0.089	0.062
S3	0.062	0.154	0.179	0.071	0.109	0.183	0.173
S4	0.082	0.198	*0.203*	0.129	0.187	0.190	0.205
S5	0.058	0.126	0.133	0.031	0.100	0.192	0.201
S6	0.066	0.148	0.164	0.067	0.105	0.157	0.126
S7	0.037	0.117	0.131	0.073	0.106	0.146	0.105
S8	0.058	0.175	0.220	0.071	0.129	0.141	0.175
MEAN	**0.062**	**0.157**	**0.148**	**0.074**	**0.126**	**0.151**	**0.155**
SD	0.015	0.032	0.055	0.030	0.033	0.038	0.052
SEM	0.005	0.011	0.020	0.011	0.012	0.014	0.018

SUM OF TCAI (mmol.kg^{-1} dw)

Subject	PRE REST	PRE 5 min	PRE Exh	POST REST	POST 5 min	POST Exh 1	POST Exh 2
S1	0.638	4.634	3.051	0.713	2.956	2.052	3.330
S2	0.526	4.144	2.577	0.414	2.998	2.495	2.319
S3	0.805	4.428	3.441	0.722	2.512	4.030	3.281
S4	0.508	4.596	*2.866*	1.079	3.742	2.592	2.576
S5	1.019	4.128	3.812	0.440	2.007	3.444	4.317
S6	0.662	4.217	3.718	0.857	3.094	2.399	3.702
S7	0.663	3.412	3.703	0.665	2.595	2.802	2.523
S8	0.446	4.991	4.745	0.639	2.131	3.396	2.876
MEAN	**0.658**	**4.319**	**3.489**	**0.691**	**2.754**	**2.901**	**3.115**
SD	0.184	0.469	0.675	0.215	0.563	0.661	0.676
SEM	0.065	0.166	0.239	0.076	0.199	0.234	0.239

LACTATE (mmol.kg^{-1} dw)

Subject	PRE REST	PRE 5 min	PRE Exh	POST REST	POST 5 min	POST Exh 1	POST Exh 2
S1	4.46	63.58	59.93	3.71	13.13	10.19	12.76
S2	3.98	61.36	17.30	2.81	22.35	18.68	*14.75*
S3	4.97	64.29	55.47	3.75	11.57	28.07	19.64
S4	3.83	76.25	*46.16*	6.97	21.96	20.07	16.68
S5	7.12	37.64	59.11	4.31	16.55	37.13	43.41
S6	4.66	67.71	57.93	4.06	20.37	10.68	35.35
S7	3.86	19.91	41.23	4.35	15.71	17.47	16.97
S8	2.66	60.25	63.74	3.97	11.78	25.00	11.59
MEAN	**4.44**	**56.37**	**50.11**	**4.24**	**16.68**	**20.91**	**21.40**
SD	1.29	18.36	15.23	1.20	4.43	9.01	11.58
SEM	0.46	6.49	5.39	0.43	1.57	3.19	4.10

PYRUVATE (mmol.kg^{-1} dw)

Subject	PRE REST	PRE 5 min	PRE Exh	POST REST	POST 5 min	POST Exh 1	POST Exh 2
S1	0.186	0.658	0.829	0.102	0.454	0.300	0.284
S4	0.167	0.632	*0.802*	0.140	0.493	0.324	0.381
S5	0.296	0.746	0.544	*0.233*	0.549	0.645	0.562
S6	0.216	0.649	1.075	0.209	0.440	0.245	0.891
S7	0.119	0.508	0.713	*0.094*	0.338	0.355	0.307
S8	0.093	0.570	0.622	*0.073*	0.482	0.461	0.211
MEAN	**0.179**	**0.627**	**0.764**	**0.142**	**0.459**	**0.388**	**0.439**
SD	0.072	0.081	0.186	0.066	0.071	0.145	0.251
SEM	0.030	0.033	0.076	0.027	0.029	0.059	0.103
Note: S2 & S3 not included in the mean							
S2		0.333	0.394		0.546	0.468	1.012
S3	0.157	0.562			0.443	0.831	0.579

ALANINE (mmol.kg^{-1} dw)

Subject	PRE REST	PRE 5 min	PRE Exh	POST REST	POST 5 min	POST Exh 1	POST Exh 2
S1	7.70	13.83	12.61	7.41	10.49	10.12	12.06
S2	6.91	11.99	7.78	7.62	11.66	14.26	14.16
S3	6.47	12.33	12.10	7.91	10.75	15.11	14.43
S4	6.00	11.08	*10.61*	9.63	13.13	12.76	11.45
S5	6.90	11.76	10.22	5.58	8.58	10.41	9.63
S6	8.92	15.43	17.00	6.67	10.14	10.16	11.90
S7	6.74	11.42	13.78	7.99	10.10	12.96	10.52
S8	5.46	12.05	12.56	6.20	10.37	15.12	15.09
MEAN	**6.89**	**12.49**	**12.08**	**7.38**	**10.65**	**12.61**	**12.41**
SD	1.06	1.44	2.72	1.25	1.32	2.15	1.96
SEM	0.37	0.51	0.96	0.44	0.47	0.76	0.69

GLUTAMATE (mmol.kg^{-1} dw)

Subject	PRE REST	PRE 5 min	PRE Exh	POST REST	POST 5 min	POST Exh 1	POST Exh 2
S1	10.24	3.14	5.31	11.24	6.78	5.61	6.18
S2	8.87	2.64	2.69	8.53	4.33	6.63	2.58
S3	10.01	3.08	4.39	13.99	8.11	5.29	5.49
S4	10.62	2.14	*3.88*	11.77	5.18	5.05	5.02
S5	10.66	4.00	4.08	8.80	5.12	3.47	4.12
S6	9.80	3.48	3.61	10.23	5.76	6.89	5.87
S7	14.35	5.76	3.75	10.95	4.93	5.49	4.19
S8	14.70	3.72	4.24	14.24	13.82	5.48	9.15
MEAN	**11.16**	**3.50**	**3.99**	**11.22**	**6.75**	**5.49**	**5.32**
SD	2.15	1.09	0.74	2.11	3.10	1.04	1.93
SEM	0.76	0.38	0.26	0.75	1.09	0.37	0.68

GLYCOGEN (mmol.kg^{-1} dw)

Subject	PRE REST	PRE 5 min	PRE Exh	POST REST	POST 5 min	POST Exh 1	POST Exh 2
S1	390.7	332.6	222.6	657.5	658.4	407.5	246.4 *
S2	437.4	361.2	2.9	*628.0*	581.6	268.4	236.3 *
S4	408.4	341.3	36.8	760.0	768.7	535.5	388.7
S5	351.0	269.7	13.0	*538.0*	488.0	454.5	92.6 *
S6	456.7	452.7	277.5	719.1	684.0	639.2	481.5
S7	445.3	366.7	257.3	*573.0*	525.3	501.6	259.5
S8	347.9	291.1	96.3	*604.2*	568.5	538.1	419.0 *
MEAN	**405.3**	**345.0**	**129.5**	**640.0**	**610.7**	**477.8**	**303.4**
SD	44.2	59.3	119.9	78.8	98.0	117.5	133.2
SEM	16.7	22.4	45.3	29.8	37.0	44.4	50.4

* Subjects reached volitional exhaustion

NOTE: S3 not included in the mean

S3		313.3			472.4		212.7

BLOOD LACTATE (mmol.L^{-1})

Subject	PRE REST	PRE 10 min	PRE 30 min	PRE 45 min	PRE 60 min	POST REST	POST 10 min	POST 30 min	POST 45 min	POST 60 min	POST 90 min
S1	0.88	5.28	5.07	6.02	4.97	0.97	3.45	2.37		2.64	3.20
S2	0.35	2.90	4.88	5.46	5.52	0.58	2.69	2.92	3.06	2.88	2.97
S3	1.15	4.74	6.83			*0.59*	2.19	2.49		3.36	2.91
S4	0.61	6.31	7.32			1.47	3.03	3.12	3.37	3.09	2.85
S5	0.53	5.46	7.40			0.34	4.52	4.25	4.75	4.17	
S6	1.41	6.30	7.96			0.75	3.19	2.25	2.11	1.86	2.48
S7	0.77	4.42	5.23			1.01	3.40	3.68	2.73	1.99	2.06
S8	0.12	5.45	7.17			0.80	2.59	2.55	2.11	1.90	
MEAN	**0.73**	**5.11**	**6.48**	**5.74**	**5.25**	**0.81**	**3.13**	**2.95**	**3.02**	**2.74**	**2.75**
SD	0.42	1.11	1.22	0.40	0.39	0.34	0.71	0.70	0.99	0.81	0.41
SEM	0.15	0.39	0.43	0.28	0.28	0.13	0.25	0.25	0.40	0.29	0.17

BLOOD GLUCOSE (mmol.L^{-1})

Subject	PRE REST	PRE 10 min	PRE 30 min	PRE 45 min	PRE 60 min	POST REST	POST 10 min	POST 30 min	POST 45 min	POST 60 min	POST 90 min
S1	3.57	4.52	4.28	4.20	5.15	3.64	4.17	3.85		3.62	3.73
S2	3.93	2.66	4.08	4.85	4.60	4.28	4.27	4.49	4.22	4.45	4.54
S3	4.41	4.45	4.18			*5.23*	4.51	4.12		4.09	3.97
S4	3.44	3.64	3.11			4.41	4.05	4.19	5.10	4.27	3.97
S5	5.08	3.37	2.83			5.85	4.33	3.56	3.23	3.57	
S6	5.73	5.41	3.71			5.97	3.87	3.65	4.14	4.29	3.75
S7	4.41	3.66	4.18			3.93	3.41	4.76	4.59	4.35	3.96
S8	4.55	4.08	3.70			3.77	3.45	4.13	4.39	4.30	
MEAN	**4.39**	**3.97**	**3.76**	**4.52**	**4.88**	**4.63**	**4.01**	**4.09**	**4.28**	**4.12**	**3.99**
SD	0.76	0.84	0.54	0.46	0.39	0.93	0.40	0.40	0.62	0.34	0.29
SEM	0.27	0.30	0.19	0.33	0.28	0.33	0.14	0.14	0.25	0.12	0.12

APPENDIX III

STATISTICAL TABLES

PAIRED T-TESTS
1 WAY & 2 WAY ANOVAS
TUKEY HSD POST HOC TESTS

CITRATE SYNTHASE (mmol.kg^{-1} ww.min^{-1})
t-Test: Paired Two Sample for Means

	PRE REST	*POST REST*
Mean	24.89	32.77
Variance	12.14	76.03
Observations	8	8
df	7	
t Stat	-2.822	
P(T<=t) two-tail	0.026	
t Critical two-tail	2.365	

ALANINE AMINOTRANSFERASE (mmol.kg^{-1} ww.min^{-1})
t-Test: Paired Two Sample for Means

	PRE REST	*POST REST*
Mean	9.53	12.92
Variance	5.30	11.80
Observations	8	8
df	7	
t Stat	-2.869	
P(T<=t) two-tail	0.024	
t Critical two-tail	2.365	

SUBJECT WEIGHT (kg)
t-Test: Paired Two Sample for Means

	PRE REST	*POST REST*
Mean	91.56	91.46
Variance	145.96	144.48
Observations	8	8
df	7	
t Stat	0.450	
P(T<=t) two-tail	0.666	
t Critical two-tail	2.365	

VO_{2max} (L.min^{-1})
t-Test: Paired Two Sample for Means

	PRE REST	*POST REST*
Mean	3.90	4.13
Variance	0.17	0.14
Observations	8	8
df	7	
t Stat	-2.107	
P(T<=t) one-tail	0.04	
t Critical one-tail	1.89	

$VO_{2\,max}$ ($ml.kg^{-1}.min^{-1}$)
t-Test: Paired Two Sample for Means

	PRE REST	*POST REST*
Mean	43.1	45.8
Variance	41.0	44.3
Observations	8	8
df	7	
t Stat	-2.235	
P(T<=t) one-tail	0.030	
t Critical one-tail	1.895	

EXERCISE WORKLOAD (W)
t-Test: Paired Two Sample for Means

	PRE REST	*POST REST*
Mean	205.6	216.9
Variance	196.0	149.6
Observations	8	8
df	7	
t Stat	-7.180	
P(T<=t) two-tail	0.0002	
t Critical two-tail	2.3646	

PERCENT VO_2 WORKED AT
t-Test: Paired Two Sample for Means

	PRE REST	*POST REST*
Mean	79.6	69.0
Variance	62.0	25.7
Observations	8	8
df	7	
t Stat	3.817	
P(T<=t) two-tail	0.007	
t Critical two-tail	2.365	

TIME TO EXHAUSTION (min)
t-Test: Paired Two Sample for Means

	PRE REST	*POST REST*
Mean	42.32	91.41
Variance	285.95	303.43
Observations	8	8
df	7	
t Stat	-11.465	
P(T<=t) two-tail	8.63E-06	
t Critical two-tail	2.36E+00	

EXERCISE STEADY STATE HEART RATE (bpm)
t-Test: Paired Two Sample for Means

	PRE REST	*POST REST*
Mean	175	157
Variance	81	4
Observations	8	8
df	7	
t Stat	6.291	
P(T<=t) two-tail	0.0004	
t Critical two-tail	2.3646	

EXERCISE V_E ($L.min^{-1}$)
t-Test: Paired Two Sample for Means

	PRE REST	*POST REST*
Mean	102.5	69.9
Variance	899.8	119.8
Observations	7	7
df	6	
t Stat	3.169	
P(T<=t) two-tail	0.019	
t Critical two-tail	2.447	

EXERCISE VO_2 ($L.min^{-1}$)
t-Test: Paired Two Sample for Means

	PRE REST	*POST REST*
Mean	3.03	2.80
Variance	0.04	0.04
Observations	7	7
df	6	
t Stat	4.27	
P(T<=t) two-tail	0.005	
t Critical two-tail	2.447	

EXERCISE VO_2 ($ml.kg^{-1}.min^{-1}$)
t-Test: Paired Two Sample for Means

	PRE REST	*POST REST*
Mean	32.7	30.3
Variance	6.8	7.6
Observations	7	7
df	6	
t Stat	3.99	
P(T<=t) two-tail	0.007	
t Critical two-tail	2.447	

EXERCISE
RER
t-Test: Paired Two Sample for Means

	PRE REST	*POST REST*
Mean	1.08	0.99
Variance	0.01	0.00
Observations	7	7
df	6	
t Stat	2.682	
P(T<=t) two-tail	0.036	
t Critical two-tail	2.447	

NET CHANGE IN ALANINE (Rest - 5 min)
(mmol.kg^{-1} dw)
t-Test: Paired Two Sample for Means

	PRE REST	*POST REST*
Mean	5.60	3.28
Variance	0.58	0.44
Observations	8	8
df	7	
t Stat	8.691	
P(T<=t) two-tail	5.3498E-05	
t Critical two-tail	2.36462256	

NET CHANGE IN GLUTAMATE (Rest - 5 min)
(mmol.kg^{-1} dw)
t-Test: Paired Two Sample for Means

	PRE REST	*POST REST*
Mean	-7.66	-4.47
Variance	2.61	3.70
Observations	8	8
df	7	
t Stat	-2.976	
P(T<=t) two-tail	0.021	
t Critical two-tail	2.365	

NET CHANGE IN GLYCOGEN (Rest - 5 min)
(mmol.kg^{-1} dw)
t-Test: Paired Two Sample for Means

	PRE REST	*POST REST*
Mean	60.3	29.3
Variance	710.8	582.2
Observations	7	7
df	6	
t Stat	2.44619	
P(T<=t) two-tail	0.0500	
t Critical two-tail	2.4469	

CREATINE (mmol.kg^{-1} dw)
Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	8	283.73	35.47	48.37
Column 2	8	616.94	77.12	129.75
Column 3	8	749.52	93.69	63.13
Column 4	8	300.96	37.62	24.38
Column 5	8	370.80	46.35	51.13
Column 6	8	583.13	72.89	187.27
Column 7	8	662.44	82.80	436.99

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
TIME	26453.0	6	4408.828	40.006	0.000
Error(TIME)	4628.6	42	110.205		

Tukey HSD test
Probabilities for Post Hoc Tests

	PRE REST	**PRE 5 min**	**PRE Exh**	**POST REST**	**POST 5 min**	**POST Exh 1**	**POST Exh 2**
MEAN	35.47	77.12	93.69	37.62	46.35	72.89	82.80
PRE REST		0.0001	0.0001	0.9996	0.3869	0.0001	0.0001
PRE 5 min	0.0001		0.0432	0.0001	0.0001	0.9832	0.9297
PRE Exh	0.0001	0.0432		0.0001	0.0001	0.0050	0.3868
POST REST	0.9996	0.0001	0.000		0.6436	0.0001	0.0001
POST 5 min	0.3869	0.0001	0.000	0.644		0.0003	0.0001
POST Exh 1	0.0001	0.9832	0.005	0.000	0.0003		0.4992
POST Exh 2	0.0001	0.9297	0.387	0.000	0.0001	0.4992	

PHOSPHOCREATINE (mmol.kg^{-1} dw)
Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	8	781.60	97.70	39.57
Column 2	8	448.39	56.05	202.14
Column 3	8	309.13	38.64	99.48
Column 4	8	764.37	95.55	35.11
Column 5	8	694.53	86.82	188.39
Column 6	8	482.20	60.27	199.59
Column 7	8	402.89	50.36	402.78

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
TIME	26864.7	6	4477.446	40.508	0.000
Error(TIME)	4642.4	42	110.533		

Tukey HSD test
Probabilities for Post Hoc Tests

	PRE REST	**PRE 5 min**	**PRE Exh**	**POST REST**	**POST 5 min**	**POST Exh 1**	**POST Exh 2**
MEAN	97.70	56.05	38.64	95.55	86.82	60.27	50.36
PRE REST		0.0001	0.0001	0.9996	0.3880	0.0001	0.0001
PRE 5 min	0.0001		0.0291	0.0001	0.0001	0.9833	0.9300
PRE Exh	0.0001	0.0291		0.0001	0.0001	0.0032	0.3018
POST REST	0.9996	0.0001	0.0001		0.6446	0.0001	0.0001
POST 5 min	0.3880	0.0001	0.0001	0.6446		0.0003	0.0001
POST Exh 1	0.0001	0.9833	0.0032	0.0001	0.0003		0.5003
POST Exh 2	0.0001	0.9300	0.3018	0.0001	0.0001	0.5003	

ATP (mmol.kg^{-1} dw)
Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	8	202.14	25.27	1.10
Column 2	8	213.08	26.63	1.90
Column 3	8	213.17	26.65	3.00
Column 4	8	195.13	24.39	2.73
Column 5	8	204.20	25.53	13.36
Column 6	8	209.41	26.18	1.67
Column 7	8	213.90	26.74	6.22

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
TIME	37.3	6	6.224	1.837	0.115
Error(TIME)	142.3	42	3.388		

MALATE (mmol.kg^{-1} dw)
Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	8	2.193	0.274	0.008
Column 2	8	22.782	2.848	0.142
Column 3	8	15.481	1.935	0.234
Column 4	8	2.128	0.266	0.010
Column 5	8	11.627	1.453	0.161
Column 6	8	11.762	1.470	0.154
Column 7	8	12.800	1.600	0.193

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
TIME	39.865	6	6.644	48.829	0.000
Error(TIME)	5.715	42	0.136		

Tukey HSD test
Probabilities for Post Hoc Tests

	PRE REST	**PRE 5 min**	**PRE Exh**	**POST REST**	**POST 5 min**	**POST Exh 1**	**POST Exh 2**
MEAN	0.274	2.848	1.935	0.266	1.453	1.470	1.600
PRE REST		0.0001	0.0001	1.0000	0.0001	0.0001	0.0001
PRE 5 min	0.0001		0.0004	0.0001	0.0001	0.0001	0.0001
PRE Exh	0.0001	0.0004		0.0001	0.1481	0.1778	0.5442
POST REST	1.0000	0.0001	0.0001		0.0001	0.0001	0.0001
POST 5 min	0.0001	0.0001	0.1481	0.0001		1.0000	0.9843
POST Exh 1	0.0001	0.0001	0.1778	0.0001	1.0000		0.9917
POST Exh 2	0.0001	0.0001	0.5442	0.0001	0.9843	0.9917	

FUMARATE (mmol.kg^{-1} dw)
Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	8	0.597	0.075	0.001
Column 2	8	6.910	0.864	0.020
Column 3	8	4.807	0.601	0.073
Column 4	8	0.612	0.076	0.000
Column 5	8	3.300	0.412	0.009
Column 6	8	3.730	0.466	0.028
Column 7	8	3.766	0.471	0.023

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
TIME	3.773	6	0.629	30.202	0.000
Error(TIME)	0.875	42	0.021		

Tukey HSD test
Probabilities for Post Hoc Tests

	PRE REST	**PRE 5 min**	**PRE Exh**	**POST REST**	**POST 5 min**	**POST Exh 1**	**POST Exh 2**
MEAN	0.075	0.864	0.601	0.076	0.412	0.466	0.471
PRE REST		0.0001	0.0001	1.0000	0.0007	0.0002	0.0002
PRE 5 min	0.0001		0.0121	0.0001	0.0001	0.0002	0.0002
PRE Exh	0.0001	0.0121		0.0001	0.1484	0.5128	0.5527
POST REST	1.0000	0.0001	0.0001		0.0007	0.0002	0.0002
POST 5 min	0.0007	0.0001	0.1484	0.0007		0.9888	0.9830
POST Exh 1	0.0002	0.0002	0.5128	0.0002	0.9888		1.0000
POST Exh 2	0.0002	0.0002	0.5527	0.0002	0.9830	1.0000	

CITRATE (mmol.kg^{-1} dw)
Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	8	1.982	0.248	0.005
Column 2	8	3.603	0.450	0.012
Column 3	8	5.929	0.741	0.023
Column 4	8	2.202	0.275	0.007
Column 5	8	6.103	0.763	0.028
Column 6	8	6.513	0.814	0.041
Column 7	8	7.113	0.889	0.029

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
TIME	3.423	6	0.571	29.831	0.000
Error(TIME)	0.803	42	0.019		

Tukey HSD test
Probabilities for Post Hoc Tests

	PRE REST	**PRE 5 min**	**PRE Exh**	**POST REST**	**POST 5 min**	**POST Exh 1**	**POST Exh 2**
MEAN	0.248	0.450	0.741	0.275	0.763	0.814	0.889
PRE REST		0.0740	0.0001	0.9997	0.0001	0.0001	0.0001
PRE 5 min	0.0740		0.0025	0.1740	0.0010	0.0002	0.0001
PRE Exh	0.0001	0.0025		0.0001	0.9999	0.9374	0.3486
POST REST	0.9997	0.1740	0.0001		0.0001	0.0001	0.0001
POST 5 min	0.0001	0.0010	0.9999	0.0001		0.9891	0.5386
POST Exh 1	0.0001	0.0002	0.9374	0.0001	0.9891		0.9289
POST Exh 2	0.0001	0.0001	0.3486	0.0001	0.5386	0.9289	

ISOCITRATE (mmol.kg^{-1} dw)
Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	8	0.495	0.062	0.000
Column 2	8	1.255	0.157	0.001
Column 3	8	1.185	0.148	0.003
Column 4	8	0.588	0.074	0.001
Column 5	8	1.005	0.126	0.001
Column 6	8	1.204	0.151	0.001
Column 7	8	1.243	0.155	0.003

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
TIME	0.078	6	0.013	15.268	0.000
Error(TIME)	0.036	42	0.001		

Tukey HSD test
Probabilities for Post Hoc Tests

	PRE REST	**PRE 5 min**	**PRE Exh**	**POST REST**	**POST 5 min**	**POST Exh 1**	**POST Exh 2**
MEAN	0.062	0.157	0.148	0.074	0.126	0.151	0.155
PRE REST		0.0001	0.0001	0.9843	0.0016	0.0001	0.0001
PRE 5 min	0.0001		0.9965	0.0002	0.3515	0.9995	1.0000
PRE Exh	0.0001	0.9965		0.0003	0.7221	1.0000	0.9988
POST REST	0.9843	0.0002	0.0003		0.0147	0.0002	0.0002
POST 5 min	0.0016	0.3515	0.7221	0.0147		0.6184	0.4091
POST Exh 1	0.0001	0.9995	1.0000	0.0002	0.6184		0.9999
POST Exh 2	0.0001	1.0000	0.9988	0.0002	0.4091	0.9999	

SUM OF TCAI (mmol.kg^{-1} dw)
Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	8	5.268	0.658	0.034
Column 2	8	34.550	4.319	0.220
Column 3	8	27.914	3.489	0.456
Column 4	8	5.530	0.691	0.046
Column 5	8	22.035	2.754	0.317
Column 6	8	23.208	2.901	0.437
Column 7	8	24.922	3.115	0.456

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
TIME	92.225	6	15.371	54.983	0.000
Error(TIME)	11.741	42	0.280		

Tukey HSD test
Probabilities for Post Hoc Tests

	PRE REST	**PRE 5 min**	**PRE Exh**	**POST REST**	**POST 5 min**	**POST Exh 1**	**POST Exh 2**
MEAN	0.658	4.319	3.489	0.691	2.754	2.901	3.115
PRE REST		0.0001	0.0001	1.0000	0.0001	0.0001	0.0001
PRE 5 min	0.0001		0.0452	0.0001	0.0001	0.0002	0.0010
PRE Exh	0.0001	0.0452		0.0001	0.1040	0.3046	0.7910
POST REST	1.0000	0.0001	0.0001		0.0001	0.0001	0.0001
POST 5 min	0.0001	0.0001	0.1040	0.0001		0.9978	0.8168
POST Exh 1	0.0001	0.0002	0.3046	0.0001	0.9978		0.9827
POST Exh 2	0.0001	0.0010	0.7910	0.0001	0.8168	0.9827	

LACTATE (mmol.kg^{-1} dw)
Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	8	35.55	4.44	1.66
Column 2	8	450.99	56.37	337.00
Column 3	8	400.87	50.11	232.06
Column 4	8	33.92	4.24	1.45
Column 5	8	133.42	16.68	19.64
Column 6	8	167.29	20.91	81.17
Column 7	8	171.16	21.40	134.16

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
TIME	20537.5	6	3422.9	30.283	0.000
Error(TIME)	4747.3	42	113.0		

Tukey HSD test
Probabilities for Post Hoc Tests

	PRE REST	**PRE 5 min**	**PRE Exh**	**POST REST**	**POST 5 min**	**POST Exh 1**	**POST Exh 2**
MEAN	4.44	56.37	50.11	4.24	16.68	20.91	21.40
PRE REST		0.0001	0.0001	1.0000	0.2675	0.0498	0.0398
PRE 5 min	0.0001		0.8983	0.0001	0.0001	0.0001	0.0001
PRE Exh	0.0001	0.8983		0.0001	0.0001	0.0002	0.0002
POST REST	1.0000	0.0001	0.0001		0.2501	0.0454	0.0362
POST 5 min	0.2675	0.0001	0.0001	0.2501		0.9841	0.9726
POST Exh 1	0.0498	0.0001	0.0002	0.0454	0.9841		1.0000
POST Exh 2	0.0398	0.0001	0.0002	0.0362	0.9726	1.0000	

PYRUVATE (mmol.kg^{-1} dw)
Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	6	1.077	0.179	0.005
Column 2	6	3.764	0.627	0.007
Column 3	6	4.585	0.764	0.035
Column 4	6	0.851	0.142	0.004
Column 5	6	2.756	0.459	0.005
Column 6	6	2.331	0.388	0.021
Column 7	6	2.636	0.439	0.063

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
TIME	1.794	6	0.299	18.215	0.000
Error(TIME)	0.492	30	0.016		

Tukey HSD test
Probabilities for Post Hoc Tests

	PRE REST	**PRE 5 min**	**PRE Exh**	**POST REST**	**POST 5 min**	**POST Exh 1**	**POST Exh 2**
MEAN	0.179	0.627	0.764	0.142	0.459	0.388	0.439
PRE REST		0.0002	0.0001	0.9985	0.0110	0.1031	0.0216
PRE 5 min	0.0002		0.5264	0.0001	0.2900	0.0423	0.1803
PRE Exh	0.0001	0.5264		0.0001	0.0046	0.0005	0.0023
POST REST	0.9985	0.0001	0.0001		0.0030	0.0330	0.0060
POST 5 min	0.0110	0.2900	0.0046	0.0030		0.9592	1.0000
POST Exh 1	0.1031	0.0423	0.0005	0.0330	0.9592		0.9924
POST Exh 2	0.0216	0.1803	0.0023	0.0060	1.0000	0.9924	

ALANINE (mmol.kg^{-1} dw)
Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	8	55.10	6.89	1.11
Column 2	8	99.89	12.49	2.08
Column 3	8	96.66	12.08	7.38
Column 4	8	59.00	7.38	1.56
Column 5	8	85.21	10.65	1.74
Column 6	8	100.90	12.61	4.63
Column 7	8	99.25	12.41	3.84

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
TIME	297.8	6	49.6	16.128	0.000
Error(TIME)	129.3	42	3.1		

Tukey HSD test
Probabilities for Post Hoc Tests

	PRE REST	**PRE 5 min**	**PRE Exh**	**POST REST**	**POST 5 min**	**POST Exh 1**	**POST Exh 2**
MEAN	6.89	12.49	12.08	7.38	10.65	12.61	12.41
PRE REST		0.0001	0.0001	0.9977	0.0019	0.0001	0.0001
PRE 5 min	0.0001		0.9992	0.0001	0.3756	1.0000	1.0000
PRE Exh	0.0001	0.9992		0.0002	0.6626	0.9964	0.9998
POST REST	0.9977	0.0001	0.0002		0.0094	0.0001	0.0002
POST 5 min	0.0019	0.3756	0.6626	0.0094		0.2985	0.4289
POST Exh 1	0.0001	1.0000	0.9964	0.0001	0.2985		1.0000
POST Exh 2	0.0001	1.0000	0.9998	0.0002	0.4289	1.0000	

GLUTAMATE (mmol.kg^{-1} dw)
Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	8	89.25	11.16	4.64
Column 2	8	27.97	3.50	1.18
Column 3	8	31.94	3.99	0.55
Column 4	8	89.74	11.22	4.46
Column 5	8	54.01	6.75	9.59
Column 6	8	43.93	5.49	1.08
Column 7	8	42.59	5.32	3.72

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
TIME	489.5	6	81.6	33.861	0.000
Error(TIME)	101.2	42	2.4		

Tukey HSD test
Probabilities for Post Hoc Tests

	PRE REST	**PRE 5 min**	**PRE Exh**	**POST REST**	**POST 5 min**	**POST Exh 1**	**POST Exh 2**
MEAN	11.16	3.50	3.99	11.22	6.75	5.49	5.32
PRE REST		0.0001	0.0001	1.0000	0.0002	0.0001	0.0001
PRE 5 min	0.0001		0.9950	0.0001	0.0026	0.1613	0.2436
PRE Exh	0.0001	0.9950		0.0001	0.0154	0.4723	0.6107
POST REST	1.0000	0.0001	0.0001		0.0002	0.0001	0.0001
POST 5 min	0.0002	0.0026	0.0154	0.0002		0.6685	0.5298
POST Exh 1	0.0001	0.1613	0.4723	0.0001	0.6685		1.0000
POST Exh 2	0.0001	0.2436	0.6107	0.0001	0.5298	1.0000	

GLYCOGEN (mmol.kg^{-1} dw)
Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	7	2837.4	405.3	1956.3
Column 2	7	2415.3	345.0	3513.0
Column 3	7	906.4	129.5	14368.9
Column 4	7	4479.7	640.0	6212.2
Column 5	7	4274.6	610.7	9599.8
Column 6	7	3344.9	477.8	13816.2
Column 7	7	2124.0	303.4	17748.2

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
TIME	1342481.4	6	223746.9	39.679	0.000
Error(TIME)	203003.5	36	5639.0		

Tukey HSD test
Probabilities for Post Hoc Tests

	PRE REST	**PRE 5 min**	**PRE Exh**	**POST REST**	**POST 5 min**	**POST Exh 1**	**POST Exh 2**
MEAN	405.3	345.0	129.5	640.0	610.7	477.8	303.4
PRE REST		0.7417	0.0001	0.0001	0.0003	0.5528	0.1760
PRE 5 min	0.7417		0.0002	0.0001	0.0001	0.0318	0.9418
PRE Exh	0.0001	0.0002		0.0001	0.0001	0.0001	0.0021
POST REST	0.0001	0.0001	0.0001		0.9897	0.0047	0.0001
POST 5 min	0.0003	0.0001	0.0001	0.9897		0.0318	0.0001
POST Exh 1	0.5528	0.0318	0.0001	0.0047	0.0318		0.0020
POST Exh 2	0.1760	0.9418	0.0021	0.0001	0.0001	0.0020	

BLOOD LACTATE ($mmol.L^{-1}$)
Anova: Two-Factor (Pre/Post Training x Time)

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	8	5.82	0.73	0.18
Column 2	8	40.86	5.11	1.23
Column 3	8	51.86	6.48	1.49
Column 4	8	6.51	0.81	0.12
Column 5	8	25.06	3.13	0.50
Column 6	8	23.63	2.95	0.49

ANOVA

Source of Variation	df Effect	MS Effect	df Error	MS Error	F	p-level
Pre/Post Training	1	39.1324	7.0000	1.1397	34.3357	0.0006
Time	2	72.4235	14.0000	0.4688	154.5024	0.0000
Training x Time	2	13.1541	14.0000	0.3421	38.4490	0.0000

Tukey HSD test
Probabilities for Post Hoc Tests

	PRE REST	**PRE 10 min**	**PRE 30 min**	**POST REST**	**POST 10 min**	**POST 30 min**
MEAN	0.73	5.11	6.48	0.81	3.13	2.95
PRE REST		0.0002	0.0002	0.9996	0.0002	0.0002
PRE 10 min	0.0002		0.0038	0.0002	0.0002	0.0002
PRE 30 min	0.0002	0.0038		0.0002	0.0002	0.0002
POST REST	0.9996	0.0002	0.0002		0.0002	0.0002
POST 10 min	0.0002	0.0002	0.0002	0.0002		0.9884
POST 30 min	0.0002	0.0002	0.0002	0.0002	0.9884	

BLOOD GLUCOSE (mmol.L^{-1})
Anova: Two-Factor (Pre/Post Training x Time)

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	8	35.12	4.39	0.58
Column 2	8	31.79	3.97	0.70
Column 3	8	30.07	3.76	0.29
Column 4	8	37.08	4.64	0.86
Column 5	8	32.06	4.01	0.16
Column 6	8	32.75	4.09	0.16

ANOVA

Source of Variation	df Effect	MS Effect	df Error	MS Error	F	p-level
Pre/Post Training	1	0.503	7.000	0.444	1.131	0.323
Time	2	1.648	14.000	0.696	2.370	0.130
Training x Time	2	0.096	14.000	0.172	0.557	0.585

APPENDIX IV

INTRA-ASSAY VARIABILITY

INTRA-ASSAY VARIABILITY

CV calculated based on mean/standard deviation (sd) of 4 – 8 repeats

CV = sd/mean x 100%

Assay	CV (%)
Creatine	**4.5**
Phosphocreatine	**2.8**
ATP	**2.2**
Malate	**3.4**
Fumarate	**7.0**
Citrate	**4.0**
Isocitrate	**19.8**
Pyruvate	**5.5**
Lactate	**1.8**
Alanine	**1.8**
Glutamate	**2.1**
Glucose (glycogen)	**1.4**
Alanine Aminotransferase	**5.1**
Citrate Synthase	**1.8**