THE EFFECT OF SHORT SPRINT INTERVAL TRAINING ON SKELETAL MUSCLE METABOLISM AND PERFORMANCE DURING INTENSE AEROBIC CYCLING

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A Thesis

Submitted to the School of Graduate Studies

In Partial Fulfillment of the Requirements

for the Degree

Master of Science

McMaster University

MASTER OF SCIENCE (2003)
(Human Biodynamics)

McMaster University
Hamilton, Ontario

TITLE: The effect of short sprint interval training on skeletal muscle metabolism and performance during intense aerobic cycling.

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NUMBER OF PAGES: x, 119

ABSTRACT

Two weeks of sprint interval training (SIT) increased maximal aerobic power (VO_2peak), however maximal anaerobic power (W_{max}; 30-sec "all out" Wingate test) was unaffected, possibly due to chronic fatigue induced by 14 daily training bouts (Rodas et al. Eur. J. Appl. Physiol. 82:480-86, 2000). The effect of fewer SIT bouts on these parameters is unknown, and no study has assessed changes in performance or muscle metabolism during intense aerobic exercise after SIT. **PURPOSE:** We examined whether 6 bouts of SIT, performed over 2 wks with 1-2 d rest between bouts, elicited changes in endurance performance or metabolism during a "challenge ride" to exhaustion @ ~80% VO_{2peak}. **METHODS:** 8 subjects (6 men; 23±2 yr) were studied before and 2-3 d following the SIT protocol (6 bouts x 4-8 Wingate tests, 4 min rest). **RESULTS:** VO_{2peak} was unchanged by SIT (Post: 45.5 ± 5 vs. Pre: 44.6 ± 3.2 ml/kg/min) as was mean power during 4 repeated Wingate tests (Post: 574.8 ± 29.9 vs. Pre: 569.8 ± 31.3 W). W_{max} during 4 repeated Wingate tests increased by 14% following SIT (Post: 1067.0 ± 234 vs. Pre: 934.3 ± 173.6 W; $p \leq 0.05$). Most strikingly, cycle time to exhaustion increased by 101% after SIT (Post: 51.1 ± 30.8 vs. Pre: 25.4 ± 14.4 min, $p \leq 0.01$). Biopsies obtained prior to, and during the challenge rides revealed that resting muscle glycogen was higher after SIT (Post: 614 ± 39 vs. Pre: 489 ± 57 mmol/kg dry wt., $p \leq 0.02$), and glycogen degradation during exercise was attenuated ($p \leq 0.05$). The maximal activity of CS was 29% higher following training (Post: 16.7 ± 2.3 vs. Pre: 11.5 ± 1.8 mmol/kg wet wt., $p \leq 0.05$) which is indicative of a higher muscle oxidative potential. **CONCLUSION:** 6 bouts of SIT (~15 min total exercise time) markedly increased intense aerobic cycling

performance and peak anaerobic power. The physiological mechanisms that account for these improvements remain to be fully elucidated, but include alterations in glycogen metabolism and an increased muscle oxidative potential.

ACKNOWLEDGEMENTS

My success in graduate school can be attributed to a large network of people who have made my educational pathway a smooth, and enjoyable one. First and foremost is my supervisor Marty Gibala. Throughout my undergraduate and graduate career you have provided leadership and instilled a drive within myself to be better. You have given me the opportunity to experience independence, but have also provided unconditional support when I have needed it, and for that I am truly thankful. However, as well as we get along, a PhD was just never an option as that would mean 4 more years with a Red Wings fan.....Go Leafs Go.....

To my boys.....Tim, Steph, and Dan. Thanks for giving me an escape from academia. Although the memories are way too numerous to list there are three things that come to mind.....sushi, golf, and the "occasional" Wednesday night. Thanks for being a huge part of my life outside the lab.

The K's: Krista, Kristen, and Kirsten. Who would have thought that Friday morning meetings could actually be enjoyable? Krista, thanks for all of your expertise in the lab, as I am positive that I couldn't have done it without you. Kristen, your support and caring nature is truly unparalleled, thank you. Kirsten, you were a huge help with my study, but more important than the help in the lab is your sarcastic, yet very amusing, sense of humour that helped lighten some of those long and frustrating nights.

My graduate thesis would have been unattainable without the technical wizardry of John Moroz, and the maternal instincts of Mary Cleland. John, you are the oil that keeps the machine going, thank you. Mary, who knows how many deadlines I would have missed and how much funding would have passed me by if it wasn't for you, thanks.

Dr's Phillips, MacDonald, Heigenhauser, and Sale, it has been a pleasure working with such intellect, but it is your genuine, diverse personalities that have made working with you so enjoyable.

Most importantly though, I would like to thank my family. Mom, Dad and T, your support has been endless despite the long....and very costly....road that I have taken. You guys have been there every step of the way, and for that, this thesis is dedicated to you.

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CHAPTER 1:

REVIEW OF LITERATURE

1.1. INTRODUCTION

Muscle adaptation to physical training is primarily dependent upon the intensity, mode, duration, and frequency of training. For example, several weeks of endurance training at moderate work intensities [e.g., 50-70% of maximal oxygen uptake (VO_{2max})] induces enzymatic adaptations which increase oxidative energy delivery and endurance performance (Tesch and Karlsson 1985). In comparison, the central and peripheral adaptations to high-intensity, "sprint"-type exercise training have been less well studied. Sprint exercise, also referred to as anaerobic or supra-maximal exercise, is characterized by a high-intensity effort sustained for a short period of time (Abernethy et al., 1990). The maximal power output generated during a single sprint bout may be equivalent to three to four times a subject's maximal aerobic power, and is at an intensity that exceeds the capacity of the musculature to derive a majority of the energy via oxidative phosphorylation (Jacobs et al., 1987). Thus, the energy for an acute bout of sprint exercise must be primarily derived from substrate phosphorylation from phosphocreatine (PCr) hydrolysis and non-oxidative glyco(geno)lysis. For example, during a 30-sec maximal sprint bout, it has been estimated that 80% of the total energy provision is derived from non-oxidative pathways (Parolin et al., 1999).

Literature describing the effects of various sprint training regimens dates back to the early 1970's (Thorstensson et al., 1975) and since then it has been well established

that sprint training increases various performance measures such as peak power (Linossier et al., 1997; MacDougall et al., 1998; Nevill et al., 1989; Sathis et al., 1994) and mean power output, or work, during an anaerobic testing protocol (Linossier et al., 1993; MacDougall et al., 1998; Sathis et al., 1994). However, the physiological rationale for these improvements have been less well studied. In addition, the effects of sprint exercise training have been shown to extend beyond the realm of anaerobic performance and non-oxidative metabolism. Recent investigations have demonstrated increases in maximal aerobic capacity (VO_{2max}) (Davies et al., 1982; Dawson et al., 1998; MacDougall et al., 1998; Rodas et al., 2000) as well as corresponding increases in many key oxidative enzymes (Cadefau et al., 1990; MacDougall et al., 1998; Rodas et al., 2000) following sprint-exercise protocols.

Despite recent attempts to identify the underlying mechanisms responsible for such adaptations, the regulation of skeletal muscle energy metabolism during high-intensity training remains elusive. Therefore the purpose of this review is to evaluate: i) the acute physiological response to high-intensity exercise; ii) the effect of sprint-exercise training on performance; iii) the physiological adaptations to sprint-exercise performance; iv) speculative mechanisms underlying these adaptations; and v) methodological considerations in sprint training.

1.2. MUSCLE METABOLIC RESPONSE TO ACUTE HIGH-INTENSITY EXERCISE

1.2.1. Overview of skeletal muscle energy metabolism during sprint exercise

The ATP requirement during a single bout of maximal sprint-exercise is so large that there is a need for maximal rates of energy provision from all ATP sources: phosphagen hydrolysis, glycogenolysis, and oxidative phosphorylation. It has been estimated that the relative contribution from these three sources are 23-28%, 50-55%, and 16-28%, respectively, during a 30-second maximal sprint (Trump et al., 1996). Energy provision is the highest during the first 6-seconds of a maximal sprint bout, during which time the rate of ATP utilization has been estimated to be as high as 14.7 mmol • kg dry wt^{-1} • s^{-1} (Bogdanis et al., 1994; Parolin et al., 1999). There is very little ATP derived from oxidative sources during this time, with nearly equal contributions from PCr hydrolysis (7.0 mmol • kg dry wt^{-1} • s^{-1}), and glyco(geno)lysis (6.2 mmol • kg dry wt^{-1} • s^{-1}). This high rate of ATP utilizations corresponds directly with the fact that peak power is generally achieved during the initial seconds of a sprint bout (Bogdanis et al., 1998; Bogdanis et al., 1996; Hargreaves et al., 1998). However, within 15-seconds of the sprint bout PCr stores have been almost completely depleted, and many negative modulators (e.g., H^+ ions) contribute to the drastic 85-95% reduction in glyco(geno)lytic rate (Parolin et al., 1999). These decreases are paralleled by a relative increase in the rate of ATP provision from oxidative metabolism during the latter half of the 30-second sprint. Since oxidative metabolism has a much lower ATP turnover rate, a greater reliance on this pathway necessitates a lower power output compared to the onset of exercise (Bogdanis et al., 1998; Bogdanis et al., 1996; Parolin et al., 1999; Trump et al., 1996).

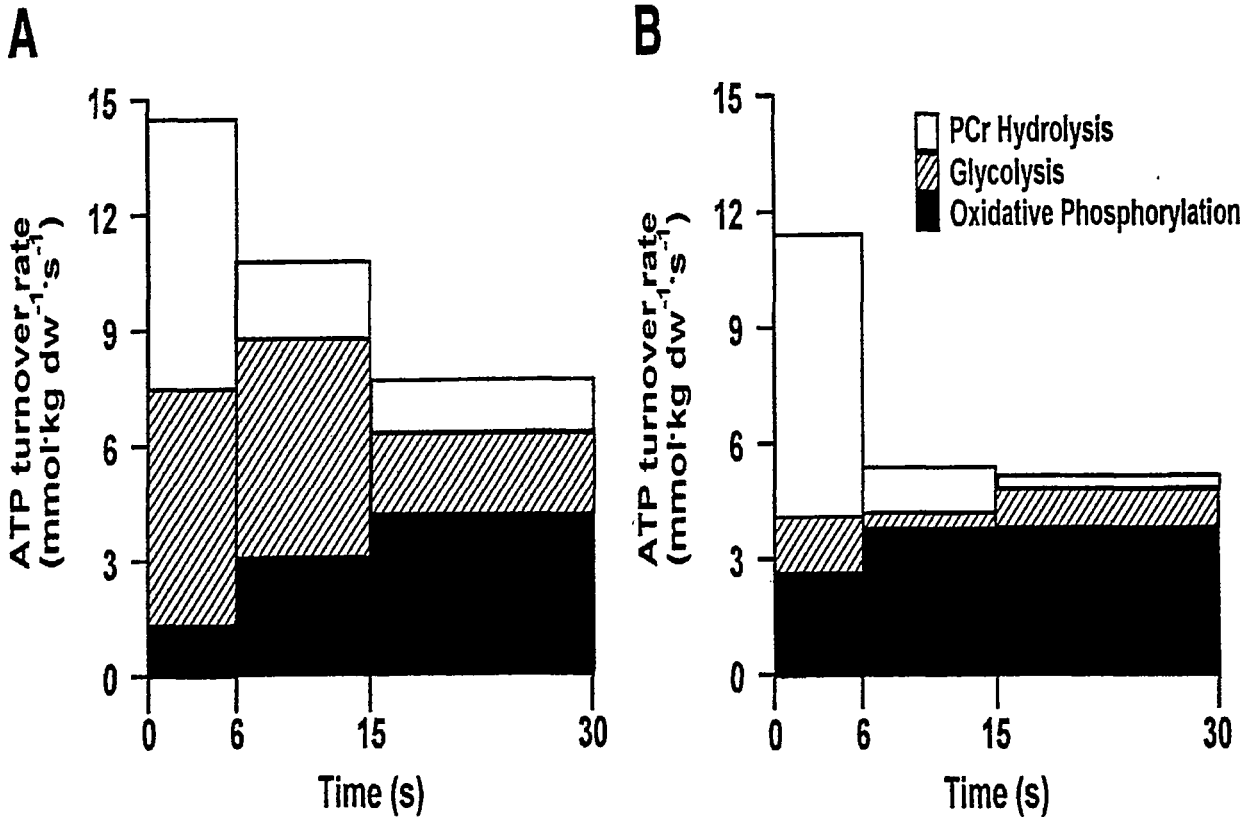

Figure 1. Schematic of relative energy contributions from PCr hydrolysis, glycolysis, and oxidative phosphorylation during first (A) and third (B) bout of maximal 30-sec sprint exercise. (Parolin et al., 1999).

1.2.1.1. Regulation of non-oxidative energy provision

The regulation of energy provision during a single bout of sprint-exercise is often hard to pinpoint since the extremely high instantaneous demand for ATP results in many concurrent metabolic perturbations. However, a couple of recent studies have employed techniques to investigate muscle metabolism during maximal sprint-exercise in the attempt to clarify the major regulatory factors associated with energy provision (Bogdanis et al., 1996; Parolin et al., 1999). Parolin et al. (1999) attempted to assess the regulation of energy provision in skeletal muscle during maximal isokinetic cycling. In response to the high energy demand of the contracting muscle, substrate level

phosphorylation by PCr degradation and glycogenolysis increased to provide rapid rates of ATP resynthesis during the initial 6 s of exercise. During these initial seconds of high intensity exercise, ADP levels begin to rise, subsequently activating creatine kinase (CK). This rapid activation of CK in response to increased [ADP] provides the initial provision of energy via PCr hydrolysis. In addition, Parolin et al. (1999) noted that within the first 15 s of cycling, glycogen phosphorylase (Phos), a key regulatory enzyme of the anaerobic energy system which is responsible for the initial breakdown of glycogen to glucose-1-phosphate, approached full activation (95%), allowing for maximal rates of glycogenolysis and pyruvate production. As the exercise bout progressed, Phos activity declined, glycogenolytic and glycolytic rates were reduced, and PCr stores became depleted. As a result, power output declined by ~38% throughout the exercise bout. Further evidence raises doubts about the regulatory significance of Phos. Significant accumulations in glucose-6-phosphate (G-6-P) and fructose-6-phosphate (F-6-P), both of which are glycolytic intermediates which are formed in the steps preceding the step catalyzed by the enzyme phosphofructokinase (PFK), are detectable at all time points during a 30-second sprint bout (Bogdanis et al., 1994; Parolin et al., 1999). An accumulation of these glycolytic intermediates suggests that there is more glycogen degradation than there is flux through the latter stages of the pathway. The inhibitory effect of an accumulation of G-6-P upon Phos appears to override positive allosteric effects (e.g., free AMP) on the flux through Phos. Although Phos may regulate the breakdown of glycogen, it seems as though PFK is the predominant rate-limiting step in anaerobic energy provision. Thus, it is clear that glycolytic flux decreases rapidly during a 30-second sprint bout in accordance with the progressive decrease observed in the

activity of key regulatory glycolytic enzymes (Bogdanis et al., 1994; Parolin et al., 1999). The plethora of positive and negative modulators acting concurrently upon these enzymes throughout a sprint bout makes it extremely difficult to define the precise regulation of this pathway.

1.2.1.2. Regulation of oxidative energy provision

Pyruvate dehydrogenase (PDH) is the enzyme that regulates oxidative energy provision during intense sprint bouts (Howlett et al., 1999; Parolin et al., 1999). PDH regulates the entry of glycolytically derived pyruvate into oxidative metabolism. The activity of this enzyme depends upon the proportion of the enzyme that is in an active form. PDH kinase catalyzes the phosphorylation of PDH resulting in inactivation, whereas PDH phosphatase dephosphorylates the enzyme concomitant with activation (Parolin et al., 1999; for a review see Weiland, 1983). During a brief bout of intense exercise, many modulating factors influence the conversion of PDH, with the majority resulting in either inhibition of PDH kinase or activation of PDH phosphatase, both of which result in a greater active fraction of PDH (Parolin et al., 1999). Parolin et al. (1999) reported that within the first 15-seconds of maximal isokinetic cycling PDH was fully active. It was hypothesized that at least 60% of the active form was stimulated by large quantities of calcium release from the sarcoplasmic recticulum at the onset of muscular contraction. Calcium is known to activate PDH by activating PDH phosphatase, and simultaneously inhibiting PDH kinase (Weiland, 1972), thus having a dual effect upon increasing the active fraction of PDH. In addition, maximal glycolytic flux at the onset of sprint-exercise results in maximal pyruvate production. Pyruvate not

only acts as the substrate for PDH, but also acts as a positive allosteric regulator stimulating the conversion to the active form (Hucho et al., 1972). Coupled with a progressive increase in hydrogen ion concentration during sprint exercise, another positive modulator of PDH phosphatase, the inhibitory effects of acetyl-CoA seem to be overridden resulting in a sustained maximal activation of PDH in the latter half of a 30-second sprint bout (Parolin et al., 1999).

1.2.1.3. Changes during repeated sprint bouts

When repeated bouts of maximal exercise are performed, the contributions from energy-producing pathways to overall energy production changes (see Fig. 1). Compared with other energy providing pathways, the contribution from PCr hydrolysis seems to be the most stable during repeated sprint bouts. Although there is between study variability, most of the variance can be attributed to inconsistent rest intervals between sprint bouts which will be addressed in a subsequent section of this review. Conversely, a marked attenuation of glyco(geno)lytic flux following the initial sprint bout (Bogdanis et al., 1996; Bogdanis et al., 1998; McCartney et al., 1986; Parolin et al., 1999; Spriet et al., 1989; Trump et al., 1996) is often the most common indication of altered energy. The energy contribution of non-oxidative glycogenolysis during the third bout of 30-second maximal cycling has been reported to be as little as 8-15% (Parolin et al., 1999; Trump et al., 1996). The reason for this marked attenuation in glycolytic flux has yet to be determined although it has been postulated that decreased glycogen stores (Hargreaves et al., 1998) and elevated [H^+] (Bogdanis et al., 1998; Parolin et al., 1999) play a prominent inhibitory role.

In order to maintain power outputs approximately 65-80% of an initial sprint bout (Bogdanis et al 1998; Bogdanis et al., 1996; Hargreaves et al., 1998) in the face of a severely decreased ATP provision from the glycolytic pathway, and a relatively constant contribution from PCr hydrolysis, oxidative phosphorylation is much more heavily relied upon for ATP resynthesis in subsequent bouts. Bogdanis et al. (1994) estimated that the contribution from aerobic energy pathways increased by 14% during the second of two 30-second sprints. The same lab group later replicated these findings and suggested that an observed mismatch between anaerobic energy provision and power output during the second 30-second sprint was partly compensated for by an increased contribution of aerobic metabolism as reflected by the 18% increase in VO_2 (Bogandis et al., 1996). Putman et al. (1995) observed a more modest, yet still significant, increase of 10% in VO_2 during the third bout of sprint exercise relative to the first bout. These results were mirrored by a 10% increase in the rate of ATP provision from oxidative phosphorylation in a later study from the same lab (Parolin et al., 1999). Parolin et al. (1999) attribute this elevation in ATP provision from oxidative metabolism to a much higher resting active fraction of PDH prior to the third sprint relative to the first. In fact, the active fraction of PDH was threefold higher before the third bout, and reached full activation only 6-seconds into the sprint. It was also proposed that the higher resting activation of PDH would provide increased levels of acetyl-CoA, thus providing increased substrate for oxidative phosphorylation during the initial 6-seconds of exercise.

1.2.1.4. Influence of recovery between sprint bouts on metabolic regulation

Recovery intervals between repeated sprint bouts allow the muscle to resynthesize metabolites and replenish energy stores that have been depleted concomitant with the removal of byproducts that have contributed to muscular fatigue. PCr resynthesis has been shown to occur very rapidly following intense sprint-exercise. Following a 2-minute recovery from a 20-second sprint, PCr levels were 76% of pre-exercise rest values (Bogdanis et al., 1998). Zhao et al. (2000) reported a complete recovery of PCr stores after 5 min of recovery from 30-seconds of sprint cycling. Subsequently, the recovery of PCr has been highly correlated with peak power outputs during repeated sprint bouts (Bogdanis et al., 1998; Bogdanis et al., 1996; Trump et al., 1996; Zhao et al., 2000). Trump et al. (1996) demonstrated this correlation using an occlusion model whereby a pressure cuff was used to occlude the blood flow to one leg while the circulation to the other leg remained in tact. The cuff was successful in significantly reducing PCr resynthesis during the 4-minute recovery interval prior to the third 30-second sprint cycle bout. Power output and total work was subsequently reduced in the cuffed leg during the third sprint bout with 70% of the reduction in total work present after only 15-seconds. The influence of PCR is strengthened by the fact that glycogenolysis contributed very little to ATP provision in either leg, subsequently supporting the correlation between PCr levels and power output while highlighting the importance of recovery intervals to PCr resynthesis. Bogdanis et al. (1995) used varying rest intervals and demonstrated a striking similarity between the time course of peak power recovery during a 30-second sprint test and the extent of PCr resynthesis.

Although recovery intervals of varying duration seem to promote an increase in PCr concentration, there seems to be no effect upon [H+] (Bogdanis et al., 1996; Bogandis et al., 1995). Despite a significant decrease in muscle lactate with as little as 3 minutes recovery following an intense sprint bout (Bogdanis et al., 1994), there is no change in muscle pH upon the cessation of exercise following as much as 5 minutes of recovery (Bogdanis et al., 1998; Bogdanis et al., 1996; Bogdanis et al., 1995). The inability of the muscle to decrease [H+], and subsequently increase muscle pH, during recovery periods may play a large role in the significantly reduced glycogenolytic flux in later sprints due to the inhibitory effects of hydrogen ions on key glycogenolytic enzymes (e.g., Phos).

Relatively brief rest intervals ($\leq$ 5-min) have also been shown to have negligible effects upon resting muscle glycogen content (Bogdanis et al., 1998; Bogdanis et al., 1996; Bogdanis et al., 1998; Hargreaves et al., 1998). This lack in glycogen resynthesis following a sprint bout results in a lower starting glycogen content for subsequent sprint bouts, and although the literature is inconclusive, there remains the possibility that less glycogenolytic substrate may contribute to the observed reduction in glycogenolytic ATP provision in the latter bouts of sprint exercise (Hargreaves et al., 1998)

1.3. EFFECT OF SPRINT-EXERCISE TRAINING ON PERFORMANCE

There is abundant evidence to suggest that the implementation of sprint training can significantly augment many performance measures. Although the majority of the literature has investigated the effects of high-intensity training upon anaerobic

performance parameters, recently there has been a vested interest in the potential aerobic performance gains following such protocols. Irrespective of potential physiological adaptations, observed elevations in performance provide a very practical rationale for the implementation of sprint training regimens in the realm of athletics.

1.3.1. Changes in "anaerobic" performance

The most common finding in the literature is an elevation in peak power and in mean power during a single high-intensity sprint bout following sprint training protocols (Esbjornsson et al., 1996; Harmer et al., 2000; Linossier et al., 1993; MacDougall et al., 1998; Nevill et al., 1989; Parra et al., 2000; Stathis et al., 1994). The increase in peak power for these studies ranged from 6-26%, whereas the range for the increase in mean power was 6-16%. Recent work has showed that 5 consecutive days of sprint exercise increased peak and mean power outputs by 17 and 10% respectively (Hughes et. al., 2002). Although power outputs are the most common indicator of improved anaerobic performance, Dawson et al. (1998) took a unique approach and analyzed anaerobic performance using time trials. Following a 6-week sprint training protocol, subjects completed a 10m and 40m sprint, a supra-maximal treadmill run to exhaustion (14 km/h, 20% gradient), and a repeated sprint test (6 x 40m sprint, 30-sec rest between each). It was found that both the 40m sprint time and the repeated sprint time significantly decreased, and the time to exhaustion in the supra-maximal run was significantly elevated, thus providing strong evidence for an effect of training in enhancing performance parameters.

The literature is not without inconsistencies. Rodas et al. (2000) trained subjects everyday for two weeks (14 total training session), with the total load increasing progressively throughout the training period. They found that peak power and mean power during a 30-sec "all-out" cycle test were unaltered. However, it must be taken into account that the 30-sec performance test was conducted immediately following the training period, and it was documented that during the last three training sessions pedaling rate and power outputs during the sprint bouts had begun to decline. This suggests that muscular fatigue could have played a major role in the absence of a training benefit. Two other investigations concluded that 6 weeks of sprint training does not elicit improvements in peak power or mean power during a single high-intensity bout cycling (Hellsten-Westing et al., 1993; Jacobs et al., 1987). Similarly, Esbjornsson et al. (1996) found no increases in peak or mean power following a 4-week sprint protocol in 6 male subjects, despite finding an elevation in the 10 female subjects following training.

MacDougall et al. (1998) were one of the first to use a repeated sprints (four 30-sec maximal cycling bouts separated by 4-min recovery) to assess power adaptations following sprint training. Although differences in power output were not significant for the first exercise bout, in each of the following three bouts, both peak power and mean power were significantly higher after training. This raises the possibility that if those studies which did not report an increase in power outputs following training had implemented a repeated sprint test, a possible enhanced power output may have been detected in the subsequent sprints. This speculation is supported by the fact that the subjects in the study by Jacobs et al. (1987), had conveyed that their feelings of nausea and exhaustion that they experienced following the pre-training sprint bout were not

existent in the post-training test. This lead to the subjective notion that there was an enhanced ability to perform repeated bouts of sprints, however this remains speculative as nothing was reported.

1.3.2. Changes in "aerobic" performance

Contrary to the vast publications documenting anaerobic performance changes, only recently has there been an interest in the changes in aerobic performance parameters secondary to sprint-exercise training. Until very recently, the lone indicator of aerobic performance had been a subject's peak oxygen uptake (VO_{2peak}), usually assessed via an incremental exercise test until exhaustion. Many authors have concluded that a strictly anaerobic training protocol can result in significant augmentations to maximal oxygen consumption and maximal aerobic power, with the percent increase ranging from 3% to 10% (Dawson et al., 1998; Harmer et al., 2000; Hughes et. al., 2002; Linossier et al., 1997; MacDougall et al., 1998; Rodas et al., 2000). Although VO_{2peak} gives a good indication of maximal aerobic capacity, surprisingly only one study has investigated the effects of sprint-exercise training on sub-maximal aerobic performance. Hughes et al. (2002) used a ride to exhaustion on a cycle ergometer at approximately 90% of the subject's previously determined VO_{2peak}, which was used as a measure of sub-maximal aerobic performance. It was found that only five sprint-exercise training bouts resulted in a substantial 21% increase in the subject's cycle time to exhaustion, thus rendering the conclusion that sprint training is capable of inducing significant increases in intense sub-maximal aerobic performance.

Similar to the inconsistencies in anaerobic performance measures, augmentations in aerobic performance parameters also lacks congruency. Although Stathis et al. (1994) found an increase of 4.1% in VO_{2peak} following a 7-week sprint training protocol, it was not deemed significant. When this protocol is juxtaposed with that of MacDougall et al. (1998), the similarities are vast. Both used a 7-week sprint exercise protocol whereby subjects trained 3-times per week, and the progressive increase in training load was almost identical between protocols. However, in the study by Stathis et al. there was a greater time between the post training assessment of VO_{2peak} and the final training bout, thus raising the possibility that the training stimulus was diminishing, and detraining had begun to set in. Linossier et al. (1993) also documented no change in VO_{2peak} following a sprint training regimen. However, this training protocol consisted of a series of 5-second maximal cycle sprints, which is much shorter in duration than the majority of other sprint-exercise protocols. Even in a series of 5-second sprints, oxidative energy provision will be extremely minimal, and this lack of stress upon the oxidative energy pathways during training may be the reason for a lack of change in peak oxygen consumption.

1.3.3. Effect of training duration

The duration of a sprint-exercise training protocol seems to have very little effect upon many performance parameters. Peak and mean power outputs during a single supra-maximal sprint bout have been shown to be increased following as much as 8-weeks of sprint training (Nevill et al., 1989), and as little as 1-week of sprint training (Hughes et al., 2002). Conversely, both 6-weeks of training (Jacobs et al., 1997) and 2-

weeks of training (Rodas et al., 2000) resulted in no change in anaerobic performance, further confounding the issue of training duration.

Upon investigation into aerobic performance adaptations, VO_{2peak} has been shown to be significantly elevated and not altered (Stathis et al., 1994) following two very similar 7-week training protocols (MacDougall et al., 1998). Although there have been only two short-term sprint training studies that have investigated aerobic performance, it is interesting to note that in response to 1-week (Hughes et al., 2002) and 2-weeks (Rodas et al., 2000) of sprint training, all measures of aerobic performance were significantly increased. As well, regardless of the performance measure, there seems to be no correlation between the duration of the training program and the magnitude of change. These inconsistencies suggest that methodological differences, other than training duration, have a more pronounced influence upon performance augmentations following sprint-exercise training.

1.3.4. Effect of recovery between training bouts

A methodological difference between studies that seems to play a major role in performance adaptations following sprint training is the amount of recovery that is allotted between training bouts. Parra et al. (2000) addressed this issue by devising a study that included two training regimens with the same muscle load (i.e., 14 exercise sessions in each trial), but a different distribution of rest periods. One group trained everyday for 2 weeks, whereas the alternate group had a two-day rest between exercise bouts, thus training for a period of 6 weeks. Although enzymatic and substrate

adaptations will be addressed in subsequent sections of this review, it is worthy to note that similar increases were found in a majority of major glycolytic enzymes between the groups; however, anaerobic performance measures were only increased in the 6-week training regimen. The novelty of this study was that the authors were able to compare adaptations to disparate rest intervals within an individual study. Thus, by limiting extraneous variables, the author's conclusion, that differing rest intervals in a sprint training protocol can sufficiently augment performance measures in an anaerobic test, is more powerful. It was speculated that the constant muscular demands of training everyday without sufficient recovery resulted in muscular fatigue which subsequently masked any potential performance increases.

It also seems that rest intervals between the final training bout and the assessment tests influence performance both anaerobic and aerobic in nature. Rodas and colleagues (2000) did not demonstrate an increase in power outputs during a 30-sec maximal cycling test, however, they did find that maximal aerobic power output was 10% higher following training. In this study, the anaerobic test was conducted one day after the last training bout, whereas the VO_{2max} test was conducted five days thereafter, thus suggesting that the five days between assessment tests allowed sufficient muscular reprieve and ultimately, enhanced performance. Another indication that a rest period is needed prior to an assessment test was demonstrated by Hellsten-Westing et al. (1993) who measured peak 5-sec power output 24-hours and 72-hours following 1-week of intensive sprint training. Once again, peak power was only significantly elevated in the sprint bout conducted 72-hours following training further supporting the notion that without adequate rest, muscular fatigue may mask performance augmentations.

1.4. PHYSIOLOGIAL ADAPTATIONS TO SPRINT-EXERCISE TRAINING

As previously discussed, sprint-exercise training often results in an array of performance adaptations. Because of this, sprint training is often used in the physical preparation of athletes for many sports; however, the physiological changes associated with these performance augmentations remain very controversial. From a mechanistic point of view, it is not sufficient to demonstrate adaptations in performance measures without rationalizing the physiological basis for the observed changes.

1.4.1. Energy metabolites

Consistent changes in the resting concentrations of key muscle metabolites in response to sprint-exercise training would provide a solid foundation to develop a physiological rationale for improved performance. In response to sprint training protocols of at least 6 weeks, there is no change in PCr stores (see Table 1). However, in response to two recent short-term training protocols, whereby subjects trained everyday for only 2 weeks, PCr stores were elevated by 31% and 39% (Parra et al., 2000; Rodas et al., 2000), thus raising the possibility that the augmentation of PCr stores is dependent upon the duration of training. Similarly, changes in the concentration of resting muscle glycogen are consistent only in short-term training studies where the increase was 32% following both training sessions (Parra et al., 2000; Rodas et al., 2000). Although increases of similar magnitude were documented following some long-term training protocols, two studies have reported no significant change in resting muscle glycogen

thereby providing inconsistencies in response to training protocols ⩾6 weeks (see Table 1). Conversely, the two short-term training studies did not demonstrate a significant change in resting ATP levels following training which corresponds well with the majority of longer term training protocols (see Table 1). Once again, the literature is not without exceptions. Stathis et al. (1994) found a significant decrease (19%) in resting ATP concentration following 7 weeks of sprint-exercise training. It was proposed that sprint training resulted in elevated IMP catabolism leading to enhanced inosine and hypoxanthine production. These two byproducts could then diffuse from the muscle ultimately resulting in a loss of purine nucleotides. This theory is further supported by the work of Bangsbo et al. (1992) who demonstrated a greater hypoxanthine efflux from muscle when intense exercise is repeated.

As Ross and Leveritt (2001) have emphasized, the influence of sprint training on metabolite concentrations is difficult to determine without careful control of dietary intake several hours prior to biopsy extraction. Without strict control of this, and many other methodological concerns, the validity of the conclusions drawn in future research may be undermined.

REFERENCE	WEEKS OF TRAINING	PCr	GLYCOGEN	ATP
Harmer et al. (2000)	7	→	→	→
Parra et al. (2000)	6	→	↑	→
Dawson et al. (1998)	6	→	n/d	→
Stathis et al. (1994)	7	→	n/d	↓
Linossier et al. (1993)	7	→	n/d	→
Cadefau et al. (1990)	32	n/d	↑	n/d
Nevill et al. (1989)	7	→	→	→

Table 1. Summary of changes in key energy metabolites in response to sprint training protocols ⩾6 weeks. PCr, phosphocreatine; ATP, adenine triphosphate. n/d: not determined.

1.4.2. Enzymes

Enzymatic adaptations are central to our understanding of the physiological effects of sprint training. These adaptations form the basis for many fundamental assumptions regarding metabolic mechanisms that underlie training induced enhancements in performance. Much of the recent literature has focused on, or at least taken into account, alterations in enzyme function in response to training. Although it makes intuitive sense that training which stresses the anaerobic pathways has the potential to increase glycolytic enzyme levels, Saltin et al. (1976) further confounded the issue of sprint-exercise training by demonstrating increased levels of oxidative enzymes secondary to anaerobic training. Thus, subsequent literature has not only focused on glycolytic enzyme adaptations but oxidative enzymes as well. Contrary to the relatively congruent enzymatic changes associated with endurance training, the glycolytic and oxidative enzyme adaptations to sprint training remain equivocal.

REFERENCE	WEEKS OF TRAINING	CK	PFK	CS
Parra et al. (2000)	6	→	↑	↑
Dawson et al. (1998)	6	n/d	→	↓
MacDougall et al. (1998)	7	n/d	↑	↑
Linossier et al. (1997)	9	→	↑	→
Linossier et al. (1993)	7	n/d	↑	→
Cadefau et al. (1990)	32	→	↑	n/d
Jacobs et al. (1987)	6	→	↑	↑

Table 2. Summary of changes in key regulatory enzymes in response to sprint training protocols ⩾6 weeks. CK, creatine kinase; PFK, phosphofructokinase; CS, citrate synthase. n/d: not determined.

1.4.2.1. Glycolytic enzyme adaptations

It has been almost completely congruent in the literature that maximal phosphofructokinase (PFK) activity is elevated in response to sprint training protocols of at least six weeks (see Table 2). Likewise, sprint training regimens of less than six weeks have also elicited substantial increases in maximal PFK activity (Parra et al., 2000; Roberts et al., 1982; Rodas et al., 2000). The observed elevations in PFK are congruent with most researchers hypotheses that increases in glycolytic enzyme activities will parallel the increases seen in anaerobic performance measures. From a mechanistic standpoint, this makes intuitive sense. However, in contrast to the relatively conclusive evidence supporting PFK adaptations, training induced changes in other major glycolytic enzymes are inconsistent.

Costill et al. (1979) were one of the first to demonstrate a training induced increase in glycogen phosphorylase (PHOS). Roberts et al. (1982) subsequently supported these findings by also demonstrating a significant elevation (~100%) in PHOS activity. Conversely, in a study whereby the rest intervals during a training program were altered, it was found that PHOS activity was not significantly altered in either group. In fact, the training protocol that consisted of training everyday for 2-weeks tended to suppress PHOS activity (Parra et al., 2000). Supporting evidence of negligible PHOS changes comes from MacDougall et al. (1998) further confounding this issue of training induced changes in PHOS activity, despite similar training protocols. In addition, changes in maximal lactate dehydrogenase (LDH) activity are also very controversial in nature. LDH has been shown to both increase following at least six weeks of training

(Linossier et al., 1997; Linossier et al., 1993), and remain relatively constant (Cadefau et al., 1990; Hellsten et al., 1996; MacDougall et al., 1998; Parra et al., 2000). However, in sprint regimens of less than six weeks, LDH activity is consistently elevated (Parra et al., 2000, Roberts et al., 1982; Rodas et al., 2000). Parra et al. (2000) nicely demonstrate the disparity in LDH adaptations. In this study, the group that had 14 training bouts over 2 weeks showed a significant rise in LDH activity, whereas those who completed 14 bouts over a 6-week period showed no increase.

1.4.2.2. Oxidative enzyme adaptations

In 1976, Saltin discovered an increased maximal succinate dehydrogenase (SDH) activity secondary to anaerobic training concomitant with an increased VO_{2max}. In light of these findings, subsequent studies have investigated the effects of sprint training on alternate mitochondrial oxidative enzymes (e.g., malate dehydrogenase, citrate synthase). At first the literature seems to be extremely controversial with regard to oxidative enzyme adaptations following sprint-exercise training protocols. However, upon further investigation, if the training regimens are grouped into those which use sprint bouts lasting 30-sec and those which use sprint bouts lasting 10-sec or less, the enzyme adaptations become much easier to explain. Cadefau et al. (1990) was the only study using sprint bouts of 10-seconds or less to report an increase in any of the major oxidative enzymes. However, it must be taken into account that the implemented training protocol was not entirely anaerobic in nature. Although sprint exercises of <10-s comprised the majority of the training regimen, during the 8 month training protocol, runs relying on the aerobic system were also implemented. Thus, the increase in SDH activity was more

than likely the result of the aerobic work, rather that the maximal sprint efforts. All other studies using brief sprint bouts have reported no change in any measured oxidative enzyme (Dawson et al., 1998; Hellsten et al., 1996; Linossier et al., 1997; Linossier et al., 1993), and in the case of Dawson et al. (1998) there was a significant decrease in maximal citrate synthase (CS) activity.

The conclusions based upon studies using sprint bouts of at least 30-seconds are in stark contrast to those using 10-second sprint protocols. Interestingly, oxidative enzyme adaptations in response to 30-s sprint training regimens have been much more consistent than glycolytic enzyme changes. In fact, increases in CS (Jacobs et al., 1987; MacDougall et al., 1998; Parra et al., 2000), and MDH (Costill et al., 1979; MacDougall et al., 1998; Roberts et al., 1982) are without conflicting evidence. Only Roberts et al. (1982) showed no changes in maximal SDH due to training whereas the majority of the literature supports the conclusion of increased SDH activity (Cadefau et al., 1990; Costill et al., 1979; MacDougall et al., 1998; Saltin, 1976).

The obvious correlation between sprint duration and oxidative enzyme changes renders the assumption that if oxidative energy provision is not utilized during training, the oxidative potential of the cell will not be altered. Conversely, despite being predominantly anaerobic in nature, the gradual shift in reliance to oxidative energy provision during repeated 30-second sprints provides enough of a stimulus to increase the maximal activity of various oxidative enzymes.

1.4.3. Substrate utilization during exercise

When assessing metabolism at the level of the muscles, pre and post-training test methodologies become a concern. Since substrate metabolism is driven by the ATP (or energy) demand placed on the muscle, training induced alterations should be assessed by keeping the muscle demand the same in both the pre and post-training tests. However, many studies have used relative pre and post-test protocols whereby the absolute amount of work being done varies if performance measures are changed. These tests are adequate when assessing maximal performance measures (e.g., peak power); however they hinder the ability to draw conclusions regarding substrate reliance.

Since the majority of the literature has focused on substrate utilisation during highly intense testing protocols, the exclusive dependence upon the glycolytic pathway has undermined the need to research changes in the contributions of lipids or amino acids to energy provision. Thus, the scope of the present review will not include lipid or amino acid metabolism; rather, the focus will centre on adaptations in carbohydrate metabolism.

1.4.3.1. High-intensity exercise

Although glycogen is by far the most predominant substrate used in any of the aforementioned sprint training protocols, the rate and extent to which it is metabolized has vast repercussions on performance measures. It has previously been shown that sprint-trained athletes tend to have higher muscle and blood lactate concentrations after high-intensity exercise than endurance-trained athletes (Costill et al., 1983). Although

this may be attributed, in part, to the greater proportion of type II muscle fibres in sprint-trained athletes, enzymatic adaptations following sprint-exercise training suggest an increase in glycolytic capacity. Nevill et al. (1989) demonstrated a 20% increase in energy production from anaerobic glycolysis during a 30-s sprint assessment test following sprint-exercise training. These observations were subsequently supported by Linossier et al. (1993), who also reported a greater ATP resynthesis from anaerobic glycolysis concomitant with elevated performance in a 30-s sprint test. However, the elevated contribution of energy provision from this pathway is likely due to the lack of work homogeneity between the compared tests.

In a more controlled study, whereby assessment measures were taken from maximal tests and work-matched tests, conflicting conclusions were drawn (Harmer et al., 2000). When work was held constant in the pre and post-tests (work-matched test:130% of pre-training VO_{2max} for a matched time period), an observed decrease in glycogen degradation indicated attenuated glyco(geno)lytic rates following training. In fact, when the post-training test continued to exhaustion (21% increase in exercise time), glycogen degradation, and anaerobic ATP production were unchanged compared to the pre-training test. Consequently, anaerobic ATP production rate (production/time) was approximated as being 25% lower subsequent to 7-week sprint training regimen. This conclusion was also indirectly drawn by Dawson et al. (1998). They found that blood lactate was lower during a 2-minute supramaximal run (110% of pre-VO_{2peak}) following a 6-week sprint training protocol. A lower blood lactate concentration led to the assumption that flux through lactate dehydrogenase was decreased as a result of attenuated glycol(geno)lysis. These work-matched analyses indicate that aerobic

adaptations to sprint training may play a larger role in elevated performance measures than was previously hypothesized. This inference is made because it is known that the exercise intensity elicited is too high to use fat as a fuel, thus carbohydrate, or glycogen, is the predominant fuel. In the face of a constant ATP demand, a lower glycol(geno)lytic rate suggests that more ATP is being produced for a given amount of glycogen. Thus, it can be deduced that a greater proportion of pyruvate is shunted into the mitochondria for oxidative phosphorylation since this pathway is known to produce a much greater ATP:glucose ratio than the opposing anaerobic pathway.

1.4.3.2. Prolonged moderate exercise

Although one study has investigated the effects of sprint-exercise training upon prolonged moderate exercise performance (Hughes et al., 2002), no study has explored the subsequent changes in substrate utilization.

1.4.4. Fibre type changes

Studies investigating fibre type changes associated with sprint-exercise training are extremely inconsistent in their findings. Type IIA fibre proportion has been shown to be increased by 8% (Jacobs et al., 1987) and 10% (Dawson et al., 1998) concomitant with a decrease in % type I proportion in response to separate 6-week sprint training protocols. Jansson et al. (1990) supported these findings by demonstrating a significant increase in % type IIA area. This fibre transition seems to make intuitive sense since sprint-trained athletes tend to have higher type II fibre proportions compared to their endurance-trained

counterparts (Costill et al., 1976). Paradoxically, Simoneau et al. (1985) observed that high-intensity intermittent training induced higher proportion of slow twitch (type I) fibres, when expressed both as a percentage and percentage area following a sprint training regimen. These results were subsequently replicated by further studies which also demonstrated an elevated type I fibre percentage together with a paralleled decrease in the percentage of type IIB fibres (Cadefau et al., 1990; Linossier et al., 1993).

The lack in congruency regarding muscle fibre type adaptations may, in part, be due to the vast methodological concerns that must be taken into account throughout the duration of the study. The huge disparity in many of the training protocols (e.g., sprint duration, rest intervals, etc.) may heavily influence fibre transition. Also, variability in muscle biopsy techniques and staining techniques, both within study and between study, may give a skewed interpretation of fibre proportion. Although controlling for all of these variables is extremely difficult, a sound effort is needed in order to clarify this issue.

1.5. POTENTIAL MECHANISMS INVOLVED WITH ADAPTIVE PHYSIOLOGICAL RESPONSE TO SPRINT EXERCISE TRAINING

5.1. Muscle metabolic mechanisms

1.5.1.1. Changes in pyruvate production and/or oxidation

Increases in many key glyco(geno)lytic and oxidative enzymes following sprint training could potentially result in a training induced augmentation in pyruvate

production, and the rate at which it is oxidized. The increased oxidative potential of the cell following sprint training raises the possibility of a better match between pyruvate production and oxidation similar to the effects following endurance training. However, the regulation of the energy pathways remains elusive as the extreme energy demand placed on the muscle during maximal sprint bouts generally exceeds the capacity of the muscle to derive energy via oxidative pathways, despite the enhanced oxidative capacity of the cell.

1.5.1.1.1. Regulation of glycolysis and glycogenolysis

Glyco(geno)lytic flux has been shown to be both attenuated, and elevated, following sprint-exercise training depending on the intensity and duration of the assessment test that is performed. It seems relatively congruent that the rate of glyco(geno)lysis is enhanced following training when a short, maximal sprint bout is performed (Jacobs et al., 1987; Linossier et al., 1993; MacDougall et al, 1998; Nevill et al., 1989). These increases are directly correlated with improvements in anaerobic performance, and suggest that sprint performance prior to training, are limited by the capacity of the muscle to derive ATP anaerobically. Alternatively, it has been directly shown (Harmer et al., 2000) and inferred through lactate analysis (Dawson et al., 1998; Nevill et al., 1989) that glycogen degradation is attenuated during work-matched assessment tests. Harmer et al. (2000) reported less glycogen degradation concomitant with depressed lactate accumulation during a cycle ride at 130% of VO_{2peak}. The major regulatory factor contributing to this attenuation was speculated as being a decreased ATP degradation at the onset of exercise. This results in a lower free AMP concentration which does not promote the activity of glycogen phosphorylase. Although the regulation

of PDH will be discussed in the following section, it was further speculated that even if PDH activity is unchanged following training, a slower rate of pyruvate presentation due to an attenuated glycolytic flux will permit a greater proportion to be oxidized. This in turn will constitute a significant energetic advantage following training.

The capacity of sprint training to elicit increases in both glycolytic potential and oxidative potential allows for several independent performance adaptations. Elevated glycolytic flux would be beneficial when attempting to maintain high power outputs for a short period of time, whereas glyco(geno)lytic attenuation is beneficial when attempting to avoid fatigue during a prolonged, intense exercise.

1.5.1.1.2. Regulation of pyruvate dehydrogenase

As previously discussed, many authors have demonstrated increases in oxidative enzymes in the tricarboxylic acid (TCA) cycle (e.g., citrate synthase, malate dehydrogenase, succinate dehydrogenase) following various sprint training protocols (Costill et al., 1979; Jacobs et al., 1987; MacDougall et al., 1998; Parra et al., 2000; Roberts et al., 1982). Conversely, no studies have examined changes in the maximal activity of PDH following sprint training. Thus, one possible explanation for the consistently observed increases in TCA cycle enzymes could be attributed to an increase in the maximal activity of PDH. An increase in the catalytic activity of PDH would likely result from exposure to consistently elevated levels of pyruvate during sprint-exercise training, whereas an increase in mitochondrial density following training would suggest a greater total PDH concentration. Although speculative, these potential adaptations could subsequently result in an increased pyruvate oxidation and flux through the TCA cycle, thus serving as a stimulus for upregulation of TCA cycle enzymes.

It has been found that the active fraction of PDH is elevated following one sprint bout and remains elevated during a repeated sprint regimen (Parolin et al., 1999). Thus, augmenting the activity of PDH following sprint training may allow for excess pyruvate that is formed during intense sprint activities to enter the mitochondria during the recovery phase of exercise. Many sprint training protocols employ resting phases of ~4 min between each successive bout, which may provide an adequate amount of time for the conversion of pyruvate to acetyl-CoA. Acetyl-CoA then serves as the substrate for TCA cycle flux resulting in the activation of mitochondrial enzymes and ultimately enhancing the oxidative potential of the cell.

1.5.1.2. Intramuscular lactate "handling"

The extreme demand placed on the muscle during sprint exercise results in many metabolic perturbations that have a detrimental effect upon ATP provision. High-intensity exercise generally elicits an imbalance between pyruvate production and oxidation concomitant with large accumulations of lactate. Although lactate is generally deemed a source of fatigue during high-intensity exercise, several studies have found that even in the presence of elevated muscle lactate concentrations following training, performance is still enhanced (Jacobs et al., 1987; Linossier et al., 1993; Nevill et al., 1989). This suggests that a potential augmentation to training is an enhanced capability of the muscle to "handle" excess lactate production through either a more efficient transport system, or an enhanced intramuscular buffering capacity.

1.5.1.2.1. Lactate transport

Recent evidence suggests that a family of monocarboxylate transporters (MCT's) are responsible for the transport of lactate across and within the muscle cell (Bonen et al., 2001). Although several MCT isoforms have been identified, two play a prominent role in lactate kinetics in the muscle. MCT1 seems to be responsible for the uptake of lactate from the circulation, whereas MCT4 seems responsible for lactate extrusion from the muscle (Bonen et al., 2001). Many authors have demonstrated an increase in the expression of these transport proteins secondary to training regimens of various durations and intensities (Bonen et al., 1998; Green et al., 2002; Pilegaard et al., 1999). These findings may help explaining the results of Harmer et al. (2000), who demonstrated that following 7-weeks of sprint training, subjects were able to significantly increase their time to exhaustion during a cycle ride at 130% of their VO_{2peak}. Upon metabolite analysis, it was found that despite the greater total exercise time post training, muscle lactate was the same, whereas plasma [lactate] was found to be higher. This suggests that there was a greater total lactate production following exercise, which corresponded with a greater time to fatigue; however, this was counter-balanced by an increased lactate extrusion from the muscle. Similar findings were found by Rodas et al. (2000) who documented an increased blood [lactate] together with a decreased intramuscular [lactate] during the recovery period of a 30-second sprint test following training.

An understanding of lactate kinetics in the muscle would be extremely beneficial in the attempt to develop a physiological rationale for performance enhancements following sprint training. However, future research is needed to clarify the characteristics

and contributions of the independent MCT isoforms and their respective responses to training regimens.

1.5.1.2.2. Muscle buffering capacity

Results from a couple of studies have supported the possibility that sprint-exercise training may elicit changes in the buffering capacity of skeletal muscle (Harmer et al., 2000; Nevill et al., 1989). During a 30-seconds sprint assessment test following an 8-week sprint training protocol, Nevill et al. (1989) demonstrated a 12% increase in peak power and a 6% increase in mean power concomitant with an elevated muscle lactate accumulation. These results suggest a greater anaerobic contribution to energy provision however, these changes occurred without a change in $[H^+]$. Harmer et al. (2000) also demonstrated an increased lactate accumulation concomitant with a decreased hydrogen ion concentration at the cessation of an exercise ride to exhaustion at 130% of the subject's VO_{2peak}. Subsequently, it has been suggested by Nevill et al. (1989) that a possible mechanism for an altered buffering capacity could be a change in membrane potential caused by a constant exposure to elevated $[H^+]$ during training. A decreased membrane potential would allow for greater $[H^+]$ efflux during exercise, thus attenuating the decrease in muscle pH and ultimately muscular fatigue. This theory is supported by Sjogaard et al. (1983) who demonstrated a greater decrease in blood pH after a 30-second sprint test following training, suggesting an increased $[H^+]$ efflux.

Regardless of the mechanism, an increased buffering capacity of skeletal muscle following training would allow for a greater contribution from anaerobic glycolysis to energy supply for a given decrease in muscle pH.

1.5.2. Non-metabolic mechanisms

1.5.2.1. Cardio-respiratory response to acute sprint exercise

The majority of studies that have assessed the changes occurring during an acute sprint bout have focused on the intramuscular metabolic adaptations, thus research centered on the cardio-respiratory responses to such exercise is scarce. However, a study by Bogdanis et al. (1996) has probably been the most informative and detailed with respect to cardio-respiratory changes during a single 30-second sprint cycling bout. It was found that the average VO_2 during the bout was 61% of the subjects VO_{2peak}. However, when the time course of VO_2 was analyzed it was found that VO_2 was 85% of peak during the final 20-seconds of the bout suggesting a relatively rapid increase in VO_2 after 10-seconds of sprinting. This corresponds well with the fact that oxidative metabolism is much more heavily relied upon during the latter stages of a sprint bout.

Oxygen uptake and other cardio-respiratory measures such as ventilation and cardiac output require further investigation as their implications during a single bout of maximal sprint-exercise remain unknown. Highlighting the potential importance of such investigations is the correlation found between maximal aerobic capacity, rate of PCr resynthesis and the ability to maintain power output during repeated sprint bouts (Bogdanis et al., 1996).

1.5.2.2. Plasma volume expansion

The somewhat paradoxical finding of an increased maximal aerobic capacity following a sprint training regimen concomitant with negligible changes in the maximal

activity of oxidative enzymes (Dawson et al., 1998; Linossier et al., 1997) raises the possibility that factors other than those within the muscle may underlie aerobic performance adaptations. One potential explanation for these findings may be adaptations in cardiovascular parameters such as plasma volume. An increase in plasma volume could theoretically increase oxygen delivery and perfusion pressure at the muscle ultimately enhancing oxygen uptake by the muscle. There is evidence to suggest that intense supra-maximal cycling serves as a potent stimulus for the expansion of plasma volume (Gillen et al., 1991; Green et al., 1984). Green et al. (1984) concluded that three sessions of supra-maximal sprinting increased plasma volume by 12%, a finding that was later supported by Gillen et al. (1991) who demonstrated a 10% increase following a single session of high-intensity intermittent exercise. Despite this evidence, it remains unclear whether increases in plasma volume necessitate an increase in oxygen utilization at the muscle level. In support of this correlation, Coyle et al. (1990) attempted to determine the effect of a hypervolemic state upon VO_{2max} and sub-maximal cycling performance. The results indicated that an artificially induced 300mL elevation in plasma volume significantly increased stroke volume by 15%. Although direct cause and effect relations were not drawn, significant increases in VO_{2max} and sub-maximal cycling performance were also reported. Evidence suggests that cardiovascular measures such as plasma volume may be augmented following sprint-exercise training; however the direct implications upon aerobic performance parameters remain elusive.

1.5.2.3. Neuromuscular adaptations

Although the primary interest of the present author is the metabolic changes associated with sprint training; changes in neuromuscular characteristics following sprint-exercise training may broaden the understanding of performance augmentations. For example, the ability to recruit more type II fibres, or simply recruit them faster, would have direct implications upon many anaerobic performance parameters. However, these mechanisms remain speculative as the neuromuscular response during either a maximal sprint, or an intense sub-maximal exercise, following high-intensity training, has not been well studied.

1.6. METHODOLOGICAL CONSIDERATIONS

Due to the relative novelty of sprint training research, many methodological considerations have arisen. Often, inconsistencies in the methodological approach between studies undermine the ability to make between study inferences; however, each little difference seems to provide more insight into the diverse effects of sprint training. Small differences in the implemented training protocol seem to have vast implications upon training induced adaptations. As well, the multitude of metabolic effects of sprint training has the potential to augment performance in a variety of ways, highlighting the need for an array of performance assessment tests.

1.6.1. Training methodology

Recent literature has demonstrated that sprint training regimens of various types have the capacity to elicit marked changes in both muscle metabolism and performance. However, each training protocol may have differential effects as was demonstrated by Parra et al. (2000) who found significantly different performance augmentations using two training protocols with the exact same training volume but different rest periods between sessions. Traditionally, training durations of 6-weeks or more were used to investigate the effects of sprint training (Jacobs et al., 1987; Linossier et al., 1997; Linossier et al., 1997; MacDougall et al., 1998; Nevill et al., 1989). However, the consistently observed augmentations to these relatively long-term protocols begged the question of how early these adaptations took place. Therefore, recent studies have tested the limits of training brevity and used training protocols as short as 5-days in the attempt to elicit performance changes (Hughes et al., 2002).

1.6.2. Performance evaluation

Performance tests not only give an indication of the performance adaptations to sprint training, but they also play an integral role in assessing the metabolic adaptations as well. Although sprint training has traditionally been associated with increases in anaerobic performance markers which have been assessed using brief maximal sprints (e.g., 30-second "all-out" cycle tests), recently there has been a vested interest into aerobic performance augmentations. However, until recently, the lone assessment test for

aerobic performance was a progressive exercise test to exhaustion which identified the subject's VO_{2peak}. This may give a good indication of maximal aerobic capacity, but there is often an altered work output which creates within subject variability and hinders the ability to assess changes in substrate utilization. Recent studies have implemented work-matched assessment tests whereby subject exercise at the same absolute workload before and after training which allows a sound comparison of metabolic differences following training (Dawson et al., 1998; Harmer et al., 2000).

In light of the numerous studies which have documented increased oxidative potential following sprint training, it is surprising that only one study has investigated the effects of sprint training upon sub-maximal exercise performance (Hughes et al., 2002). Hughes et al. (2002) documented a significant increase (21%) in time to exhaustion during a cycle ride at 90% VO_{2peak} following 1-week of sprint training. Further investigation into performance and metabolic regulation during sub-maximal exercise is required as the implications of such research would extend to a variety of athletes. Although work-matched assessment tests allow for thorough metabolic analyses, it is rare for an athlete to work at the exact same workload following a training period. Thus, from a practicality standpoint, it would be interesting to use a sub-maximal time trial assessment test whereby would have to travel a given distance (e.g., 10 kilometers) as fast as they can. If oxidative potential is improved following training, it would theoretically allow the subject to work at a greater workload (i.e., greater % of their VO_{2peak}), subsequently improving their time to complete the distance.

Once again, the novelty of sprint training research leaves many questions unanswered, subsequently providing a solid foundation for future research.

1.7. CONCLUSION

Sprint training has been traditionally associated with enhancements in anaerobic performance markers, and as result, has been a popular training method for athletes participating in explosive sports or events. However, recent research suggests that the effects of sprint training are much more diverse than initially believed.

Increases in peak power and mean power during a short, maximal sprint bout are a common adaptation to sprint training regimens. Until very recently, the only indication of enhanced aerobic performance was a training induced elevation in maximal aerobic capacity, or VO_{2peak}. Two studies have subsequently used assessment tests where the workload is held constant to investigate potential augmentations in the exercise time to fatigue following training. Harmer et al. (2000) were the first to implement this type of assessment test, however they used a workload equivalent to 130% of the subjects VO_{2peak} which is considered supramaximal, and relies primarily on anaerobic energy derivation. Although the time to fatigue significantly increased, few conclusions could be drawn regarding the augmentations in aerobic performance. In light of this, Hughes et al. (2002) implemented a sub-maximal exercise test to exhaustion at a workload equivalent to 90% VO_{2peak}. Although the significant increase in time to fatigue suggests another novel implication of sprint training; to date, this has been the only study to investigate the effects of sprint training upon intense sub-maximal exercise performance, thus future studies are needed to support and validate the findings. Nonetheless, most literature pertaining to performance adaptations seems to be relatively congruent, unlike

the disparities that arise when assessing skeletal muscle metabolism following sprint training regimens.

High-intensity sprint bouts place an extreme demand upon skeletal musculature such that energy provision is maximal from all ATP providing pathways. Although PCr hydrolysis and anaerobic glyco(geno)lysis are the prominent energy deriving pathways at the onset of sprint-exercise, oxidative metabolism becomes relied upon in the latter stages of the sprint bout. This reliance becomes even more pronounced during repeated sprint bouts when oxidative metabolism has been shown to contribute as much as 70% of ATP turnover (Trump et al., 1996). This extreme metabolic stress is believed to be the stimulus for enhancements in many key regulatory enzymes in both the non-oxidative and oxidative energy pathways. Although increases in many enzymes are often documented following sprint training, the plethora of positive and negative modulators, which arise as a result of the metabolic and ionic disturbances during a sprint bout, hinder the ability to define the regulation of energy providing pathways. Future research will be essential to clarify the regulation of ATP provision in order to accurately deduce a physiological rationale for performance enhancements.

Although muscle metabolism, and to a lesser extent performance, following sprint training remains equivocal, many of the disparities can be attributed to methodological inconsistencies. Upon investigation into the effects of sprint training, the details of the training protocol must be carefully planned. Although it seems that training duration has very little effect upon the adaptations to sprint training, Parra et al. (2000) demonstrated the significant influence of rest intervals upon performance and metabolism. Similarly, the duration of the sprint bouts also seems to play a major role in enzymatic changes

associated with sprint training. These metabolic and performance incongruities, which are found as a result of methodological differences, may make it difficult to compare between studies, but they also give a sense of the diverse effects of sprint training.

In summary, the novelty of sprint-interval training, and specifically its effects upon oxidative metabolism and aerobic performance, lends itself to many unanswered questions. Speculative mechanisms underlying performance augmentations such as changes in plasma volume, intramuscular lactate handling, and PDH activity are just a few of the many unresolved mechanisms that may play an integral role in understanding the effects of sprint training. Although the scope of this review was limited primarily to the metabolic and cardiovascular changes secondary to sprint training, neuromuscular adaptations (e.g., muscle fibre recruitment) may play an equally important role in deducing a physiological rationale for performance adaptations. Thus, the use of an integrative approach, where changes in skeletal muscle metabolism, muscle morphology, and cardiovascular regulation are assessed, may provide a more complete picture regarding the adaptive processes of skeletal muscle following sprint-exercise training.

CHAPTER 2:

THE EFFECT OF SHORT SPRINT INTERVAL TRAINING ON SKELETAL MUSCLE METABOLISM AND PERFORMANCE DURING INTENSE AEROBIC CYCLING

2.1. INTRODUCTION

Sprint interval training (SIT) has been implemented as part of athletic training regimens since the early 1970's (Thorstensson et al., 1975). SIT has traditionally been practiced by athletes who participate in sports that are predominately "anaerobic" in nature since this type of training is characterized by repeated bouts of brief, high intensity exercise. The intensity at which this type of training is typically performed elicits power outputs that are 3-4 times higher than an individual's maximal aerobic power. Since the capacity of the musculature to derive energy from oxidative sources is exceeded, there is a large reliance on non-oxidative pathways for ATP generation (Abernathy et al., 1990; Jacobs et al., 1987). Not surprisingly then, much of the early literature focused upon the changes in anaerobic performance and non-oxidative metabolism in response to various sprint training regimens. It is generally accepted that training protocols of 6-8 weeks in duration result in an improved ability to perform sprint exercise, however, the underlying physiological rationale for this improvement is unclear. Phosphofructokinase (PFK) is one of the only glycolytic enzymes that has been shown to be consistently elevated following sprint exercise training (Cadefau et al., 1990; Jacobs et al., 1987; MacDougall et al., 1998), whereas the reported changes in glycogen phosphorylase (PHOS), and

lactate dehydrogenase (LDH) are variable. These inconsistencies undermine the ability to determine the regulation of non-oxidative energy provision following sprint interval training and subsequently provide a viable rationale for improvements in sprint performance.

Recent evidence suggests that although the reliance upon non-oxidative sources is necessary to meet the ATP demand placed on the muscle during the initial stages of a short sprint bout, ATP generation via oxidative phosphorylation begins to play a prominent role in the latter stages of a brief sprint-exercise bout, and continues to be heavily relied upon during repeated sprint bouts (Parolin et al., 1999; Trump et al., 1996). In recognition of this, several recent sprint training studies have investigated potential changes in aerobic performance and the underlying adaptations in oxidative metabolism. Saltin et al. (1976) was one of the first to demonstrate an increase in maximal aerobic capacity (VO_{2peak}) concomitant with an increase in the maximal activity of succinate dehydrogenase (SDH), an enzyme associated with oxidative metabolism. Since then, there has been abundant evidence to suggest that 6-8 week sprint training regimens have the capacity to elicit marked elevations in many key oxidative enzymes and maximal aerobic capacity (Dawson et al., 1998; Harmer et al., 2000; Linossier et al., 1997; MacDougall et al., 1998).

Irrespective of the recent advances in sprint training research, there remains many potential implications that have not been well investigated. Sprint training protocols of less than 6-weeks have rarely been implemented. In fact, only two studies have used a training protocol as short as 2-weeks, and in both studies, subjects trained every day for a total of 14 sprint exercise bouts (Parra et al., 2000; Rodas et al., 2000). Although an

increase in VO_{2max} was reported, there was no change in anaerobic performance measures, possibly due to chronic fatigue caused by 14 consecutive days of training. The effect of fewer sprint training bouts on peak anaerobic or aerobic power is unknown, thus a short-term training (*i.e.*, ≤ 2 weeks) study is warranted to determine how quickly these adaptations begin to occur. In addition, no study has examined the effect of sprint interval training on aerobic performance measures other than maximal aerobic capacity. A sub-maximal assessment test (e.g., a prolonged ride at 85% of the subject's VO_{2peak}) would allow a researcher to investigate the effects of sprint training on the regulation of energy provision at sub-maximal work intensities, while providing further insight into aerobic performance augmentations.

The primary purpose of the present study was to examine the effect of short-term sprint interval training on skeletal muscle metabolism and performance during intense aerobic cycling. We hypothesized that 6 bouts of sprint interval training would elicit marked increases in both anaerobic and aerobic performance measures while concomitantly elevating the maximal activity of key oxidative enzymes (e.g., citrate synthase). This increase in the oxidative potential of the cell following sprint training would also permit a better match between pyruvate production and oxidation, subsequently increasing performance during intense aerobic exercise.

2.2. METHODS

2.2.1. Subjects

Eight healthy individuals (six men, two women) from the McMaster University undergraduate student population were recruited to take part in the study. The group had a mean age, weight, and height (± SD) of 21.9 ± 1.1yrs, 83.1 ± 15.1 kg, and 179.6 ± 10.0 cm, respectively. Three of the male subjects were varsity water polo players, however they were not sprint trained and would be classified as recreationally active. The remaining five subjects were all recreationally active and had not participated in a training program at least 6 months prior to the experiment The experimental procedure and potential risks were fully explained to the subjects prior to 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. Experimental Protocol

Subjects performed a series of baseline performance tests prior to the actual experiment. The main experiment consisted of two "Invasive Exercise Trials" (including venous blood and muscle biopsy sampling) which were performed prior to and following 2-weeks of sprint exercise training protocol. Details of all testing and training procedures are outlined below and summarized in fig. 2.

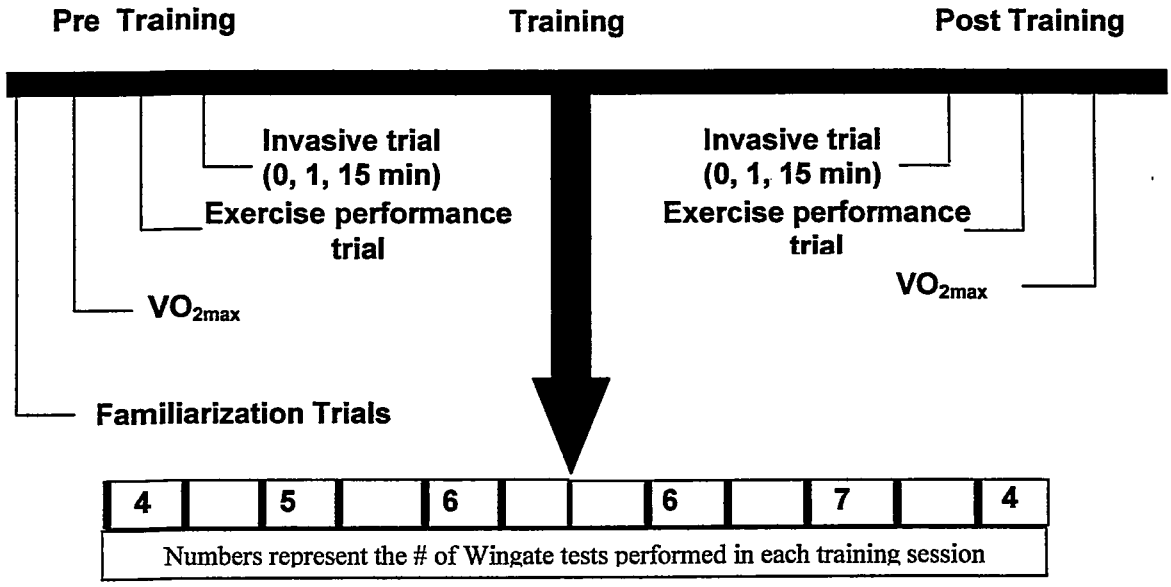

Fig. 2. Schematic of the order of pre and post assessment tests and training protocol.

2.2.2.1. Baseline Performance Tests

Three baseline tests were conducted over a 5 day period prior to the actual experiment:

1. Maximal Aerobic Power (VO_{2peak} Test)

An incremental protocol on an electronically braked cycle ergometer (Lode BV, Excalibur Sport V2.0, The Netherlands) was used to determine maximal oxygen uptake using an on-line gas collection system (Moxus modular oxygen uptake system, AEI Technologies, Pittsburg, PA). The first three stages of the VO_{2peak} test consisted of 2-min intervals at 50, 100, and 150 W, respectfully, and thereafter the workload was increased by 25 W every minute until volitional exhaustion. The inability of the subject to maintain a pedal cadence above 40 rpm was used to determine the end of the test since according to manufacturer's specifications power output is not constant below 40 RPM.

2. Wingate Test

Peak power, mean power, fatigue index, and anaerobic capacity were determined during a 30-sec bout of maximal sprint cycling on an electronically braked cycle ergometer (Lode BV, Excalibur Sport V2.0, The Netherlands) using appropriate software supplied by the manufacturer (V1.1). The workload was set at 7.5% body mass.

3. Exercise Performance Trial

Subjects cycled on an electronically braked cycle ergometer (Lode BV, Excalibur Sport V2.0, The Netherlands) at a workload designed to elicit ~80% VO_{2max} until volitional fatigue. The test was terminated when the subjects were unable to maintain a pedal cadence of 40 RPM since this is when power outputs are no longer guaranteed by the manufacturer. Verbal encouragement and sporadic music was used as motivation, however subjects were given no temporal or physiological feedback throughout the test.

2.2.2.2. Reproducibility of Baseline Tests

Our laboratory conducted extensive baseline tests to determine the day-to-day variability in the performance testing in the absence of the training intervention. Six subjects performed a VO_{2max} test, a challenge ride to exhaustion at 80% VO_{2max}, and a series of 4 Wingate tests separated by 4 minutes of passive recovery. Each test was then repeated one week later to determine the reproducibility of the results. The coefficient of variation for the various tests was as follows: VO_{2max} test = 3.7%; exercise performance trial = 14.6%; Wingate peak power (test 1 of 4) = 5.5%; Wingate peak power (test 4 of 4) = 5.0%; Wingate mean power (test 1 of 4) = 2.3%; Wingate mean power (test 4 of 4) = 2.3%. For individual subject data see appendix II.

2.2.2.3. Invasive Exercise Trial

Upon arrival at the laboratory, a catheter was inserted into an anticubital vein and the lateral portion of each thigh was prepared for needle biopsy sampling as previously described by Bergström (1975). Three small incisions were made through the skin and fascia over the vastus lateralis muscle under local anesthesia (2% lidocaine). A biopsy and blood sample were obtained at rest and then subjects moved to an electronically braked cycle ergometer (Lode BV, Excalibur Sport V2.0, The Netherlands) and began pedaling to exhaustion at a workload designed to elicit 80% VO_{2max}. Venous blood samples were drawn every 5 minutes during exercise. Muscle biopsies were obtained after 1 min and 15 min of exercise. Expired gases were collected during the 7 - 10 min period of exercise during 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 determined using a telemetry monitor (Polar Electro, Woodbury, NY).

2.2.2.4. Exercise Training Protocol

Two days following the first Invasive Exercise Trial, subjects performed 6 bouts of sprint interval training over a period of 2 weeks. Subjects trained three times per week on alternate days (i.e., Mon., Wed., Fri.). A training interval consisted of one Wingate test (i.e., a 30-sec "all-out" sprint cycle test against a constant resistance equivalent to 7.5% of the subject's body mass) followed by 4 min of passive cycling. Intervals were increased from 4 to 7 during the first five training bouts and then back down to 4

intervals on the sixth training bout (i.e., 4, 5, 6, 6, 7, 4). The number of intervals was decreased on the final training bout in order to have a viable comparison between the first day of training and the last. After 2 weeks of training, the second Invasive Exercise Trial was performed, as well as an Exercise Performance Trial and a VO_{2max} test.

2.2.3. Analyses

2.2.3.1. 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 prior to analysis.

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. 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. Creatine (Cr), phosphocreatine (PCr), ATP, lactate (La), malate and glycogen (glucose assay) concentrations were determined with a Hitachi F-2500 fluorescence spectrophotometer, using fluorometric enzyme assays described 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.

2.2.3.2. Blood analyses

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

2.2.4. Statistics

All data that consisted of single pre and post measurements, including VO_{2peak}, challenge ride times, and muscle enzyme activities, were analyzed using paired t-tests. Muscle and blood metabolites were analyzed using a two-way analysis of variance (ANOVA) (condition x time). When a significant main effect or interaction was identified, data were subsequently analyzed using a Tukey HSD post hoc test. Significance for all analyses was set at $P \leq 0.05$. All values are presented as means ± standard error of the mean (SEM).

2.3. RESULTS

2.3.1. Performance

2.3.1.1. Anaerobic Capacity

Peak power during each of the four Wingate tests was significantly elevated following training ($P<0.05$)(Fig. 3). However, mean power did not change during any of the sprint bouts (Fig. 4) which is the result of an increased % fatigue during each of the Wingate tests ($P<0.05$)(Table 4).

Table 3. ***Anaerobic performance averaged across 4 pre and post-TR sprint bouts***

	Pre Training	**Post Training**
Peak power (Watts)	934 ± 61	1067 ± 83 *
Mean power (Watts)	570 ± 31	575 ± 30
% Fatigue	27 ± 2	31 ± 3 *

Values are mean ± SEM. $n = 8$. * $P \leq 0.05$ vs. pre-TR.

Table 4. ***Anaerobic performance during each pre and post-TR sprint bout***

	Pre Training				Post Training			
	1	**2**	**3**	**4**	**1**	**2**	**3**	**4**
Peak Power	1023 ± 67	963 ± 64	899 ± 72	852 ± 63	1176 ± 80 *	1062 ± 69 *	1040 ± 99 *	990 ± 95 *
Mean Power	642 ± 26	585 ± 33	535 ± 32	520 ± 36	632 ± 24	576 ± 30	543 ± 35	548 ± 30
Fatigue Index	29 ± 2	27 ± 3	26 ± 3	24 ± 2	31 ± 2	33 ± 4 *	33 ± 4 *	29 ± 4 *

Values are mean ± SEM. $n = 8$. * $P \leq 0.05$ vs. same pre-TR sprint bout.

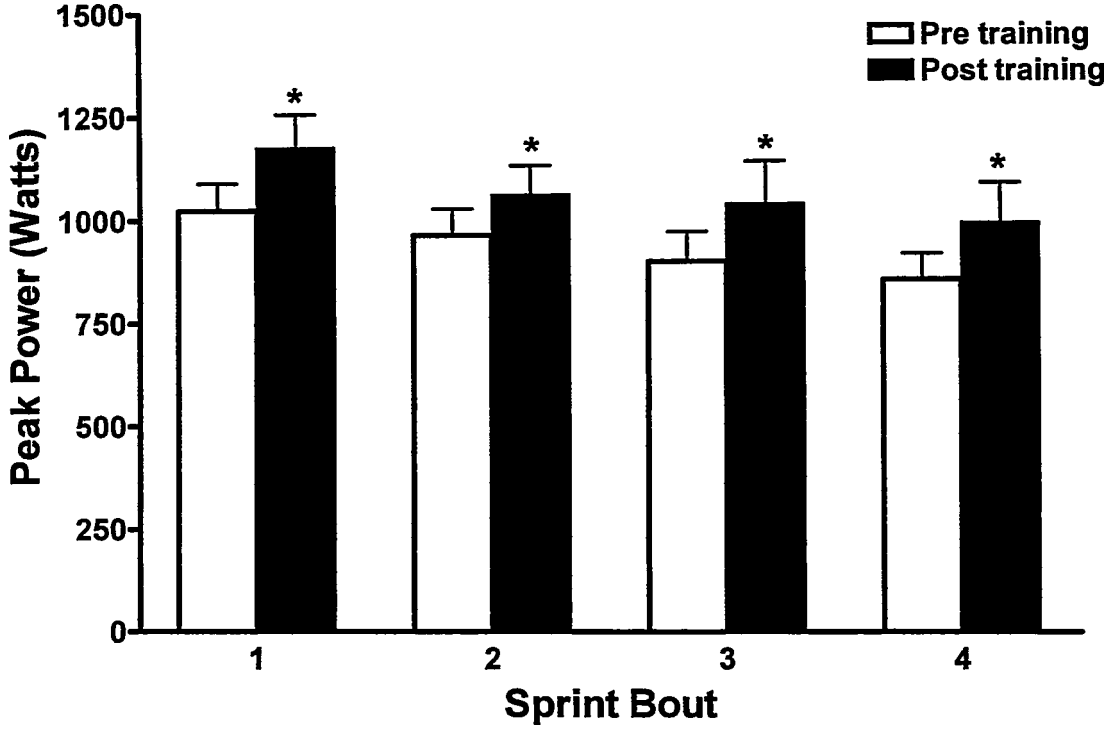

Fig 3. Peak power during 4 sprint bouts, pre vs. post-TR. * $P \leq 0.05$ vs. pre-TR.

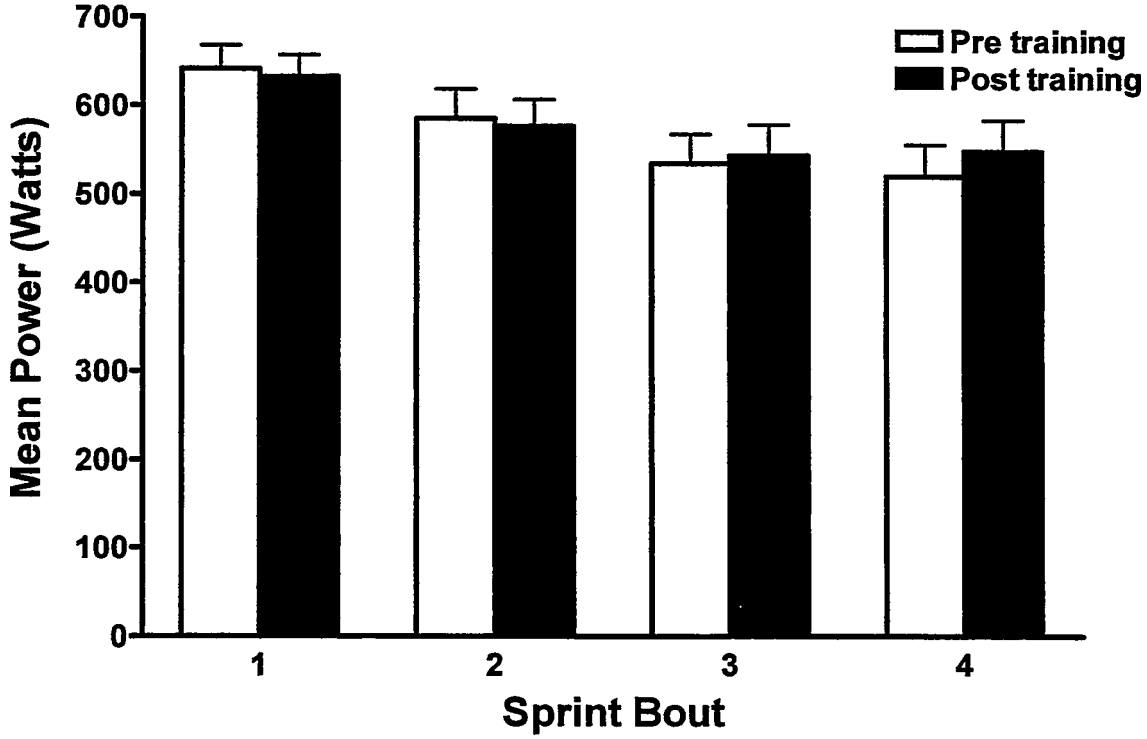

Fig 4. Mean power during 4 sprint bouts, pre vs. post-TR.

2.3.1.2. Maximal Aerobic Capacity

VO_{2peak} did not change following training. Likewise, the subject's maximum heart rate and ventilation achieved during the VO_{2peak} testing protocol remained unchanged (Table 5).

Table 5. ***Maximal aerobic performance***

	Pre Training	**Post Training**
VO_{2peak} (L/min)	3.66 ± 0.2	3.71 ± 0.2
VO_{2peak} (ml/kg/min)	44.6 ± 1	45.5 ± 2
HR max (b/min)	191 ± 4	194 ± 3
V_E max (L/min)	143 ± 12	155 ± 14

Values are mean ± SEM. $n = 8$.

2.3.1.3. Intense Aerobic Cycling Performance

Mean challenge ride time to exhaustion increased by 100% ($P<0.05$) after training, despite the fact that the pre determined workload elicited the same percentage of the subject's VO_{2peak} during both the pre training and post training assessments (Table 6). Ventilation and RER averaged over a 3 minute span during the invasive exercise trial were reduced following training ($P< 0.05$)(Table 6). Individual times to exhaustion are displayed in Table 7 to illustrate the relatively parallel increases in performance between subjects during the challenge ride, except for subject 7 who suffered an ankle injury prior to the challenge ride consequently hindering his performance.

Table 6. ***Cardio-respiratory and performance results during the challenge ride and invasive exercise trial***

	Pre Training	Post Training
Time to exhaustion (min)	26 ± 5	51 ± 10 *
Workload (Watts)	218 ± 14	218 ± 14
% VO_{2peak}	80 ± 2	77 ± 3
V_E (L/min)	104 ± 9	91 ± 7 *
RER	1.24 ± 0.02	1.18 ± 0.02 *

Values are mean ± SEM. $n = 8$. * $P \leq 0.05$ vs. pre-TR. $^{+}$ Values measured from 6-9 min during invasive exercise trial then averaged. $^{\#}$ Values measured during the challenge ride.

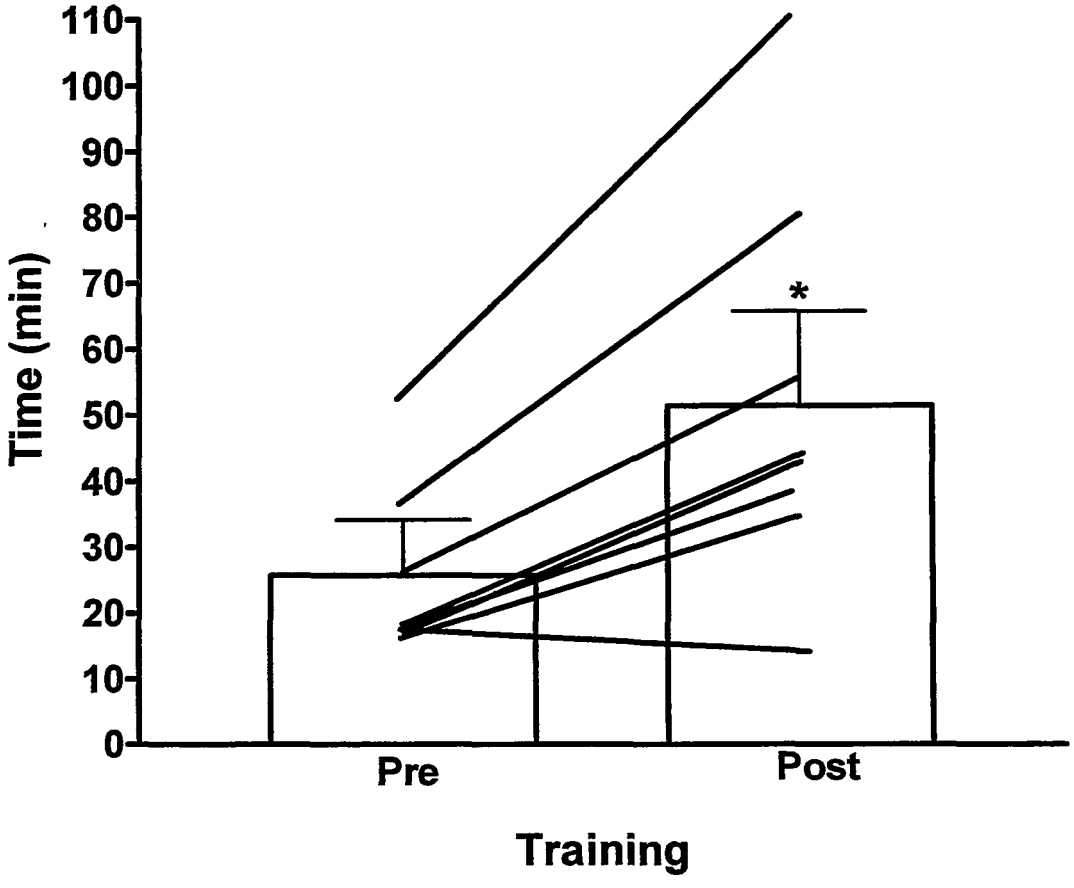

Fig 5. Time to exhaustion during challenge ride, pre vs. post-TR. Solid lines represent individual subject times, pre vs. post-TR. * $P \leq 0.05$ vs. pre-TR.

Table 7. ***Individual times to exhaustion during challenge ride***

Subject	Pre Training	Post Training	% Change
S1	38.8	81.0	109
S2	55.3	110.0[a]	99
S3	15.9	32.5	104
S4	15.9	42.7	169
S5	18.6	42.9	131
S6	26.9	55.0	104
S7	15.5	13.0	-17
S8	18.8	34.0	81

[a] Exercise was terminated when subject doubled their pre-TR time to exhaustion with a relatively low perceived exertion. Times are in minutes.

2.3.2. Muscle Metabolites

Muscle glycogen concentration was 20% higher at rest ($P<0.05$) following training. However, net muscle glycogen utilization from 0-15 min of exercise was lower ($P<0.05$) following training (Fig. 6). Muscle [lactate] increased during exercise in both trials, and while [lactate] was ~ 15% lower after 15 min of exercise post-TR, there was no significant difference between trials (Fig. 7). Similarly, there was no significant difference in muscle [PCr], [creatine], [ATP], or [malate] at any time during the invasive exercise trial pre vs. post-TR.

Table 8. ***Muscle metabolite summary at 0, 1, 15 min of invasive exercise trial***

	Pre Training			Post Training		
	0 min	**1 min**	**15 min**	**0 min**	**1 min**	**15 min**
Glycogen	489 ± 57	n/d	343 ± 33	614 ± 39	n/d	547 ± 47
Lactate	8 ± 2	42 ± 7	94 ± 9	7 ± 1	42 ± 11	85 ± 12
PCr	81 ± 3	54 ± 4	31 ± 8	84 ± 3	56 ± 6	34 ± 7
Creatine	30 ± 2	56 ± 4	79 ± 6	27 ± 3	54 ± 5	76 ± 6
ATP	24 ± 1	32 ± 2	28 ± 3	24 ± 2	24 ± 1	27 ± 4
Malate	0.3 ± 0.1	2.0 ± 0.4	2.3 ± 0.2	0.3 ± 0.1	2.0 ± 0.4	3.1 ± 0.5

Values are mean ± SEM. $n = 8$. Values are in mmol/kg ww/min.

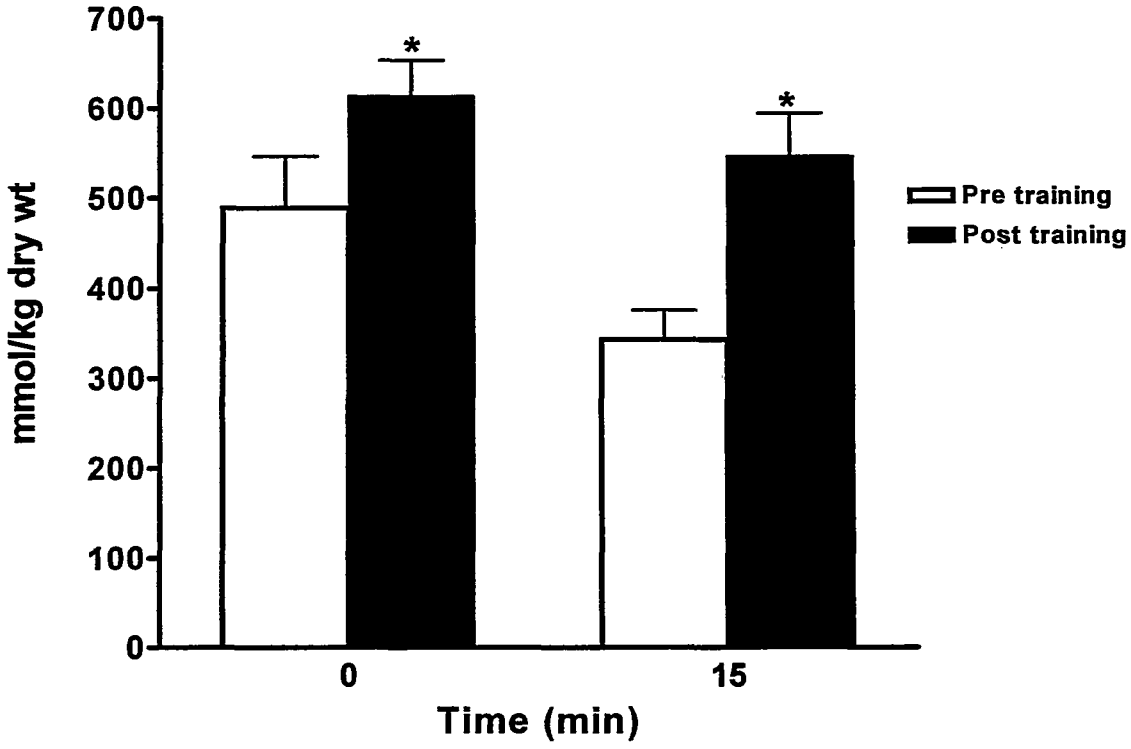

Fig. 6. Muscle [glycogen] at 0 and 15 min of exercise, pre vs. post-TR. * $P \leq 0.05$ vs. pre-TR. Glycogen degradation from 0 – 15 min was significantly less post-TR.

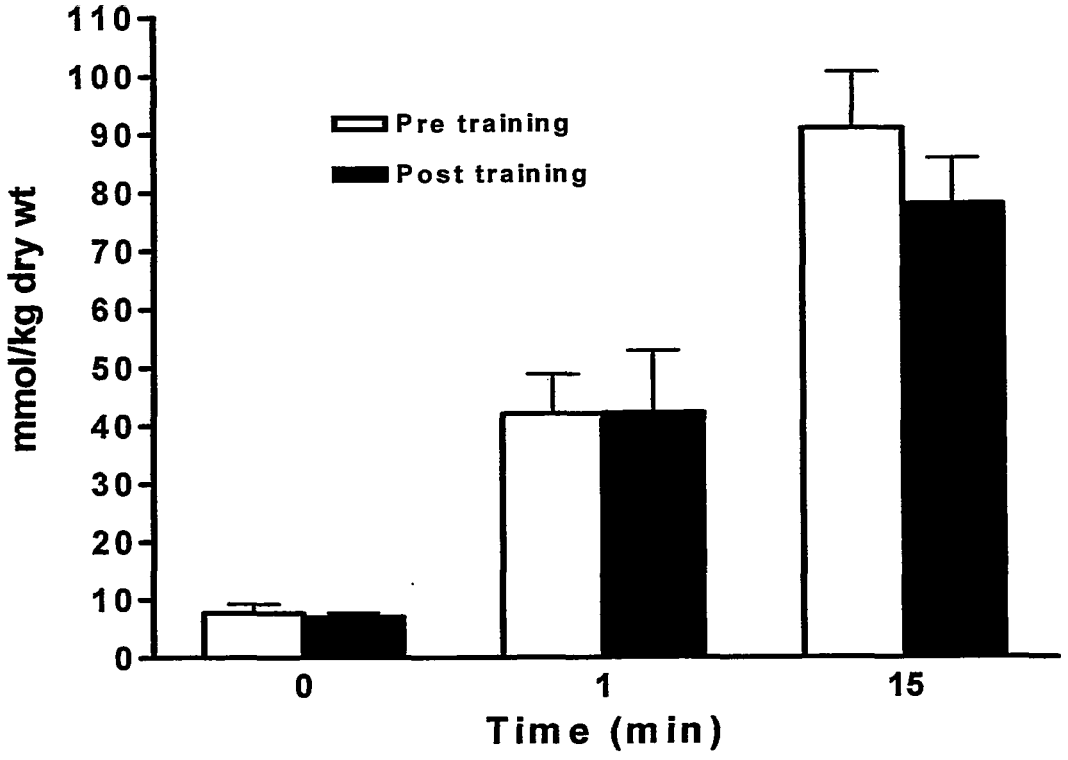

Fig. 7. Muscle [lactate] at 0, 1, and 15 min of exercise, pre vs. post-TR.

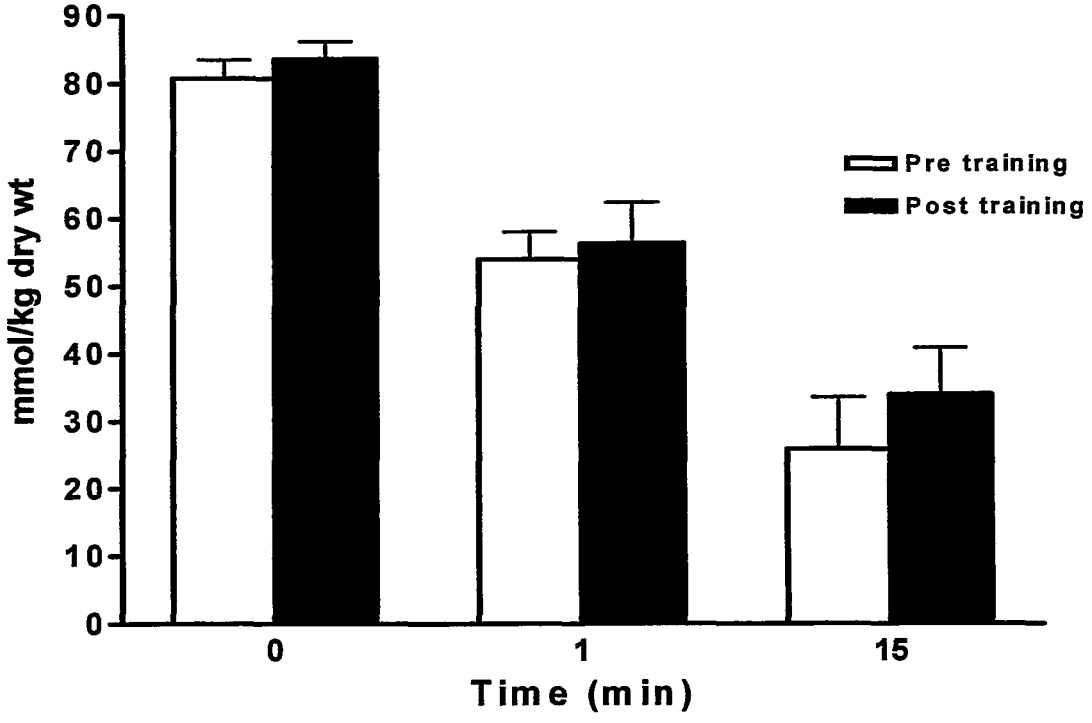

Fig 8. Muscle [phosphocreatine] at 0, 1, and 15 min of exercise, pre vs. post-TR.

2.3.3. Muscle Enzymes

The maximal activity of citrate synthase and alanine aminotransferase increased from pre-TR to post-TR (Fig 9 and Fig 10). There was a 29.3% increase ($P<0.05$) in citrate synthase, whereas alanine aminotransferase increased by 27.4% ($P<0.05$) following the training protocol (Table 9).

Table 9. ***Maximal activity of muscle enzymes***

	Pre Training	Post Training	% Change
CS	11.5 ± 1.8	16.7 ± 2.3*	29 ± 11
AAT	2.6 ± 0.6	3.2 ± 0.5*	28 ± 11

Values are mean ± SEM. $n = 8$. * $P \leq 0.05$ vs. pre-TR. Values are in mmol/kg ww/min.

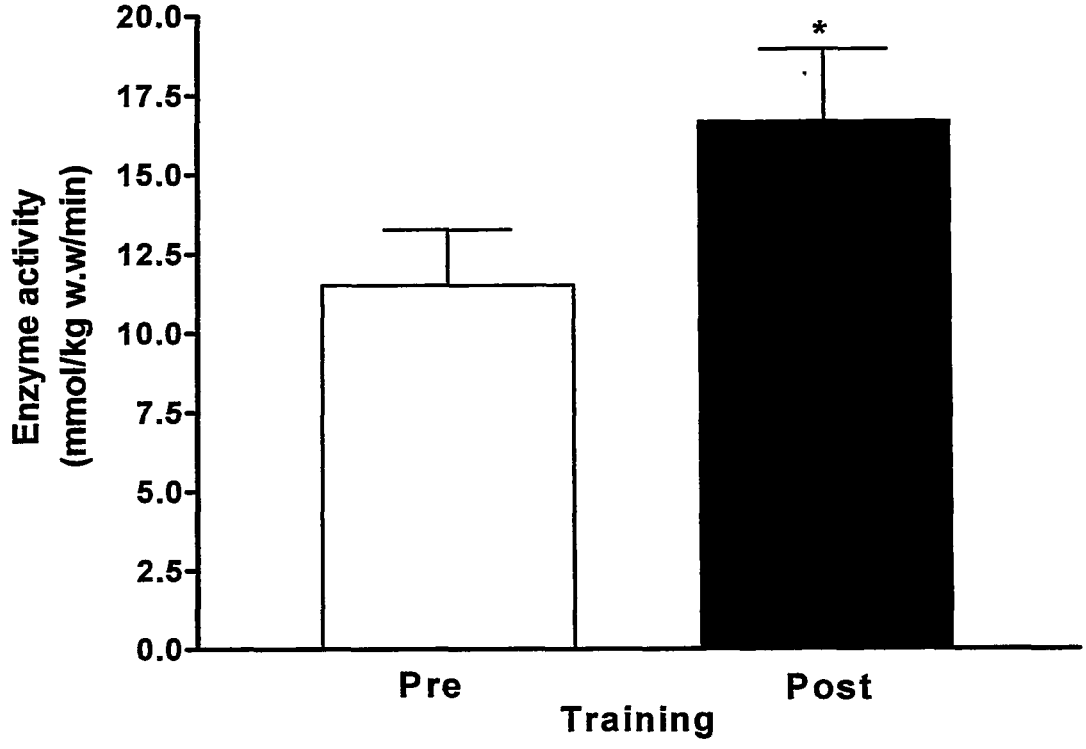

Fig. 9. Change in the maximal activity of muscle citrate synthase, pre vs. post-TR. $P \leq 0.05$ vs. pre-TR.

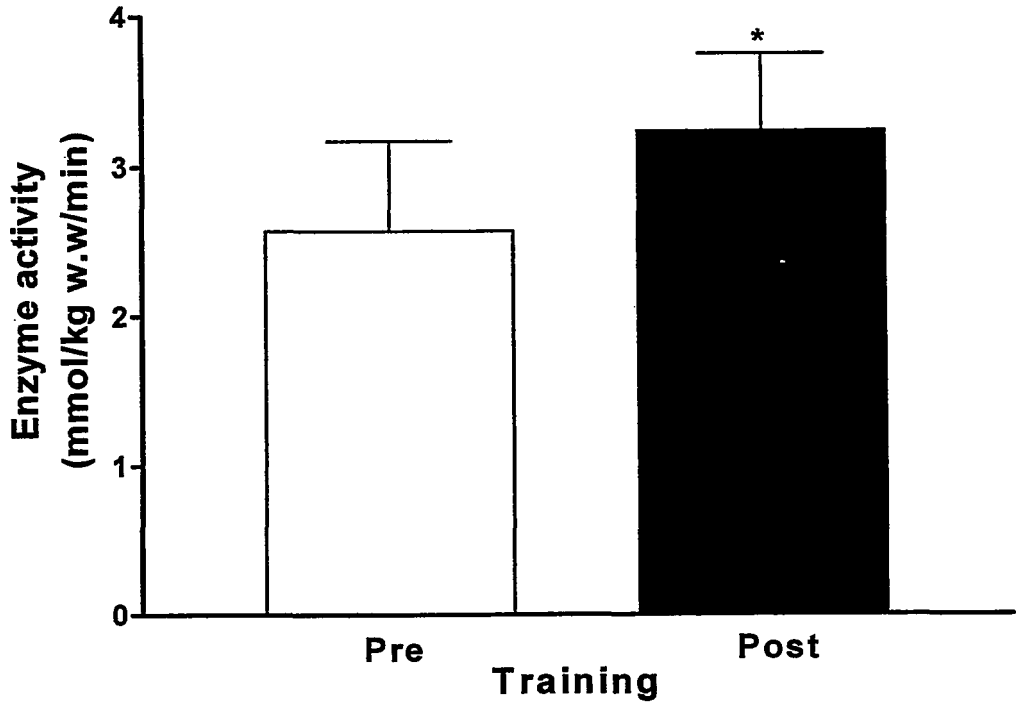

Fig. 10. Change in the maximal activity of muscle alanine aminotransferase, pre vs. post-TR. * $P \leq 0.05$ vs. pre-TR.

2.3.4. Blood lactate and blood glucose

Blood [lactate] increased throughout both trials, however there were no significant differences between time points in each trial (Fig. 11). Similarly, there were no differences in blood [glucose] at each time point, pre vs. post-TR (Fig. 12).

Table 10. ***Blood metabolite summary at rest, 5, 10, and 15 min of invasive exercise trial.***

	Pre training				Post training			
	0min	**5min**	**10min**	**15min**	**0min**	**5min**	**10min**	**15min**
Blood Glucose	5.1 ± 0.4	4.1 ± 0.2	4.2 ± 0.2	4.4 ± 0.3	4.8 ± 0.1	4.6 ± 0.2	4.6 ± 0.2	4.6 ± 0.2
Blood Lactate	0.6 ± 0.2	2.6 ± 0.2	5.9 ± 0.5	6.3 ± 0.5	0.4 ± 0.1	2.9 ± 0.3	6.0 ± 0.5	7.0 ± 0.6

Values are mean ± SEM. $n = 7$.

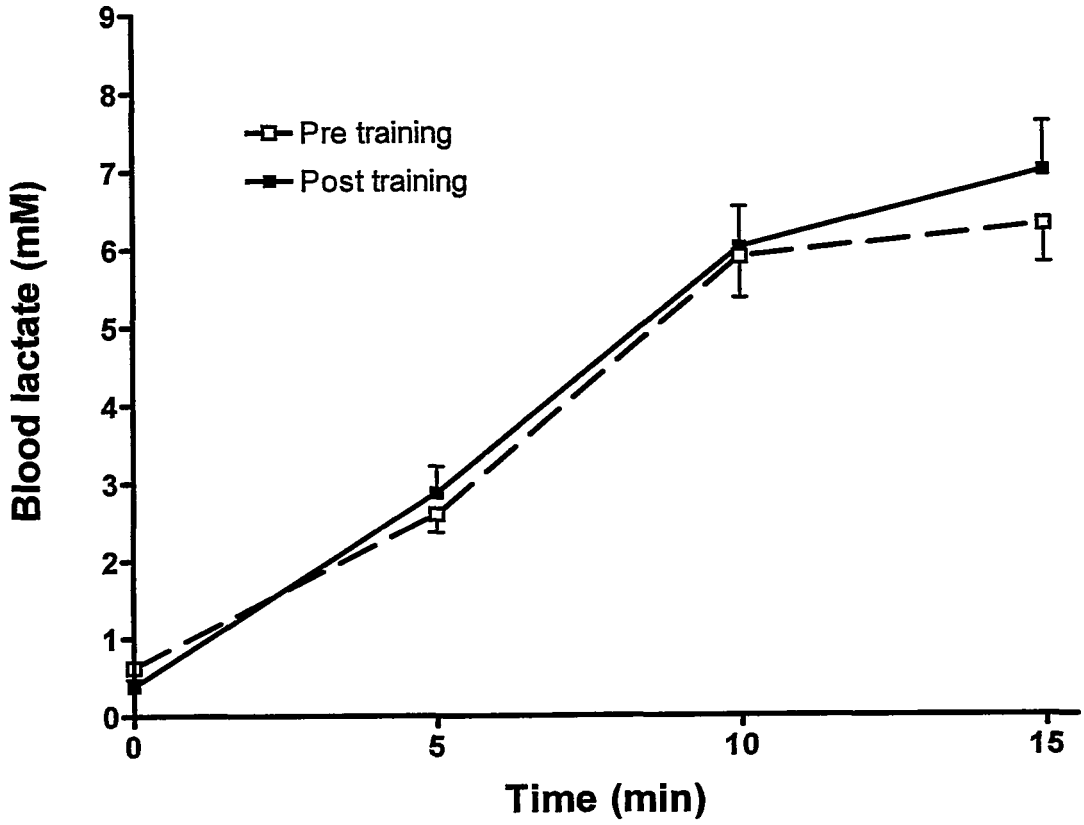

Fig 11. Changes in blood [lactate] during invasive exercise trial, pre vs. post-TR.

2.4. DISCUSSION

The primary novel finding from the present investigation was that, subjects were able to increase their time to exhaustion during an intense aerobic task by 100% following a short training program that consisted of 6 sprint interval sessions over a period of 2 weeks. In addition, anaerobic performance, as indicated by peak power generated during a 4-30sec Wingate test challenge, was also increased following training. Finally, muscle biopsy samples revealed alterations in muscle oxidative potential and muscle glycogen metabolism at rest and during a fixed work bout performed at 80% VO_{2peak}.

Changes in Aerobic and Anaerobic Performance Following Short Sprint Training

To date, changes in sub-maximal aerobic performance and metabolism are unknown as no study has investigated these effects. Similarly, no study has used a training protocol of such minimal volume as the present investigation, however, comparable changes in peak power have been observed in studies using longer training protocols (i.e., 6-8 weeks)(Harmer et al., 2000; MacDougall et al., 1998; Nevill et al., 1989). In each of these studies peak power increased more than mean power, the ranges are 12-26% and 6-16% respectively, which is consistent with the fact that peak power increased more than mean power in the present investigation. However, the lack of any increase in mean power in the present study necessitates an explanation since previous literature suggests that there is either an increase in both peak and mean power (Esbjornsson et al., 1996; Harmer et al., 2000; Linossier et al., 1993; MacDougall et al., 1998; Nevill et al., 1989; Parra et al., 2000; Stathis et al., 1994), or no change in either of the performance variables (Hellsten-Westing et al., 1993; Jacobs et al., 1987; Rodas et

al., 2000). Our laboratory is primarily interested in the metabolic adaptations to training, however we appreciate that many other factors may play an integral role when attempting to explain changes in performance following training. For example, it could be rationed that an increased ability to recruit type II muscle fibres following training would significantly augment power outputs and alter the metabolic profile of the muscle group during exercise. Type II muscle fibres are more metabolically suited to sprint efforts by virtue of their having higher myosin ATPase activity than type I fibres, and a very high glycolytic dependence (Dawson et al., 1998). Subsequently, if a greater proportion of type II fibres are recruited at the onset of cycling following training, a greater power output would be obtained; however, the greater glycolytic flux in these fibres would result in a greater production of lactate, thus rendering the muscle more susceptible to fatigue during the exercise bout. This could possibly explain the observed increase in peak power concomitant with no change in mean power following training in the present investigation. Methodological differences (i.e. time course of pre and post training assessment tests) between studies may provide an alternate explanation. Rodas et al. (2000) trained subjects everyday for two weeks (14 total training session), with the total load increasing progressively throughout the training period. They found that peak power and mean power during a 30-sec "all-out" cycle test were unaltered. However, the 30-sec performance test was conducted immediately following the training period, and it was documented that during the last three training sessions pedaling rate and power outputs during the sprint bouts had begun to decline. This suggests that muscular fatigue could have played a major role in the absence of a training benefit. Likewise, in the present investigation, only 1 day of rest separated the final training session from the

repeated Wingate assessment test. If this rest interval was not sufficient to allow for the dissipation of muscular or neural fatigue that may have developed during the training period, improvements in performance may have been hindered, possibly explaining the lack of a change in mean anaerobic power.

Skeletal Muscle Metabolic Adaptations to Short Sprint Training

The changes in aerobic and anaerobic performance in the present study were accompanied alterations in oxidative and non-oxidative metabolism. Similar to the findings of Parra et al. (2000), we observed that resting muscle [glycogen] was increased following the training protocol. In addition, glycogen degradation was 20% lower from 0 – 15 minutes of exercise during the post-TR invasive exercise trial. This attenuated glyco(geno)lytic rate during a work-matched assessment test is consistent with the findings of Harmer et al. (2000), however a lack of change in muscle and blood [lactate] during intense aerobic cycling in the present investigation is rather surprising. The lower glycogen degradation would suggest less pyruvate formation and potentially less total flux through lactate dehydrogenase. However, muscle [lactate] was not different at any time point pre vs. post-TR. It is of interest that while the increase in muscle lactate is almost identical from rest to 1 minute of exercise in the pre vs. post-TR trials, it appears that muscle lactate levels begin to diverge by 15 minutes of exercise. Although not significant, there seems to be a trend for muscle [lactate] to be lower at 15 minutes of exercise in the post-TR invasive exercise trial than the pre-TR trial. Coupled with the fact that this trend is paralleled by a similar increase in blood [lactate] at 15 minutes of exercise following training, it raises the possibility that an up-regulation in lactate transport may begin to play an important role in the latter stages of intense exercise.

Bonen et al. (unpublished observations) have suggested that a single acute bout of intense exercise is sufficient to increase MCT 1 and 4 expressions in skeletal muscle, and Green et al. (2002) reported increases in MCT 2 days after a single session of prolonged moderate exercise in humans. Therefore, it is very possible that MCT isoform expression, particularly MCT 4 which is primarily responsible for lactate extrusion from muscle, is elevated following 6 sessions of sprint interval training, subsequently contributing to the extended time to fatigue. Another plausible explanation extends from the work of Harmer et al. (2000) who were the first to demonstrate an enhanced H+ regulation following 7 weeks of sprint cycle training. It was found that muscle [H+] was significantly lower in a post-TR cycle ride to exhaustion at ~130% VO_{2max} than in the pre-TR trial despite a similar [lactate] accumulation and an increased time to exhaustion. Although [H+] was not assessed in the present study, an enhanced buffering capacity that allows for a steady muscle pH during exercise would explain the present data, which suggests that intense aerobic exercise performance improves dramatically despite a similar intramuscular lactate accumulation.

Muscle Enzymatic Adaptations

The observed 29% increase in the maximal activity of citrate synthase is consistent with previous reports in studies using sprint bouts of 30 seconds (Jacobs et al., 1987; MacDougall et al., 1998; Parra et al., 2000). In addition, the 28% increase in AAT activity in the present study is similar to that reported by Cadefau et al. (1990) following a sprint training protocol, although to our knowledge this is the only other study that has reported the activity of AAT following sprint exercise training. One limitation in reporting AAT activity is that it is present in both the cytosol and the mitochondria,

subsequently making it difficult to distinguish where the adaptation is occurring. However, knowing that CS is expressed exclusively in the mitochondria, the relatively parallel increase in maximal CS and AAT activity suggest that the majority of the increase in AAT is occurring within the mitochondria, possibly due to an increase in mitochondrial volume. Regardless of where the adaptation occurred, an increase in these oxidative enzymes contributes to an enhanced oxidative potential of the muscle. Although the mechanism by which this enhancement occurs is not precisely defined, a large proportion of the adaptation probably occurs because these ATP provision pathways are challenged when oxidative metabolism becomes an important source of ATP during repeated 30 second all-out efforts (Bogandis et al., 1996; Trump et al., 1996). In addition, the short rest intervals between sprints in the present study (i.e., 4 minutes) may not be sufficient to allow a complete recovery of anaerobic energy stores, thus resulting in a greater reliance upon aerobic pathways during the sprints. Furthermore, consistent maximal pyruvate production during the sprint bouts may serve as a stimulus for an increase in the catalytic activity of PDH. Although PDH activity was not measured, one can speculate that had it increased, it would have resulted in an increased entry rate of pyruvate into the mitochondria, subsequently serving as a stimulus for the enhanced mitochondrial enzyme activity following training. Even if the active fraction of PDH was unchanged, the decreased glycogenolytic rate following training during intense aerobic exercise would result in a slower presentation of pyruvate which may allow for a greater proportion of pyruvate to be oxidized.

Maximal Oxygen Uptake after Short Sprint Training

Despite the increased maximal oxidative potential of the cell, the training protocol did not elicit a significant change in VO_{2peak} (Pre: 44.6 ml/kg/min vs. Post: 45.5 ml/kg/min). It is unclear whether sprint training protocols increase maximal aerobic capacity as it seems dependent upon many of the protocol parameters (e.g., training duration, rest intervals, sprint duration). Even though there was no change in maximal oxygen consumption during the progressive exercise test, there was a significant increase in the peak power obtained (Pre: 313 vs. Post: 341 Watts). Notably, all 8 subjects increased their power output in the post-TR test, whereas only 4 increased their maximal oxygen consumption. This increase in peak aerobic power and peak anaerobic power suggests that the subject's leg strength had increased following training. Thus, it would be reasonable to assume that although the workload during the pre and post-TR intense aerobic exercise rides may have elicited a similar percentage of maximal aerobic capacity (i.e., ~80% VO_{2peak}), the same absolute workload would have certainly been a smaller percentage of peak power, or leg strength following training. Even though the imposed load may have demanded the same oxygen uptake at the muscle, the exercise may have felt easier to perform, thus enabling subject's to extend their time to exhaustion.

An increase in leg strength as a result of training could be attributed to muscle hypertrophy, an alteration in the muscle recruitment pattern, or a shift in the distribution of fibre types such that the muscle becomes more prolific at producing force. Several authors have documented that sprint interval training results in a greater percentage of type II fibres and type II fibre area, particularly type IIA (Dawson et al., 1998; Jacobs et al., 1987). Since type IIA fibres are characterized by relatively high activities of both

glycolytic and oxidative enzyme activities compared to other type II fibre types, this shift remains consistent with the observed increase in CS activity in the present investigation, and the well documented increase in PFK following various sprint training protocols (Cadefau et al., 1990; MacDougall et al., 1998; Parra et al., 2000).

Other Potential Physiological Adaptations Induced by Short Sprint Training

Another possible adaptation to sprint interval training may be changes in the cardiovascular or cardio-respiratory profile of the subjects during exercise. There is evidence to suggest that intense supra-maximal cycling serves as a potent stimulus for the expansion of plasma volume (Gillen et al., 1991; Green et al., 1984). Green et al. (1984) concluded that three sessions of supra-maximal sprinting increased plasma volume by 12%, a finding that was later supported by Gillen et al. (1991) who demonstrated a 10% increase following a single session of high-intensity intermittent exercise. Coyle et al. (1990) reported a 15% increase in stroke volume following an artificial elevation of plasma volume, suggesting that blood flow, and consequently oxygen delivery, to the working muscles is enhanced. However, there is no empirical evidence to suggest that an increased plasma volume would directly result in either enhanced intense aerobic performance or sprint performance. An interesting alternate possibility may be changes in the distribution of oxygen uptake in the body. During the post-TR exercise performance trial, V_E was significantly decreased which may suggest that respiratory muscle VO_2 was also decreased. If this is true, since whole body $V0_2$ was unchanged, it renders the possibility that $V0_2$ of the working muscles was increased. Unfortunately whole body $V0_2$ measures do not give us the ability to distinguish where oxygen is being taken up, thus this theory remains speculative.

Future Directions

The novelty of sprint training research lends itself to a plethora of potential future investigations. Simple manipulations of the training protocol or assessment tests may provide a completely different perspective of sprint training and its vast effects. For example, although the present investigation used a very small training load, it would be interesting to determine what physiological and performance adaptations would occur if this load was reduced even further, possibly down to just 1 training session. Another possibility may be to examine the effect of sprint training upon low intensity exercise (e.g., 60% VO_{2max}). Although this may seem to be just a minor variation from the present study, it would provide vastly different information regarding the effects of sprint training, particularly the regulation of oxidative metabolism. It would also be exciting to determine the effects of creatine supplementation when combined with sprint training. The extremely high demand placed upon the musculature during training would suggest that an elevated total creatine content may be beneficial; however, its effects during an exercise bout which is predominantly fueled by oxidative metabolism is less clear. From a practicality standpoint, it would be interesting to see what effect sprint training would have upon a subject's performance in a race of a certain distance. For example, an improved 10k race time may be a lot more impressive and useful to a coach and an athlete then an understanding of the physiological adaptations underlying the improvement.

The novelty of sprint training investigations not only lends itself to many future investigations, it is also susceptible to many limitations. Although an integrative approach to a study which combines metabolic, cardiovascular and neuromuscular factors

would be ideal, pragmatic issues such as time, money and laboratory expertise often render these studies not feasible. Nonetheless, sprint training remains a very fertile and exciting topic for further examinations.

2.5 CONCLUSIONS

The main finding from this study was that only six sessions of sprint interval training (~ 15 min total exercise time) resulted in a marked increase in intense aerobic cycling performance. In addition, there was an increase in peak anaerobic power during 4 repeated Wingate tests. These performance changes were accompanied by a host of metabolic changes. Resting muscle glycogen was increased following training, while net muscle glycogenolysis was reduced during exercise following training. Furthermore, increased maximal activity of citrate synthase (CS) suggests an increased oxidative potential of the cell, which is consistent with the observed increase in intense aerobic exercise performance. Thus, we conclude that two weeks of sprint interval training (6 training bouts) can increase intense aerobic cycling performance and peak anaerobic power; resting muscle [glycogen] and maximal CS activity.

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APPENDIX 1

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 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 accompanying forms are entitled "Invasive 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 PRIMARY 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
Scott Hughes, B.Kin.	Kinesiology, A103	905-525-9140 ext. 27037
Maureen MacDonald, Ph.D.	Kinesiology, AB115	905-525-9140 ext. 23580

PROJECT TITLE

"The Effect of Sprint Exercise Training on Skeletal Muscle Energy Metabolism"

PURPOSE OF THE STUDY

Our laboratory is interested in the regulation of energy metabolism in human skeletal muscle. It is known that repeated, brief bouts of high-intensity, "sprint"-type exercise increase the maximal activity of various enzymes and the concentration of fuels such as muscle glycogen. These adaptations are usually associated with an improvement in sprint exercise performance (e.g., power output). Our study is unique in that we intend to investigate whether changes in muscle enzymes and metabolites secondary to sprint training are also associated with improved energy metabolism and performance during strenuous aerobic exercise. We will also document performance adaptations which take place during sprint-type exercise.

DESCRIPTION OF TESTING AND EXPERIMENTAL PROCEDURES

Following routine medical screening and the completion of a health questionnaire, you will be required to make 16 visits to the laboratory over a period of approximately 4 weeks. Specifically, the study will consist of 5 pre-training (baseline) experimental exercise tests, 6 training sessions and 5 post-training tests (see below for summary of complete schedule).

VO_2max Test. This test involves cycling on a stationary bike (cycle ergometer) at progressively higher workloads while the amount of oxygen taken up by your body is determined from a mouthpiece connected to a gas analyzer.

Cardiovascular Function Test. This test is also similar to the VO_2max test, except that a small probe will be used to non-invasively determine the amount of blood which is being ejected from your heart. The probe (which looks like a hand-held microphone) will be placed over your chest while you are cycling on the ergometer.

Wingate Test: Single and Repeated. A Wingate test is a 30-second "all out" sprint exercise test on a cycle ergometer and is used to determine your maximal power output. For the repeated Wingate test, 4 x 30 sec bouts of maximal cycling will be performed with 4 min of recovery in between each bout.

Performance Trial. This test involves cycling at a workload designed to elicit ~80% of your VO_2max to volitional fatigue (i.e., until you cannot maintain the required power output).

Experimental Exercise Trial. Upon arrival at the laboratory, a catheter will be inserted into a forearm vein for blood sampling and one leg will be prepared for the extraction of muscle biopsy samples. The details of the blood sampling and muscle biopsy procedures and associated risks are thoroughly described on the attached forms entitled "Description of Medical Procedures." A blood sample and muscle biopsy will be obtained at rest. You will then perform cycle exercise for 15 min at a workload designed to elicit ~80% of your VO_2max. Heart will be monitored non-invasively 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 5 minutes during exercise, and needle biopsy samples will be obtained after 1 min and 15 min of exercise. Upon completion, you will be permitted to leave the laboratory following ~30 min of routine, post-exercise monitoring. Shower and change facilities are available should you require them.

Training Protocol. The training protocol will consist of two weeks of sprint interval training three times per week (six sprint training sessions in total). Training will be peformed on alternate days (i.e., Mon., Wed., Fri.). The program will begin with four intervals per session at the start of the first week and progress to eight invervals per session by the end of the second week. Recovery intervals will be four minutes in duration. Training will be performed on a cycle ergometer whereby one interval will be the equivalent to one Wingate test (30 sec all-out sprint cycle test against a constant resistance). The resistance will be set at 7.5% of the subject's body mass for the duration of the test.

Post-Testing Procedures Following the training protocol, you will perform a series of five post-training tests (i.e., VO_2max test, Repeated Wingate test, Cardiovascular Function Test, Performance Trial, Experimental Exercise Trial). These tests, including invasive procedures, will be identical in all respects to the first series of tests described above.

SUMMARY: LABORATORY VISITS, TESTING AND TRAINING PHASES:

(A) PRE-TRAINING PHASE: *5 tests will be conducted over 7-10 days:*

1. VO_2max Test (~ 1 hour)
2. Single Wingate Test (~ 15 min)
3. Cardiovascular Function Text (~ 30 min)
4. Performance Trial #1 (~ 15-60 min)
5. Experimental Exercise Trial #1 (~ 2 hours)

(B) TRAINING PHASE: *6 lab visits (30-60 min each) over 2 weeks (3x / week)*

(C) POST-TRANING PHASE: *5 tests will be conducted over ~7 days:*

1. VO_2max Test (~ 1 hour)
2. Repeated Wingate Test (~ 30 min)
3. Cardiovascular Function Text (~ 30 min)
4. Performance Trial #2 (~ 15-60 min)
5. Experimental Exercise Trial #2 (~ 2 hours)

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 $350.00 in order to compensate for your time commitment and effort. Remuneration is normally provided within one week following 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:

McMaster University

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

DESCRIPTION OF INVASIVE MEDICAL PROCEDURES

The study in which you are invited to participate involves several invasive medical procedures. Prior to your involvement in the study, 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 the muscle to be sampled will be cleaned and a small amount of local anesthetic ("freezing") will be injected into and under the skin over the vastus lateralis (quadriceps) muscle. 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 the muscle. 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. During the time that the sample is being taken (~5 sec), you may feel the sensation of deep pressure in the muscle 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 (stitch) 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 limb 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 muscle may feel uncomfortable upon movement, e.g., 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 ~1,000 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 ~2,000 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. There is also a theoretical but extremely small risk of damage to a small motor nerve branch leading to the muscle which is being sampled.

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: _____

Address: _____

Phone Number: _____ E-mail: _____

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 have kidney problems?

   YES NO

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

   YES NO

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

   YES NO

6. Have you ever had any allergies to medication?

YES NO

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

YES NO

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

YES NO

12. Are you currently taking ANY medication or have you taken any medication in the last two days? If yest, please indicate medication(s).

YES NO

13 Have you ever taken part is a study which involved needle biopsy sampling or the placement of arterial or venous catheters? If yes, please provide details in space below

YES NO

Please use the space below to transmit any additional comments you may have.

APPENDIX II

REPRODUCIBILITY OF BASELINE TESTS

REPRODUCIBILITY OF BASELINE TESTS
DAY-TO-DAY

VO2MAX ML/KG/MIN		DAY 1	DAY 2	DIFF
SUBJECT	S1	53.70	56.40	-2.7
	S2	49.10	53.90	-4.8
	S3	53.70	56.20	-2.5
	S4	49.60	50.30	-0.7
	S5	50.90	49.30	1.6
	S6	49.90	47.70	2.2
MEAN		**51.2**	**52.3**	**-1.2**
SD		**2.1**	**3.7**	**2.7**
METHOD ERROR				**1.9**
VARIABILITY				**3.7%**

CHALLENGE RIDE @ 85% SECONDS		DAY 1	DAY 2	DIFF
SUBJECT	S1	677	563	114.0
	S2	475	492	-17.0
	S3	1229	1460	-231.0
	S4	683	843	-160.0
	S5	262	240	22.0
	S6	333	300	33.0
MEAN		**609.8**	**649.7**	**-39.8**
SD		**349.0**	**450.9**	**129.8**
METHOD ERROR				**91.8**
VARIABILITY				**14.6%**

PEAK POWER (1 OF 1) WATTS		DAY 1	DAY 2	DIFF
SUBJECT	S1	978.00	1142.00	-164.0
	S2	1320.00	1247.00	73.0
	S3	1354.00	1322.00	32.0
	S4	713.00	691.00	22.0
	S5	1096.00	1042.00	54.0
	S6	1161.00	1144.00	17.0
MEAN		**1103.7**	**1098.0**	**5.7**
SD		**237.2**	**221.5**	**85.7**
METHOD ERROR				**60.6**
VARIABILITY				**5.5%**

PEAK POWER (4 OF 4) WATTS		DAY 1	DAY 2	DIFF
SUBJECT	S1	664.00	655.00	9.0
	S2	978.00	1067.00	-89.0
	S3	811.00	916.00	-105.0
	S4	551.00	570.00	-19.0
	S5	894.00	847.00	47.0
	S6	899.00	927.00	-28.0
MEAN		**799.5**	**830.3**	**-30.8**
SD		**161.8**	**185.2**	**57.8**
METHOD ERROR				**40.8**
VARIABILITY				**5.0%**

MEAN POWER (1 OF 1) WATTS		DAY 1	DAY 2	DIFF
SUBJECT	S1	649.00	646.40	2.6
	S2	731.10	738.50	-7.4
	S3	683.10	703.60	-20.5
	S4	452.90	487.30	-34.4
	S5	683.40	656.20	27.2
	S6	654.00	657.40	-3.4
MEAN		**642.3**	**648.2**	**-6.0**
SD		**97.3**	**86.3**	**21.0**
METHOD ERROR				**14.8**
VARIABILITY				**2.3%**

MEAN POWER (4 OF 4) WATTS		DAY 1	DAY 2	DIFF
SUBJECT	S1	649.00	646.40	2.6
	S2	731.10	738.50	-7.4
	S3	683.10	703.60	-20.5
	S4	452.90	487.30	-34.4
	S5	683.40	656.20	27.2
	S6	654.00	657.40	-3.4
MEAN		**642.3**	**648.2**	**-6.0**
SD		**97.3**	**86.3**	**21.0**
METHOD ERROR				**14.8**
VARIABILITY				**2.3%**

APPENDIX III

RAW DATA – SUBJECT CHARACTERISTICS

THESIS SUBJECT CHARACTERISTICS

SUBJECT	SEX	AGE	WEIGHT (kg)	HEIGHT (cm)
1	M	22	67	181.5
2	F	23	60	160.5
3	M	21	98	193.5
4	M	21	87.5	185
5	M	22	96	183
6	M	23	90.4	183
7	M	20	96	180
8	F	23	70	170
MEAN		**21.9**	**83.1**	**179.6**
STDV		**1.1**	**15.1**	**10.0**
SEM		**0.40**	**5.33**	**3.55**

APPENDIX IV

RAW DATA – ANAEROBIC AND AEROBIC PERFORMANCE

VO2 max test data

	VO2 rel (ml/kg/min)	
	Pre	Post
1	51.3	55.7
2	41.1	39.9
3	45.3	48.6
4	41.6	45.8
5	44.5	41.8
6	45.3	48.6
7	46.3	43
8	41	40.5
MEAN	44.6	45.5
SEM	1.1	1.8
STDEV	3.22	5.00

	VO2 abs (l/min)	
	Pre	Post
1	3.443	3.587
2	2.463	2.392
3	4.102	4.299
4	3.647	3.987
5	4.13	3.918
6	4.102	4.299
7	4.486	4.292
8	2.867	2.876
MEAN	3.655	3.706
SEM	0.2	0.2
STDEV	0.65	0.67

	RER max	
	Pre	Post
1	1.34	1.59
2	1.49	1.48
3	1.37	1.56
4	1.9	1.44
5	1.37	1.84
6	1.37	1.56
7	1.26	1.39
8	1.54	1.55
MEAN	1.46	1.55
SEM	0.1	0.0
STDEV	0.19	0.13

	Peak Power (Watts)	
	Pre	Post
1	250	275
2	300	325
3	350	375
4	325	375
5	250	275
6	350	375
7	350	375
8	325	350
MEAN	312.5	340.6
SEM	14.0	14.6
STDEV	39.53	41.34

	HR max (b/min)	
	Pre	Post
1	194	195
2	210	208
3	175	183
4	190	191
5	194	194
6	175	183
7	194	197
8	197	200
MEAN	191.1	193.9
SEM	3.8	2.8
STDEV	10.82	7.85

	VE max (l/min)	
	Pre	Post
1	112.1	113.6
2	111.6	104.4
3	136.9	172.6
4	154.3	151.5
5	187.1	223.5
6	136.9	172.6
7	204	187.6
8	102.2	115
MEAN	143.1	155.1
SEM	12.2	13.8
STDEV	34.41	39.15

Exercise Performance Trial

	Time to exhaustion (min)		
	Pre exh	Post exh	% CHNG
1	38.8	81.0	108.6
2	55.3	110.0	99.0
3	15.9	32.5	104.0
4	15.9	42.7	169.3
5	18.6	42.9	131.0
6	26.9	55.0	104.6
7	15.5	12.9	-16.6
8	18.8	33.9	81.0
MEAN	**25.7**	**51.4**	**99.9**
SEM	**14.4**	**30.7**	**53.2**
STDEV	**5.1**	**10.9**	**18.8**

ANAEROBIC PERFORMANCE

Peak Power - pre

SUBJECT	1	2	3	4	AVG
1	980	1019	704	777	870
2	778	679	601	532	647.5
3	1266	940	964	1021	1047.75
4	1201	1051	1004	952	1052
5	1231	1155	1199	1099	1171
6	976	1154	997	810	984.25
7	959	990	1016	890	963.75
8	795	716	704	738	738.25
MEAN	1023.3	963.0	898.6	852.4	934.3
SEM	67.4	63.7	72.4	63.2	61.4
STDEV	190.5	180.1	204.7	178.8	173.6

Peak Power - post

SUBJECT	1	2	3	4	AVG	% CHNG
1	1242	1063	939	926	1042.5	16.55
2	918	795	628	567	727	10.94
3	1599	1221	1383	1296	1374.75	23.79
4	1211	1129	1035	1072	1111.75	5.37
5	1301	1310	1357	1309	1319.25	11.24
6	1205	1208	1213	1083	1177.25	16.39
7	1007	985	1077	1012	1020.25	5.54
8	925	782	684	655	761.5	3.05
MEAN	1176.0	1061.6	1039.5	990.0	1067	11.61
SEM	80.1	69.2	99.7	95.1	82.8	2.5
STDEV	226.5	195.7	281.9	269.0	234.3	7.02

Mean Power - pre

SUBJECT	1	2	3	4	AVG
1	626	538	473	454	522
2	527	436	373	343	419
3	729	706	615	645	673
4	671	605	546	566	597
5	722	670	640	603	658
6	646	614	569	523	588
7	665	635	601	582	620
8	546	476	462	441	481
MEAN	641.5	585.0	534.9	519.6	569.8
SEM	26.1	33.2	32.3	35.5	31.3
STDEV	73.7	94.0	91.4	100.3	88.59

Mean Power - post

SUBJECT	1	2	3	4	AVG	% CHNG
1	649	545	487	503	546	4.40
2	556	462	424	395	459	8.71
3	688	636	579	615	630	-6.83
4	622	561	546	573	575	-3.83
5	728	716	697	694	709	7.19
6	693	635	593	585	626	6.07
7	582	579	611	582	588	-5.44
8	539	476	408	436	465	-3.44
MEAN	632.1	576.3	543.1	547.9	574.8	0.9
SEM	24.4	30.1	34.8	34.6	29.9	2.2
STDEV	69.1	85.2	98.3	97.8	84.64	6.33

% Fatigue - pre

SUBJECT	1	2	3	4	AVG
1	26.6	30.6	16.1	19.3	23.2
2	25.7	15.3	15.5	12.3	17.2
3	39.9	17.1	32.1	33.5	30.7
4	32.7	32.6	34.6	28.1	32.0
5	31.2	30.6	32.4	28	30.6
6	31.3	42.8	25.3	26	31.4
7	27.8	22.7	29.4	23.1	25.8
8	20.5	23.8	22.8	25.6	23.2
MEAN	29.5	26.9	26.0	24.5	26.7
SEM	2.0	3.2	2.6	2.3	1.9
STDEV	5.75	9.03	7.39	6.41	5.30

% Fatigue - post

SUBJECT	1	2	3	4	AVG	% CHNG
1	33.7	32.1	27.2	24.5	29.4	21.2
2	20.7	19.6	16.8	13.4	17.6	2.4
3	38.3	56.4	52.2	48.8	48.9	37.4
4	33.4	33.2	29.6	29.8	31.5	-1.6
5	33.9	34.3	38.7	33.4	35.1	12.9
6	32.9	36.9	40.2	33.4	35.9	12.6
7	27.8	31.8	31.8	27.9	29.8	13.7
8	25	20	25.6	19.5	22.5	-2.9
MEAN	30.7	33.0	32.8	28.8	31.3	11.9
SEM	2.0	4.0	3.8	3.7	3.3	4.7
STDEV	5.73	11.43	10.80	10.60	9.38	13.25

Anaerobic Power - pre

SUBJECT	1	2	3	4	AVG
1	14.6	15.2	10.5	11.6	10.3
2	13	11.3	10	8.9	9.1
3	12.9	9.6	9.8	10.4	8.8
4	13.6	11.9	11.4	10.8	10.2
5	12.6	11.8	12.2	11.2	10.4
6	10.8	12.8	11.1	8.9	10.2
7	10	10.3	10.6	9.3	9.5
8	11.4	10.2	10.1	10.5	9.9
MEAN	12.4	11.6	10.7	10.2	9.8
SEM	0.5	0.6	0.3	0.4	0.2
STDEV	1.52	1.78	0.81	1.04	0.60

Anaerobic Power - post

SUBJECT	1	2	3	4	AVG	% CHNG
1	18.5	15.9	14	13.8	15.6	33.6
2	15.3	13.3	10.5	9.4	12.1	25.2
3	12.5	16.3	14.1	13.2	14.0	37.1
4	13.8	12.8	11.8	12.2	12.7	19.2
5	13.3	13.4	13.8	13.4	13.5	22.8
6	13.4	13.4	13.5	12	13.1	22.2
7	10.5	10.3	11.2	10.5	10.6	10.8
8	13.2	11.2	9.8	9.4	10.9	8.9
MEAN	13.8	13.3	12.3	11.7	12.8	22.5
SEM	0.8	0.7	0.6	0.6	0.6	3.5
STDEV	2.32	2.05	1.72	1.77	1.62	9.81

Anaerobic Capacity - pre

SUBJECT	1	2	3	4	AVG
1	9.3	8	7.1	6.8	7.8
2	8.8	7.3	6.2	5.7	7.0
3	7.4	7.2	6.3	6.6	6.9
4	7.6	6.9	6.2	6.4	6.8
5	7.4	6.8	6.5	6.2	6.7
6	7.2	6.8	6.3	5.7	6.5
7	6.9	6.6	6.3	6.1	6.5
8	7.8	6.8	6.6	6.3	6.9
MEAN	7.8	7.1	6.4	6.2	6.9
SEM	0.3	0.2	0.1	0.1	0.1
STDEV	0.83	0.45	0.30	0.39	0.41

Anaerobic Capacity - post

SUBJECT	1	2	3	4	AVG	% CHNG
1	9.7	7.9	7.3	7.5	8.1	3.70
2	9.3	7.7	7.1	6.6	7.7	8.79
3	6.5	7	5.9	6.3	6.4	-7.00
4	7.1	6.4	6.2	6.5	6.6	-3.44
5	7.4	7.3	7.1	7.1	7.2	6.92
6	7.7	7.1	6.6	6.5	7.0	6.81
7	6.1	6	6.4	6.1	6.2	-5.28
8	7.7	6.8	5.8	6.2	6.6	-3.77
MEAN	7.7	7.0	6.6	6.6	7.0	0.8
SEM	0.4	0.2	0.2	0.2	0.2	2.2
STDEV	1.25	0.63	0.57	0.48	0.67	6.35

Invasive exercise trial (data averaged over 6-9min of exercise)

	VO2 rel (ml/kg/min)	
	Pre	Post
1	45.6	41.3
2	34.2	30.6
3	32.1	32.1
4	32.6	34.6
5	35.3	36.4
6	36.3	36.7
7	34.4	31.1
8	33.3	35.3
MEAN	**35.5**	**34.8**
SEM	**1.5**	**1.2**
STDEV	**4.3**	**3.5**

	% VO2max	
	Pre	Post
1	88.9	74.1
2	83.2	76.7
3	70.9	66.0
4	78.4	75.5
5	79.3	87.1
6	80.1	75.5
7	74.3	72.3
8	81.2	87.2
MEAN	**79.5**	**76.8**
SEM	**5.5**	**7.2**
STDEV	**1.9**	**2.5**

	RER	
	Pre	Post
1	1.17	1.17
2	1.15	1.04
3	1.32	1.26
4	1.28	1.22
5	1.25	1.19
6	1.26	1.17
7	1.31	1.22
8	1.2	1.13
MEAN	**1.24**	**1.18**
SEM	**0.02**	**0.02**
STDEV	**0.06**	**0.07**

	VE (l/min)	
	Pre	Post
1	81.3	73
2	70.9	59.1
3	107.5	104.1
4	123.1	105.5
5	127.6	113.4
6	104.7	96
7	135.7	102
8	78.5	75.3
MEAN	**103.7**	**91.1**
SEM	**8.7**	**6.8**
STDEV	**24.5**	**19.3**

APPENDIX V

RAW DATA – MUSCLE METABOLITES, BLOOD METABOLITES, MUSCLE ENZYMES

MUSCLE METABOLITES

CREATINE (mmol/kg dw)

Subject	PRE - 0	PRE - 1	PRE - 15	POST - 0	POST - 1	POST - 15
S1	34.5	62.4	97.3	36.7	62.4	94.3
S2	35.5	47.0	76.1	29.6	40.0	56.4
S3	38.2	73.9	57.9	39.5	66.6	85.6
S4	22.0	56.1	97.3	19.0	57.8	66.2
S5	33.2	72.2	91.2	31.4	58.4	93.6
S6	25.9	47.6	89.8	21.1	30.1	73.6
S7	24.0	50.6	64.7	21.2	70.6	92.6
S8	22.5	40.3	56.0	14.1	45.6	47.2
MEAN	**29.5**	**56.3**	**78.8**	**26.6**	**53.9**	**76.2**
SD	**6.5**	**12.3**	**17.4**	**9.1**	**14.0**	**18.2**
SEM	**2.3**	**4.3**	**6.1**	**3.2**	**5.0**	**6.4**

PCR (mmol/kg dw)

Subject	PRE - 0	PRE - 1	PRE - 15	POST - 0	POST - 1	POST - 15
S1	77.3	49.4	14.5	75.1	49.4	17.5
S2	87.9	76.4	47.3	93.8	83.4	67.0
S3	93.1	57.4	29.3	91.8	64.7	45.7
S4	85.5	51.4	10.2	88.5	49.7	41.3
S5	74.8	35.8	16.8	76.6	49.6	14.4
S6	80.0	58.3	16.1	84.8	75.8	32.3
S7	77.9	51.3	37.2	80.7	31.3	9.3
S8	68.9	51.1	35.4	77.3	45.8	44.2
MEAN	**80.7**	**53.9**	**25.9**	**83.6**	**56.2**	**34.0**
SD	**7.8**	**11.4**	**21.5**	**7.2**	**17.1**	**19.5**
SEM	**2.7**	**4.0**	**7.6**	**2.6**	**6.1**	**6.9**

TOTAL CREATINE (mmol/kg dw)

Subject	PRE - 0	PRE - 1	PRE - 15	POST - 0	POST - 1	POST - 15
S1	111.8	111.8	111.8	111.8	111.8	111.8
S2	123.4	123.4	123.4	123.4	123.4	123.4
S3	131.3	131.3	131.3	131.3	131.3	131.3
S4	107.5	107.5	107.5	107.5	107.5	107.5
S5	108.0	108.0	108.0	108.0	108.0	108.0
S6	105.9	105.9	105.9	105.9	105.9	105.9
S7	101.9	101.9	101.9	101.9	101.9	101.9
S8	91.4	91.4	91.4	91.4	91.4	91.4
MEAN	**110.1**	**110.1**	**110.1**	**110.1**	**110.1**	**110.1**
SD	**12.4**	**12.4**	**12.4**	**12.4**	**12.4**	**12.4**
SEM	**4.4**	**4.4**	**4.4**	**4.4**	**4.4**	**4.4**

ATP (mmol/kg dw)

Subject	PRE - 0	PRE - 1	PRE - 15	POST - 0	POST - 1	POST - 15
S1	24.9	29.7	25.5	18.6	21.6	18.3
S2	22.9	***28.5***	***24.8***	24.7	23.6	24.3
S3	21.9	***27.2***	26.7	27.2	25.3	***26.4***
S4	23.4	30.2	18.8	30.6	22.3	27.0
S5	26.8	25.3	18.9	21.1	19.6	16.5
S6	24.3	26.1	25.3	27.5	28.4	27.8
S7	25.3	***32.6***	33.7	25.9	25.6	18.6
S8	21.9	34.3	32.4	18.3	27.0	36.3
MEAN	**23.9**	**29.3**	**25.8**	**24.2**	**24.2**	**24.4**
SD	**1.7**	**5.1**	**7.5**	**4.5**	**2.9**	**10.8**
SEM	**0.6**	**1.8**	**2.7**	**1.6**	**1.0**	**3.8**

LACTATE (mmol/kg dw)

Subject	PRE - 0	PRE - 1	PRE - 15	POST - 0	POST - 1	POST - 15
S1	4.5	32.8	131.1	6.2	59.5	96.2
S2	6.2	30.5	97.7	10.2	24.2	60.1
S3	6.3	43.4	105.5	8.7	19.1	150.5
S4	7.4	47.8	72.2	8.3	41.0	79.2
S5	17.9	34.0	98.5	7.7	31.0	61.3
S6	8.6	14.9	98.5	5.2	10.0	47.5
S7	7.2	53.1	41.8	5.2	47.2	69.5
S8	3.3	79.7	105.8	5.1	105.5	113.3
MEAN	**7.7**	**42.0**	**93.9**	**7.1**	**42.2**	**84.7**
SD	**4.5**	**19.3**	**26.5**	**1.9**	**30.1**	**34.0**
SEM	**1.6**	**6.8**	**9.4**	**0.7**	**10.7**	**12.0**

MALATE (mmol/kg dw)

Subject	PRE - 0	PRE - 1	PRE - 15	POST - 0	POST - 1	POST - 15
S1	0.15	2.84	1.81	0.14	1.34	1.23
S2	0.17	1.29	1.69	0.45	1.15	1.31
S3	0.12	1.77	1.56	0.20	1.27	4.51
S4	0.50	1.23	1.79	0.27	1.28	2.77
S5	0.47	1.27	3.56	0.49	1.71	2.05
S6	0.22	0.85	2.48	0.32	2.10	4.52
S7	0.48	4.29	2.63	0.27	3.45	4.07
S8	0.16	2.76	2.58	0.34	3.55	4.61
MEAN	**0.28**	**2.04**	**2.26**	**0.31**	**1.98**	**3.13**
STDV	**0.17**	**1.17**	**0.68**	**0.12**	**0.99**	**1.47**
SEM	**0.06**	**0.41**	**0.24**	**0.04**	**0.35**	**0.52**

GLYCOGEN (mmol/kg dw)

SUBJECT	pre - 0	pre -15	post - 0	post - 15
S1	567.5	533.4	653.3	599.8
S2	493.5	***363.0***	682.8	679.2
S3	456.7	331.1	732.5	***652.3***
S4	459.4	255.7	711.1	579.0
S5	838.4	393.2	621.3	417.4
S6	325.8	284.7	387.3	280.7
S7	441.8	335.4	559.9	553.5
S8	328.6	248.7	563.2	611.2
MEAN	**489.0**	**343.2**	**613.9**	**546.7**
STDEV	**162.5**	**92.0**	**111.3**	**133.2**
SEM	**57.4**	**32.5**	**39.4**	**47.1**

BLOOD METABOLITES

BLOOD GLUCOSE (mmol/L)

Subject	pre - 0	pre - 5	pre - 10	pre - 15	post - 0	post - 5	post - 10	post - 15
S1	6.06	4.79	5.22	5.23	4.58	4.79	5.23	5.35
S2								
S3	4.43	3.89	3.45	3.44	4.75	4.27	4.08	4.07
S4	3.82	3.36	3.78	4.24	4.79	4.16	4.36	4.49
S5	4.94	4.24	4.25	3.93	4.13	4.75	4.37	4.37
S6	6.72	4.28	3.76	3.67	4.98	4.62	3.94	3.66
S7	4.51	3.93	4.23	4.40	4.61	4.30	4.29	4.48
S8	5.30	4.86	4.99	5.56	5.42	5.39	5.73	5.60
MEAN	**5.11**	**4.19**	**4.24**	**4.35**	**4.75**	**4.61**	**4.57**	**4.58**
STDEV	**1.00**	**0.53**	**0.66**	**0.79**	**0.39**	**0.42**	**0.65**	**0.69**
SEM	**0.38**	**0.20**	**0.25**	**0.30**	**0.15**	**0.16**	**0.25**	**0.26**

BLOOD LACTATE (mmol/L)

SUBJECT	pre - 0	pre - 5	pre - 10	pre - 15	post - 0	post - 5	post - 10	post - 15
S1	0.75	3.18	8.11	7.79	0.19	3.37	7.86	9.78
S2								
S3	0.39	2.07	4.01	4.88	0.45	1.99	4.25	5.15
S4	0.79	2.19	7.35	7.54	0.48	3.69	6.55	6.47
S5	1.30	2.82	5.99	6.51	0.64	3.43	7.09	7.80
S6	0.69	3.10	4.95	4.45	0.04	1.93	4.63	5.04
S7	0.23	1.70	5.66	5.84	0.45	1.88	5.01	7.24
S8	0.18	3.13	5.25	7.13	0.41	3.86	6.76	7.52
MEAN	**0.62**	**2.60**	**5.90**	**6.31**	**0.38**	**2.88**	**6.02**	**7.00**
SEM	**0.15**	**0.23**	**0.53**	**0.49**	**0.07**	**0.34**	**0.52**	**0.62**
STDEV	**0.39**	**0.60**	**1.41**	**1.30**	**0.20**	**0.90**	**1.38**	**1.65**

MUSCLE ENZYMES

CITRATE SYNTHASE (mmol/kg wwt)

Subject	PRE - 1	POST - 1
S1	11.76	10.78
S2	11.21	15.81
S3	9.50	14.77
S4	15.75	22.06
S5	15.01	18.14
S6	11.03	18.38
S7	5.94	27.02
S8	0.67	6.50
MEAN	**10.11**	**16.68**
STDEV	**4.89**	**6.37**
SEM	**1.73**	**2.25**

ALANINE AMINOTRANSFERASE (mmol/kg wwt)

Subject	PRE - 1	POST - 1
S1	1.57	2.02
S2	1.78	2.62
S3	2.91	3.29
S4	2.97	4.82
S5	2.68	3.73
S6	5.07	5.58
S7	1.09	2.73
S8	0.06	1.14
MEAN	**2.27**	**3.24**
STDEV	**1.51**	**1.45**
SEM	**0.53**	**0.51**

APPENDIX VI

STATISTICAL TABLES

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

CREATINE (mmol/kg dwt)

ANOVA: two factor (Pre/Post training x Time)

Summary of all Effects

1-TRAINING, 2-TIME

	df Effect	MS Effect	df Error	MS Error	F	p-level
1	1	80.53127	7	140.9388	0.571392	0.47437
2	2	9815.477	14	149.8109	65.51912	*7.81E-08*
12	2	0.30435	14	82.15115	0.003705	0.996303

Tukey HSD test

Probabilities for Post Hoc Tests

	PRE - 0	PRE - 1	PRE - 15	POST - 0	POST - 1	POST - 15
MEAN	**29.45527**	**56.25349**	**78.76511**	**26.57932**	**53.92810**	**76.19479**
PRE - 0		*0.000557*	*0.000158*	0.986307	*0.001164*	*0.000158*
PRE - 1	*0.000557*		*0.002381*	*0.000286*	0.994795	*0.006483*
PRE - 15	*0.000158*	*0.002381*		*0.000158*	*0.001028*	0.991759
POST - 0	0.986307	*0.000286*	*0.000158*		*0.00048*	*0.000158*
POST - 1	*0.001164*	0.994795	*0.001028*	*0.00048*		*0.002612*
POST - 15	*0.000158*	*0.006483*	0.991759	*0.000158*	*0.002612*	

ATP (mmol/kg dwt)

ANOVA: two factor (Pre/Post training x Time)

Summary of all Effects

1-TRAINING, 2-TIME

	df Effect	MS Effect	df Error	MS Error	F	p-level
1	1	87.44682	7	51.68001	1.692082	0.234508
2	2	73.46667	14	33.66474	2.182303	0.149632
12	2	79.03458	14	26.34871	2.999562	0.08238

Tukey HSD test

Probabilities for Post Hoc Tests

	PRE - 0	PRE - 1	PRE - 15	POST - 0	POST - 1	POST - 15
MEAN	**23.93358**	**31.97849**	**27.74278**	**24.24272**	**24.17333**	**27.14034**
PRE - 0		0.064897	0.679018	0.999996	0.999999	0.806102
PRE - 1	0.064897		0.582064	0.080185	0.076485	0.449054
PRE - 15	0.679018	0.582064		0.746654	0.731827	0.999883
POST - 0	0.999996	0.080185	0.746654		1	0.861494
POST - 1	0.999999	0.076485	0.731827	1		0.849825
POST - 15	0.806102	0.449054	0.999883	0.861494	0.849825	

MALATE (mmol/kg dwt)

ANOVA: two factor (Pre/Post training x Time)

Summary of all Effects

1-TRAINING, 2-TIME

	df Effect	MS Effect	df Error	MS Error	F	p-level
1	1	0.949925	7	0.656451	1.447062	0.268103
2	2	24.45467	14	0.858197	28.49541	**1.16E-05**
12	2	1.054464	14	0.475626	2.217003	0.145733

Tukey HSD test

Probabilities for Post Hoc Tests

	PRE - 0	PRE - 1	PRE - 15	POST - 0	POST - 1	POST - 15
MEAN	.2843150	2.037418	2.261743	.3102370	1.983151	3.134151
PRE - 0		0.001953	0.000711	1	0.002553	0.000163
PRE - 1	0.001953		0.984706	0.002216	0.999984	0.05981
PRE - 15	0.000711	0.984706		0.00079	0.961342	0.180344
POST - 0	1	0.002216	0.00079		0.002906	0.000163
POST - 1	0.002553	0.999984	0.961342	0.002906		0.045135
POST - 15	0.000163	0.05981	0.180344	0.000163	0.045135	

LACTATE (mmol/kg dwt)

ANOVA: two factor (Pre/Post training x Time)

Summary of all Effects

1-TRAINING, 2-TIME

	df Effect	MS Effect	df Error	MS Error	F	p-level
1	1	240.5739	7	179.0065	1.343939	0.284352
2	2	23779.08	14	558.9364	42.54345	***1.12E-06***
12	2	216.8625	14	170.3886	1.272753	0.310553

Tukey HSD test

Probabilities for Post Hoc Tests

	PRE - 0	PRE - 1	PRE - 15	POST - 0	POST - 1	POST - 15
MEAN	**7.682434**	**42.03594**	**90.83858**	**7.065629**	**42.18839**	**77.87051**
PRE - 0		*0.001456*	*0.000158*	0.999999	*0.001403*	*0.000158*
PRE - 1	*0.001456*		*0.00018*	*0.001248*	1	*0.001013*
PRE - 15	*0.000158*	*0.00018*		*0.000158*	*0.000181*	0.395627
POST - 0	0.999999	*0.001248*	*0.000158*		*0.001198*	*0.000158*
POST - 1	*0.001403*	1	*0.000181*	*0.001198*		*0.001049*
POST - 15	*0.000158*	*0.001013*	0.395627	*0.000158*	*0.001049*	

PHOSPHOCREATINE (mmol/kg dwt)

ANOVA: two factor (Pre/Post training x Time)

Summary of all Effects

1-TRAINING, 2-TIME

	df Effect	MS Effect	df Error	MS Error	F	p-level
1	1	80.53127	7	140.9388	0.571392	0.47437
2	2	9815.477	14	149.8109	65.51912	***7.81E-08***
12	2	0.30435	14	82.15115	0.003705	0.996303

Tukey HSD test

Probabilities for Post Hoc Tests

	PRE - 0	PRE - 1	PRE - 15	POST - 0	POST - 1	POST - 15
MEAN	**80.69224**	**53.89401**	**31.38239**	**83.56818**	**56.21939**	**33.95271**
PRE - 0		*0.000557*	*0.000158*	0.986307	*0.001164*	*0.000158*
PRE - 1	*0.000557*		*0.002381*	*0.000286*	0.994795	*0.006483*
PRE - 15	*0.000158*	*0.002381*		*0.000158*	*0.001028*	0.991759
POST - 0	0.986307	*0.000286*	*0.000158*		*0.00048*	*0.000158*
POST - 1	*0.001164*	0.994795	*0.001028*	*0.00048*		*0.002612*
POST - 15	*0.000158*	*0.006483*	0.991759	*0.000158*	*0.002612*	

GLYCOGEN (mmol/ kg dwt)

ANOVA: two factor (Pre/Post training x Time)

Summary of all Effects

1-TRAINING, 2-TIME

	df Effect	MS Effect	df Error	MS Error	F	p-level
1	1	215769.8	7	21886.5	9.858577	***0.016383***
2	1	90789.2	7	9798.366	9.265748	***0.018742***
12	1	12338.94	7	2259.147	5.46177	0.052071

Tukey HSD test; variable Var.1

Probabilities for Post Hoc Tests

	PRE - 0	PRE - 15	POST - 0	POST - 15
MEAN	**488.9583**	**343.1553**	**613.9146**	**546.6575**
PRE - 0		*0.002197*	*0.005116*	0.158646
PRE - 15	*0.002197*		*0.000275*	*0.000454*
POST - 0	*0.005116*	*0.000275*		0.093661
POST - 15	0.158646	*0.000454*	0.093661	

BLOOD GLUCOSE (mmol/L)

ANOVA: two factor (Pre/Post training x Time)

Summary of all Effects

1-TRAINING, 2-TIME

	df Effect	MS Effect	df Error	MS Error	F	p-level
1	1	0.326538	6	0.290432	1.124319	0.329796
2	3	0.913231	18	0.305049	2.993715	0.058157
12	3	0.433806	18	0.106203	4.084691	***0.0224***

Tukey HSD test

Probabilities for Post Hoc Tests

	PRE - 0	PRE - 1	PRE - 5	PRE - 15	POST - 0	POST - 1	POST - 5	POST - 15
MEAN	**5.112946**	**4.194213**	**4.240833**	**4.352007**	**4.751476**	**4.613483**	**4.570895**	**4.575034**
PRE - 0		*0.00119*	*0.001994*	*0.007179*	0.464602	0.138682	0.088902	0.092924
PRE - 1	*0.00119*		0.999993	0.981621	0.075433	0.295222	0.415899	0.403118
PRE - 5	*0.001994*	0.999993		0.997719	0.123706	0.428535	0.570749	0.556469
PRE - 15	*0.007179*	0.981621	0.997719		0.348229	0.797455	0.903234	0.894764
POST - 0	0.464602	0.075433	0.123706	0.348229		0.991471	0.96203	0.966368
POST - 1	0.138682	0.295222	0.428535	0.797455	0.991471		0.999996	0.999998
POST - 5	0.088902	0.415899	0.570749	0.903234	0.96203	0.999996		1
POST - 15	0.092924	0.403118	0.556469	0.894764	0.966368	0.999998	1	

BLOOD LACTATE (mmol/L)

ANOVA: two factor (Pre/Post training x Time)

Summary of all Effects

1-TRAINING, 2-TIME

	df Effect	MS Effect	df Error	MS Error	F	p-level
1	1	0.63973	6	0.310043	2.063357	0.2009
2	3	115.3895	18	1.029884	112.0412	***7.8E-12***
12	3	0.524626	18	0.330287	1.588392	0.227016

Tukey HSD test

Probabilities for Post Hoc Tests

	PRE - 0	PRE - 1	PRE - 5	PRE - 15	POST - 0	POST - 1	POST - 5	POST - 15
MEAN	**.6187232**	**2.597679**	**5.901840**	**6.305274**	**.3792165**	**2.878731**	**6.021186**	**6.999436**
PRE - 0		*0.000252*	*0.000162*	*0.000162*	0.992233	*0.000184*	*0.000162*	*0.000162*
PRE - 1	*0.000252*		*0.000162*	*0.000162*	*0.000188*	0.980574	*0.000162*	*0.000162*
PRE - 5	*0.000162*	*0.000162*		0.882334	*0.000162*	*0.000162*	0.999911	*0.036376*
PRE - 15	*0.000162*	*0.000162*	0.882334		*0.000162*	*0.000162*	0.979389	0.36493
POST - 0	0.992233	*0.000188*	0.000162	*0.000162*		*0.000163*	*0.000162*	*0.000162*
POST - 1	*0.000184*	0.980574	0.000162	*0.000162*	*0.000163*		*0.000162*	*0.000162*
POST - 5	*0.000162*	*0.000162*	0.999911	0.979389	*0.000162*	*0.000162*		0.07755
POST - 15	*0.000162*	*0.000162*	*0.036376*	0.36493	*0.000162*	*0.000162*	0.07755	

CITRATE SYNTHASE
ANOVA: one factor (Pre/ Post training)

Summary of all Effects
1-TRAINING

	df Effect	MS Effect	df Error	MS Error	F	p-level
1	1	172.7485	7	20.44294	8.450277	0.02276

ALANINE AMINOTRANSFERASE
ANOVA: one factor (Pre/ Post training)

Summary of all Effects
1-CONDITION

	df Effect	MS Effect	df Error	MS Error	F	p-level
1	1	3.798077	7	0.147933	25.67429	0.001452

VO2peak TEST

VO2peak

T-test (dependent samples)

	Mean	Std.Dv.	N	Diff.	Std.Dv.Dif	t	df	p
VAR1	44.55	3.44259						
VAR2	45.4875	5.340529	8	-0.9375	3.198633	-0.829	7	0.434461

HRmax

T-test (dependent samples)

	Mean	Std.Dv.	N	Diff.	Std.Dv.Dif	t	df	p
VAR1	191.125	11.56889						
VAR2	193.875	8.391109	8	-2.75	3.615443	-2.15138	7	0.068474

VEmax

T-test (dependent samples)

	Mean	Std.Dv.	N	Diff.	Std.Dv.Dif	t	df	p
VAR1	143.1375	36.78738						
VAR2	155.1	41.85181	8	-11.9625	21.46046	-1.57662	7	0.158887

RERmax

T-test (dependent samples)

	Mean	Std.Dv.	N	Diff.	Std.Dv.Dif	t	df	p
VAR1	1.455	0.199786						
VAR2	1.55125	0.135482	8	-0.09625	0.26997	-1.00839	7	0.346844

AEROBIC POWER

T-test (dependent samples)

	Mean	Std.Dv.	N	Diff.	Std.Dv.Dif	t	df	p
VAR1	312.5	42.25771						
VAR2	340.625	44.19417	8	-28.125	8.838835	-9	7	**4.27E-05**

INVASIVE EXERCISE TRIAL

AVG VO2 (6-9 min)

T-test (dependent samples)

	Mean	Std.Dv.	N	Diff.	Std.Dv.Dif	t	df	p
VAR1	35.475	4.316						
VAR2	34.7625	3.53187	8	0.7125	2.609016	0.772419	7	0.465141

AVG VE (6-9 min)

T-test (dependent samples)

	Mean	Std.Dv.	N	Diff.	Std.Dv.Difi	t	df	p
PRE	103.6625	24.49384						
POST	91.05	19.33797	8	12.6125	9.856896	3.619145	7	**0.00852**

AVG RER (6-9 min)

T-test (dependent samples)

	Mean	Std.Dv.	N	Diff.	Std.Dv.Difi	t	df	p
PRE	1.2425	0.063189						
POST	1.175	0.067401	8	0.0675	0.032842	5.813321	7	***0.000655***

CHALLENGE RIDE

TIME TO EXHAUSTION (min)

T-test (dependent samples)

Summary of all Effects

1-CONDITION

	Mean	Std.Dv.	N	Diff.	Std.Dv.Difl	t	df	p
PRE	25.6975	14.36489						
POST	51.36375	30.73832	8	-25.6663	17.38514	-4.1757	7	**0.004159**