Metabolic responses to supramaximal exercise and training
A GENDER COMPARISON

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DECLARATION

This work has not previously been submitted for a degree or diploma in any university. To the best of my knowledge and belief, the thesis contains no material previously published or written by another person except where due references is made in the thesis itself.

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Clare L. Weber
“It is considered that a woman is only about 50 per cent. as efficient athletically as a man, and there are plenty of avenues for her energy without invading the spheres which men have developed. I would seriously recommend those [women] who will take part in sports to practice regularly a set of scientifically designed exercises everyday to build up any weak sections.”

PROFESSOR CLARENCE A. WEBER
VICTORIAN HEALTH & STRENGTH COLLEGE
Many experts in the field argued that the early 1900’s was the most significant era for women in sports. At that time, there was not only an increase in participation, but women’s involvement in sports increased at the Olympic level. Indeed, since 1930, female athletes have narrowed the gender gap in athletic performance. However, my interest in the area of gender differences in metabolism arose from observations of the remaining ~10% margin that exists between male and female performance in sprint-type events.

Sydney, Australia, 2000; Marion Jones had barely finished her victory lap when Maurice Greene joined her as an Olympic 100-m champion. I was curious as to why the fastest female sprinter over 100 m was taller than the fastest male sprinter, yet weighed 10 kg lighter. Could the 0.88 s margin in their medal winning performances be solely accounted for by a difference in lean body mass? Of even greater interest to me was the wider margin evident between men and women in the middle-distance events; events that virtually exhaust the capacity of the anaerobic energy systems while demanding a maximal rate of oxygen uptake.

Intuitively, it seems reasonable to suggest that investigations into gender differences in metabolism are important. In reality, evidence is required to support the suggestion that differences in both anaerobic and aerobic metabolism exist between men and women during exercise. Metabolic disparity between men and women may contribute to the varying success of exercise training programs and to athletic performance. Consequently, there is a need for additional studies that examine the effects of gender on the metabolic response to exercise and training. As researchers have previously asked whether what is true of men is also true of women, the answer has been virtually the same in every case - not precisely.

When I began this work, the purpose was to compare the maximal capacity of the anaerobic energy systems between men and women. After examining the data collected before and after training, it was apparent that aside from the anaerobic energy systems, aerobic metabolism made an important contribution to successful performance during sprint-type activity. At that point, I considered the rate response of pulmonary oxygen uptake in an effort to gain a greater understanding of aerobic
metabolism at the onset of exhaustive, short-duration exercise. Thus, the focus of this work is across two principal areas of measurement: anaerobic capacity and oxygen uptake kinetics. Furthermore, the two key themes that are consistent throughout this thesis are gender and supramaximal exercise. These four expressions are described below.

**Anaerobic capacity** is the maximum amount of adenosine triphosphate that can be regenerated anaerobically during a single bout of exercise. Here, anaerobic capacity is determined by measuring the maximal accumulated oxygen deficit during exhaustive, short-duration exercise. **Oxygen uptake kinetics** reflect the readjustment of oxidative phosphorylation to meet the new ATP demand after a step increase in work rate. In the present thesis, pulmonary oxygen uptake is described using mathematical modeling. **Gender** is used in preference to 'sex' throughout this thesis. There has been some discussion as to the terminology of 'sex' versus 'gender'. However, I am not aware of the technically correct expression. **Supramaximal exercise**: to the well-established cardiorespiratory physiologist, heavy or severe exercise may be characterized by exercise performed at any intensity that is above the work rate achieved at the ventilatory threshold. Thus, I have chosen to use the term 'supramaximal' specifically to describe exercise that is performed at an intensity greater than that which corresponds to the maximal rate of oxygen uptake.

This thesis is written in six parts: Chapter 1 provides an overview to anaerobic and aerobic metabolism. The maximal accumulated oxygen deficit and the oxygen uptake response to supramaximal exercise are explored as indirect measures of anaerobic and aerobic ATP production, respectively. Gender-specific differences in the mechanisms that drive anaerobic and aerobic metabolism is the primary focus and the research aims are presented in the final section of Chapter 1. Chapter 2, 3 and 4 represent three research experiments that systematically address the research aims. These chapters include independent introduction, method, result and discussion sections. Chapter 2 is a comprehensive evaluation of the reliability of the maximal accumulated oxygen deficit measured at two exercise intensities in both men and women. Chapter 3 examines the changes in the maximal accumulated...
oxygen deficit and compares anaerobic and aerobic metabolism before and after short-duration, high-intensity interval training in both men and women. Chapter 4 focuses on the oxygen uptake response in men and women at the onset of moderate-intensity and supramaximal exercise before and after 8 wk of high-intensity interval training. Chapter 5 presents a statement of conclusions that summarize the findings of all the experiments. Chapter 6 provides information to assist the reader in setting up a laboratory, in testing a subject, and making the necessary calculations. This will help to enhance the reader's understanding of the technical aspects of the measurements and calculations used in this thesis. In addition, the reader will find justification for the methods used throughout this thesis and a description of any limitations or assumptions accepted in this thesis.

In summary, when the current exercise physiology curriculum is reconsidered, it should not simply add paragraphs to every section of the textbook describing women's unique response to exercise, nor should it simply offer a single lesson on the female athlete presented in a chapter entitled 'special population groups'. Rather, it should challenge and revise existing views on the response to exercise and training for each gender, having demonstrated that it will truly make a difference to current practice. However, if we are going to move ahead, it is essential to respond to the most important question asked by the skeptic: “Where is the evidence?”

Clare L. Weber
I am delighted to acknowledge my colleagues, friends and family for the support and inspiration I have received over the period of my doctoral candidature. To my chief supervisor, Dr Donald Schneider, thank you for this productive partnership. Your perseverance for good research is exceptional and your ability to absorb my frustrations is remarkable. To Professor Gregory Gass, thank-you for creating this wonderful environment that facilitates inquiry and discovery. I truly enjoyed our conversations on the topics of altitude training and national politics. The commitment that you offer your students is outstanding.

I would like to thank Professor Lewis Adams for making positive criticisms and offering valuable encouragement during the critical stages of this work. To the School of Physiotherapy and Exercise Science administration staff, thank you for always finding time for me. In addition, I will be forever grateful to the participants
involved in each of these experiments for their dedication, exertion and genuine interest.

To my colleagues Justin Keogh, Dale Lovell, and Surendran Sabapathy, I will always cherish the time we spent in deep discussion, drowned in laughter or simply sharing the harmony of our office. To my precious friends overseas and interstate, your detailed descriptions of the African Horizon, Greek Island Sunsets, and the mystery of the Egyptian Pyramids at times had me restless for adventure. Thank-you for believing in me and offering continual encouragement and support that helped me to remain focused.

Finally, I would like to thank those closest to me. I am forever grateful to my parents for their absolute confidence in me. Your guidance and enthusiasm offer the perfect balance between providing direction and encouraging independence. Most importantly, I thank Shaun, who has shown untiring patience and support, reminding me of my priorities and keeping things in perspective. Your unique way of seeing the world kept me optimistic and made me happy.

For the errors that may have crept into this thesis, I alone am responsible.
The primary aim of this thesis was to investigate the gender-specific responses to supramaximal cycling and to examine the changes in anaerobic and aerobic metabolism that occur in response to high-intensity interval training (HIT). All subjects in the present experiments were untrained, healthy young adults aged between 18 and 35 yr. Cycle ergometry was used for all experimental test procedures and training programs. The accumulated oxygen ($\text{AO}_2$) deficit was used to quantify the production of adenosine triphosphate (ATP) via anaerobic metabolism during supramaximal cycling. In addition, pulmonary oxygen uptake ($\text{VO}_2$) measured at the onset of exercise was described using mathematical modeling to determine the rate response of the aerobic energy system during exercise.

The purpose of experiment one was to examine the test-retest reliability of the maximal accumulated oxygen deficit (MAOD) measured at 110% and 120% of peak
oxygen uptake ($\dot{V}O_2$peak) for cycling in seven untrained male and seven untrained female subjects. After one familiarization trial, all subjects performed two MAOD tests at a power output corresponding to 110% and two tests at 120% of $\dot{V}O_2$peak in random order. MAOD was calculated for each subject as the difference between the estimated AO$_2$ demand and the AO$_2$ uptake measured during the exercise bout. The mean±standard error time to exhaustion (TE) for the group was not significantly different between trial one (226±13 s) and trial two (223±14 s) of the 110% test. Likewise, the difference in the TE between trial one (158±11 s) and trial two (159±10 s) was not significant for the 120% test. The intra-class correlation coefficients for the TE were 0.95 for the 110% test and 0.98 for the 120% test. The mean MAOD value obtained in trial one (2.62±0.17 L) was not significantly different from the mean value obtained in trial two (2.54±0.19 L) for the 110% test. Additionally, the mean values for the two trials did not differ significantly for MAOD (2.64±0.21 L for trial one and 2.63±0.19 L for trial two) in the 120% test. The intra-class correlation coefficients for MAOD were 0.95 for the 110% test and 0.97 for the 120% test. All intra-class correlation coefficients were significant at p < 0.001. When conducted under standardized conditions, the determination of MAOD for cycling was highly repeatable at both 110% and 120% of $\dot{V}O_2$peak in untrained male and female subjects.

The results observed in experiment one suggest that the MAOD may be used to compare the anaerobic capacity (AC) of men and women and to examine changes in the contribution of the anaerobic energy systems before and after training. Experiment two examined the gender-specific differences in MAOD before and after 4 and 8 wk of HIT. Untrained men (n=7) and women (n=7) cycled at 120% of pre-training $\dot{V}O_2$peak to exhaustion (MAOD test) pre-, mid-, and post-training. A post-training timed test was also completed at the MAOD test power output, but this test was stopped at the TE achieved during the pre-training MAOD test. The 14.3±5.2% increase in MAOD observed in males after 4 wk of training was not different from the 14.0±3.0% increase seen in females (p > 0.05). MAOD increased by a further 6.6±1.9% in males and this change was not different from the additional 5.1±2.3% increase observed in females after the final 4 wk of training. Peak $\dot{V}O_2$ measured during incremental cycling increased significantly (p < 0.01) in male but not in female
subjects after 8 wk of training. Moreover, the AO\textsubscript{2} uptake was higher in men during the post-training timed test compared to the pre-training MAOD test (p < 0.01). In contrast, the AO\textsubscript{2} uptake was unchanged from pre- to post-training in female subjects. The increase in MAOD with training was not different between men and women suggesting an enhanced ability to produce ATP anaerobically in both groups. However, the increase in VO\textsubscript{2} peak and AO\textsubscript{2} uptake obtained in male subjects following training indicates improved oxidative metabolism in men but not in women. It was concluded that there are basic gender differences that may predispose males and females to specific metabolic adaptations following an 8-wk period of HIT.

Increases in AO\textsubscript{2} uptake during supramaximal cycling demonstrated in men after training led to the hypothesis that VO\textsubscript{2} kinetics are speeded in male subjects with short-term HIT. It was suggested that training does not improve VO\textsubscript{2} kinetics in women as no change in AO\textsubscript{2} uptake was found after 8 wk of HIT in female subjects. The purpose of experiment three was to examine VO\textsubscript{2} kinetics before and after 8 wk of HIT in six men and six women during cycling at 50% (50% test) and 110% (110% test) of pre-training VO\textsubscript{2} peak. A single-term exponential equation was used to model the VO\textsubscript{2} response (after phase I) during the 50% and 110% tests pre- and post-training. In addition, phase II and III of the VO\textsubscript{2} response during the 110% tests were examined using a two-term equation. The end of the phase I VO\textsubscript{2} response was identified visually and omitted from the modeling process. The duration of phase I determined during all experimental tests was not different between men and women and did not change with training in either group. Before training, men obtained a phase II VO\textsubscript{2} time constant (\(\tau_2\)) of 29.0±3.3 s during the 50% test which was not different to the \(\tau_2\) of 28.8±2.2 s attained by women. In addition, the \(\tau_2\) determined during the 50% test was unchanged after 8 wk of HIT in both groups. The VO\textsubscript{2} kinetics examined during the 110% tests before training were well described by a single-term model in all male and female subjects. The \(\tau_2\) determined before training for the 110% test was significantly faster in men than in women. Furthermore, VO\textsubscript{2} peak was unchanged in female subjects and the \(\tau_2\) remained unaltered with 8 wk HIT (pre 45.5±2.2; post 44.8±2.3 s). In contrast, male subjects achieved a significantly higher VO\textsubscript{2} peak after training and the \(\tau_2\) determined for men during the 110% test was faster after training (36.4±1.6 s) than before training (40.1± 1.9 s).
Improved model fits were obtained with the two-term equation compared to the single-term equation in two of the six male subjects during the 110% test post-training. It was found that the onset of the \( \dot{VO}_2 \) slow component occurred at a mean time of 63.5±2.5 s and the \( \tau_2 \) was reduced to 18.4±1.7 s. Using a Wilcoxon Signed Ranks z-test, the \( \tau_2 \) described by the single-term equation in the remaining four subjects was determined to be significantly faster after training than before training, thus confirming the results obtained from the original group (n=6) of male subjects.

End exercise heart rate (HREE) values obtained during the 50% and 110% tests were not different between men and women. During the 50% test, HREE values were unchanged, whereas HREE was significantly decreased during the 110% test after training in both groups. These data show that HIT might improve oxidative metabolism in men but not in women as reflected by a greater \( \dot{VO}_2 \) peak and faster \( \dot{VO}_2 \) kinetics during supramaximal work rates. We further suggest that the faster \( \dot{VO}_2 \) kinetics demonstrated in men after training are probably not due to an improvement in cardiac function. Finally, the augmentation of oxidative metabolism during exercise after HIT in men might be dependent on the intensity of the exercise bout at which the \( \dot{VO}_2 \) response is examined.

The findings presented in this thesis suggest that MAOD is a reliable measure in both male and female subjects and can be used to monitor changes in anaerobic ATP production during supramaximal cycling. Moreover, these data suggest that 4 and 8 wk of HIT produce similar changes in anaerobic ATP generation in men and women. Finally, 8 wk of HIT results in the increase of \( \dot{VO}_2 \) peak and \( AO_2 \) uptake as well as the speeding of \( \dot{VO}_2 \) kinetics during supramaximal cycling in male subjects. There was no evidence to suggest that oxidative metabolism was improved in women after short-term HIT. In conclusion, improvement in supramaximal exercise performances should be examined specifically for changes in the anaerobic and aerobic contributions to energy production. In addition, it is suggested that gender should be of primary consideration when designing exercise-training programs where improvement in both anaerobic and aerobic metabolism is required.
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CHAPTER 1: Overview

The liberation and harnessing of energy is central to maximal performance during athletic events. Energy is required constantly during contraction for cross-bridge movement and release, during relaxation to pump calcium (Ca\(^{2+}\)) back into the sarcoplasmic reticulum, and after excitation-contraction coupling to restore sodium (Na\(^{+}\)) and potassium (K\(^{+}\)) to the extracellular and intracellular compartments, respectively. Whether the physical activity is one of power or endurance, energy demands for muscle contraction are met through the degradation of adenosine triphosphate (ATP). However, ATP stores in the muscle are small and only sufficient to maintain muscle contraction for a few seconds (Sahlin and Henriksson, 1984). For continued muscle work, high-energy phosphate bonds must be regenerated by either aerobic and/or anaerobic metabolic pathways.
1.1 ANAEROBIC ENERGY SYSTEMS

The phosphagen system. The phosphagen system produces ATP when energy demands are in excess of the ATP that can be provided from mitochondrial respiration and glycolysis. The release of energy from creatine phosphate (CrP) is facilitated by the separation of inorganic phosphate (P\textsubscript{i}) from creatine (Cr). The energy released is used to regenerate ATP by coupling P\textsubscript{i} to an adenosine diphosphate (ADP) molecule. The enzyme responsible for transferring the phosphate group is creatine kinase (CK). de Groof et al. (2002) highlight that the CK system is essential for efficient delivery of ATP to the sarcoplasmic reticulum Ca\textsuperscript{2+}-ATPase pumps during moderate and maximal contractile activity. The adenylate kinase (AK) system also provides a rapid source of ATP during intense exercise. In the presence of AK, two moles of ADP are converted to one mole of ATP and one mole of adenosine monophosphate (AMP). Robergs (2001) states that the production of AMP is important as it stimulates rate-limiting enzymes that drive glycogenolysis and glycolysis. The metabolic importance of ATP resynthesis via the AK reaction on muscle function has been reviewed previously by Savabi (1994). Quantitatively, ATP and CrP are the most important immediate energy sources. However, the amount of ATP stored as well as the resynthesis of ATP via the phosphagen system are only sufficient to sustain activities for 3-15 s during an all-out sprint (Brooks et al., 1996).

Glycogenolysis and anaerobic glycolysis. Phosphorylase (PHOS) is the primary enzyme involved in the regulation of glycogenolysis and is responsible for the conversion of glycogen and P\textsubscript{i} to glucose 1-phosphate. The activation of PHOS is mediated primarily by intracellular Ca\textsuperscript{2+} concentrations and by the action of epinephrine binding to cell surface beta (\(\beta\))-receptors that activate the subsequent signal cascade. Glycogenolysis is able to provide a high production rate of glucose 6-phosphate, which is the first intermediate of anaerobic glycolysis. In this thesis, anaerobic glycolysis specifically refers to the biochemical pathway that serves to reoxidize nicotinamide adenine dinucleotide (NADH) and hydrogen to NAD\textsuperscript{+} with a net increase in lactate (La\textsuperscript{-}) production.
CHAPTER 1: Overview

The first stage of anaerobic glycolysis requires activation by a primary regulating enzyme phosphofructokinase (PFK). The enzyme PFK catalyzes the production of fructose 1, 6-biphosphate and ADP from fructose 6-phosphate and ATP. Phosphofructokinase catalyzes one of the three proton releasing reactions of glycolysis. At rest, when ATP concentrations are high, PFK activity is minimized due to the allosteric regulation by ATP. However, during exercise, ATP is hydrolyzed and free ADP, AMP and P_i accumulate, decreasing ATP binding to the regulatory site. This results in an increased flux through PFK. The second stage of glycolysis involves the phosphorylation and oxidation of glyceraldehyde 3-phosphate that releases two hydrogen ions (H^+) and two electrons. During the final reactions of anaerobic glycolysis, NADH + H^+ react with pyruvate. Lactate dehydrogenase (LDH) catalyzes this reaction producing NAD^+ and La^- to complete anaerobic glycolysis.

Blood and muscle La^- are useful, indirect markers of perturbations in cellular metabolism that cause acidosis and a non-steady-state cellular metabolic milieu (Robergs, 2001). However, Robergs (2001) clearly demonstrates that there is no biochemical evidence to suggest that the production of La^- releases a proton. In fact, Robergs (2001) suggests that a greater capacity to produce La^- would delay the onset of acidosis as the LDH reaction consumes a proton. Anaerobic glycolysis can be summarized as presented in the equation below (1).

\[
\text{Glucose} + 2 \text{ADP} + 2 \text{P}_i \rightarrow 2 \text{lactate} + 2 \text{ATP} + 2 \text{H}_2\text{O} \quad (1)
\]

In contrast to anaerobic glycolysis, pyruvate may be transported into the mitochondria and oxidized to acetyl coenzyme A (acetyl CoA) as shown in the equation below (2). In this instance, the H^+ from NADH + H^+ are shuttled into the mitochondria for participation in aerobic ATP production.

\[
\text{Pyruvate} + \text{CoA} + \text{NAD}^+ \rightarrow \text{acetyl CoA} + \text{CO}_2 + \text{NADH} + \text{H}^+ \quad (2)
\]
1.2 AEROBIC ENERGY SYSTEMS

Oxygen supply to the active musculature. Ventilation and the pumping of blood are important mechanisms that supply the musculature with oxygen during rest and exercise. The supply of oxygen via the cardiorespiratory system plays a key role in cellular oxidation.

Pulmonary ventilation and blood hemoglobin concentration. The mechanisms that induce and maintain increased ventilation during exercise remain uncertain (Vander et al., 1994). Brooks et al. (1996) has described the integrated neural-humoral theory that suggests a fast-slow ventilatory response at the onset of exercise. At the onset of exercise, the respiratory center receives neural inputs from the motor cortex and from peripheral mechanoreceptors and chemoreceptors that result in an abrupt rise in ventilation. As exercise continues, fluctuations in the partial pressure of oxygen (PO$_2$) as well as an increase in the partial pressure of carbon dioxide (PCO$_2$) and H$^+$ concentration are sensed by the peripheral chemoreceptors (humoral). Peripheral chemoreceptors act to maintain elevated ventilation rates throughout the exercise period. Nevertheless, the ability to increase ventilation during exercise is greater than the body's capacity to increase cardiac output ($\dot{Q}$) or oxygen utilization. Thus, for apparently healthy young adults, ventilation is not usually considered as a rate-limiting factor to the supply of oxygen during exercise at sea level altitudes (Bassett and Howley, 2000; Hoppeler and Weibel, 2000).

The process of ventilation results in a relatively higher PO$_2$ in the alveoli than in the venous blood draining the metabolizing tissues. The PO$_2$ of arterial blood is the primary determinant of the amount of oxygen that binds to hemoglobin; the oxygen-binding protein in red blood cells that establishes the oxygen-carrying capacity of the blood. At a PO$_2$ of 100 mmHg in arterial blood, hemoglobin is nearly 100% saturated with oxygen. This level of saturation is maintained during rest at sea-level altitude as well as during maximal exercise (Powers et al., 1989). The total amount of oxygen carried by hemoglobin in the blood depends on not only the percent saturation of hemoglobin but also on how much hemoglobin is in each liter of blood (Ekblom et al., 1976). Blood hemoglobin concentration may depend on gender (Freedson, 1981).
and/or training status (Lindstedt et al., 1988). Thus, the arterial oxygen content or the oxygen-carrying capacity of the blood will vary among individuals.

**Cardiac output.** Central oxygen delivery is dependent on both arterial oxygen content and \( \dot{Q} \). Cardiac output represents the volume of blood flowing through either the systemic or the pulmonary circuit per minute. Heart rate (HR) and stroke volume (SV) act to increase \( \dot{Q} \) under conditions of increased oxygen demand. It is well known that, during a step increase in work rate, \( \dot{Q} \) is immediately regulated by an increase in HR. Heart rate increases linearly in proportion to work rate (Rowell, 1993) and reaches a peak value during maximal exercise work rates. Stroke volume also increases at the onset of exercise in the upright posture and has been suggested to plateau during exercise that exceeds 50% of maximum in untrained subjects (Brooks et al., 1996). Maximal \( \dot{Q} \) has been proposed as the major determinant of oxygen supply to the active musculature during exhaustive exercise. Hossack and Bruce (1982) as well as Hagberg et al. (1985), indicate that a reduced maximal \( \dot{Q} \) with ageing explains in part, the age related decline in maximal \( \dot{VO}_2 \).

**Control of cardiac function and blood flow.** Heart rate and SV are under constant control by nervous and hormonal influences. At the onset of exercise, withdrawal of parasympathetic (vagus) nerve activity causes the immediate rise in HR. Further increases in HR require the activation of the sinoatrial (SA) node via sympathetic stimulation. In addition, epinephrine that is liberated from the adrenal medulla speeds HR by acting on the \( \beta \)-adrenergic receptors in the SA node as does norepinephrine that is released neurally. Innervation of cardiac sympathetic nerves and circulating epinephrine also act to increase ventricular contractility, thereby causing a rise in SV.

The rise in active muscle blood flow occurs immediately after the onset of exercise (Delp, 1999). The control of blood flow is of primary importance during exercise to meet elevated metabolic demands of the tissues. At a constant \( \dot{Q} \), tissue blood flow is regulated by increasing or decreasing the size or resistance of the vasculature. Tschakovsky et al. (1996) indicated that the mechanical muscle pump effect might only contribute partially to the increase in skeletal muscle blood flow at the onset of
exercise. They suggested that chemical changes in the extracellular fluid surrounding the arterioles of the active muscle must also contribute to the rapid increase in muscle blood flow at the onset of exercise. Decreased arterial oxygen content as well as increased carbon dioxide, $\text{H}^+$ and $\text{K}^+$ that occur in response to exercise have been suggested to augment arteriolar dilation. Wunsch et al. (2000) suggested that $\text{K}^+$ released into the interstitium during muscle contraction might be the most important locally released vasodilator to be involved in the mediation of blood flow 4-5 s after the onset of exercise. Furthermore, exercise bouts of moderate or greater intensities are accompanied by large increases in circulating epinephrine levels. Aside from having powerful effects on metabolism, epinephrine secretion from the adrenal medulla results in the stimulation of $\beta$ receptors that effect cardiac function and vasomotor tone. The arterioles in skeletal muscle possess large numbers of $\beta$ receptors and the presence of circulating epinephrine might contribute to vasodilatation in these vascular beds (Vander et al., 1994).

Moreover, systemic adjustments must also occur to ensure that a greater volume of blood reaches the active muscles during exercise. In a reflex response to decreased arterial pressure, sympathetic nerve stimulation results in vasoconstriction in the tissues of the spleen, kidneys, gastrointestinal track and inactive muscles. Blood is diverted away from these regions temporarily, ensuring that more blood reaches the active muscles and blood pressure is maintained. Ho and colleagues (1997) demonstrated that a greater redistribution of blood flow from splanchnic and renal circulations might be dependent on age and training status.

*Peripheral oxygen diffusion.* The transport of oxygen from blood hemoglobin, across the capillary membrane and finally into the mitochondria is an important determinant of oxygen supply. The primary factor that determines the diffusion of oxygen across the capillary wall is the difference in the $\text{PO}_2$ between the arterial plasma and interstitial fluid (Vander et al., 1994). Increased cellular utilization of oxygen lowers the mitochondrial concentration of oxygen relative to the capillary and therefore, increases the diffusion gradient between plasma and cell. Furthermore, Hoppeler and Weibel (2000) suggest that the microvasular supply of oxygen is related to the volume of capillaries in skeletal muscle. In particular, capillary length effects
peripheral diffusion by influencing the amount of time oxygen has to diffuse across the capillary wall into the muscle tissue. Interestingly, the number and length, but not the diameter of capillaries, changes with body-size and/or adaptation to a stimulus such as exercise training or altitude (Hoppeler and Weibel, 2000).

**Oxygen utilization by the active musculature.** The capacity of skeletal muscle to utilize oxygen is related to the amount of oxygen that is extracted from arterial blood. The amount of oxygen that is extracted from arterial blood can be determined by measuring the arteriovenous oxygen content difference \(( (a - \bar{v})O_2 \text{ difference} )\).

*Tricarboxylic acid cycle.* The tricarboxylic acid cycle (TCA cycle) is a continuous cycle that produces ATP, high-energy electrons and carbon dioxide. This pathway was first described by Sir Hans A. Krebs, hence the TCA cycle is also commonly known as the Krebs cycle. Pyruvate dehydrogenase (PDH) regulates the conversion of pyruvate to acetyl CoA into the TCA cycle. The TCA cycle begins when the two-carbon acetyl unit enters the cycle by combining with a four-carbon molecule of oxaloacetate to form citrate. This reaction is catalyzed by the enzyme citrate synthase (CS). Citrate synthase activity has been suggested as an indicator of aerobic fitness (Russell et al., 2002) and the CS reaction is considered a key regulatory point in the TCA cycle (Vander et al., 1994). The resulting six-carbon citrate molecule goes through a series of reactions until it completes the cycle as another oxaloacetate molecule that becomes available to accept another acetyl group. In addition to producing carbon dioxide, most of the energy released by the reactions of the TCA cycle is captured as high-energy electrons by three NADH and one flavin adenine dinucleotide (FADH\(_2\)). The hydrolysis of guanosine triphosphate (GTP) can also provide energy; P\(_i\) is transferred to guanosine diphosphate (GDP) to form GTP. The net result of the catabolism of one acetyl group from acetyl CoA by way of the TCA cycle (3) can be written as:

\[
\text{Acetyl CoA} + 3 \text{NAD}^+ + \text{FAD} + \text{GDP} + P_i + 2 H_2O \rightarrow \\
2 \text{CO}_2 + \text{CoA} + 3 \text{NADH} + 3 H^+ + \text{FADH}_2 + \text{GTP} \quad (3)
\]
The primary function of the TCA cycle is to generate hydrogen atoms for their subsequent passage to the electron transport chain (ETC) by means of NAD$^{+}$ and FAD.

*Electron transport chain.* The ETC represents a series of linked carrier molecules called cytochromes that remove electrons from NADH and FADH$_2$ and eventually transfer these electrons to oxygen. The process by which ATP is synthesized during the transfer of electrons from NADH and FADH$_2$ to oxygen has been termed oxidative phosphorylation. According to the chemiosmotic hypothesis (Maughan et al., 1997; Vander et al., 1994), as electrons are transferred along the cytochromes chain, the energy released is used to move H$^+$ from the mitochondrial matrix, across the mitochondrial membrane into the cytosolic side of the inner mitochondrial membrane space. A high concentration of positively charged H$^+$ in this outer chamber produces a source of potential energy across the membrane. As the H$^+$ flow back into the mitochondrial matrix, the potential energy is transferred and used to drive the formation of ATP from ADP and P$_i$. Cytochrome oxidase is an enzyme complex found on the inner mitochondrial membrane and catalyzes the transfer of electrons from ferrocytochrome c to molecular oxygen in order to complete oxidative phosphorylation. Samelman et al. (2000) found significantly higher levels and enzymatic activities of cytochrome oxidase in rats after 16 wk of endurance training, suggesting that improved aerobic power is related to an increased potential for oxidative phosphorylation. The net reaction of oxidative phosphorylation (4) is (Vander et al., 1994):

$$0.5 \text{Oxygen} + \text{NADH} + \text{H}^+ + 3 \text{ADP} + 3 \text{P}_i \rightarrow \text{H}_2\text{O} + \text{NAD}^+ + 3 \text{ATP}$$  \hspace{1cm} (4)

*Oxygen stores of the body.* In the transition from rest-to-exercise, the oxygen uptake ($\dot{\text{VO}}_2$) measured at the mouth may underestimate oxygen that is consumed by the tissues. This is due to changes in the oxygen stores of the body, which are comprised of oxygen bound to hemoglobin and myoglobin, oxygen dissolved in the body fluids and oxygen present in the lungs (Medbø et al., 1988). Medbø et al. (1988) has previously suggested that oxygen stores of the body may be reduced by
about 0.25 mmol·kg\(^{-1}\) (~ 9%) at the onset of supramaximal exercise. A reduction in the oxygen stores of the body contributes significantly to the production of ATP, yet is often quantified as part of the ATP produced via anaerobic metabolism. Consequently, when anaerobic ATP production is quantified, ATP produced using oxygen stored in the body should be accounted for to give a more accurate estimation of the true whole body anaerobic energy release.

1.3 INDIRECT MEASURES OF ATP TURNOVER

Measuring the metabolic response to exercise may be the most important means of evaluating the effects of exercise. The direct measurement of ATP turnover is difficult. Thus, indirect methods of measuring ATP turnover have been developed. During steady-state submaximal exercise, pulmonary \(\dot{V}O_2\) measured using open-circuit spirometry reflects the relationship between oxygen utilization and ATP resynthesis via aerobic metabolism. The accurate measurement of \(\dot{V}O_2\) during exercise is simple and can provide information on the maximal power of the aerobic energy system as well as the rate response of \(\dot{V}O_2\) at the onset of exercise. However, similar information on anaerobic metabolism is more difficult to obtain in humans.

The accumulated oxygen deficit. The methodology used to determine the accumulated oxygen (AO\(_2\)) deficit takes advantage of the ease and precision by which \(\dot{V}O_2\) can be measured in the laboratory. For exercise performed at a work rate below the ventilatory threshold (VT), steady-state \(\dot{V}O_2\) reflects the total rate of energy release (oxygen demand) during exercise. The relationship between work rate and \(\dot{V}O_2\) is called the \(\dot{V}O_2\)-power relationship and appears to remain linear within a range of intensities (Medbø et al., 1988). Once the \(\dot{V}O_2\)-power relationship has been established, the oxygen demand can be estimated for supramaximal exercise intensities by extrapolating the regression model. The AO\(_2\) demand represents the total energy required to fuel the supramaximal exercise bout and is the product of the
oxygen demand and exercise duration. The AO$_2$ deficit can then be determined as the difference between the AO$_2$ demand and the total VO$_2$ (L) (AO$_2$ uptake) that is measured during the exercise bout. The AO$_2$ deficit represents energy that is derived anaerobically from intramuscular phosphagens (ATP and CrP) as well as energy derived via anaerobic glycolysis. Figure 1 is a schematic of the calculation procedure for AO$_2$ deficit.

![Diagram of the calculation procedure for the accumulated oxygen (AO$_2$) deficit. A: Extrapolation of the relationship between oxygen uptake and power output. The triangles represent six, 10-min submaximal exercise bouts used to establish the VO$_2$-power relationship. B: Supramaximal exercise bout with the oxygen demand calculated according to the VO$_2$-power relationship determined in A. The AO$_2$ deficit is the difference between the total oxygen demand (total area in B) and the AO$_2$ uptake (shaded area). Adapted from Medbø et al. (1988).](image)

**The maximal accumulated oxygen deficit.** Medbø and colleagues (Medbø et al., 1988, 1989, 1990, 1993) presented an elegant series of studies that clearly describe the use of the AO$_2$ deficit to quantify anaerobic ATP production. The assumptions and limitations to the AO$_2$ deficit method were presented and the use of the AO$_2$
deficit method to examine changes in anaerobic ATP production before and after training was explored. In addition, it was demonstrated that the AO$_2$ deficit is limited and can reach a maximum value during exhaustive, supramaximal exercise of between 2 and 3 min. Medbø defined this maximum value as the *maximal AO$_2$ deficit* (MAOD) and suggested that MAOD represented an individual’s anaerobic capacity (AC). Since this work was first presented by Medbø, several studies have used the AO$_2$ deficit method to quantify anaerobic ATP production and have measured MAOD to determine AC (Scott et al., 1991; Gastin et al., 1995; Naughton et al., 1997; Weber and Schneider, 2001). However, the fundamental assumptions surrounding the AO$_2$ deficit concept are a matter of considerable debate (Bangsbo 1992, 1998; Medbø et al., 1988) and therefore, require further consideration. A discussion that addresses the validity of quantifying anaerobic ATP production using MAOD has been detailed in Chapter 6 of this thesis.

**Oxygen uptake kinetics during constant-load exercise.** An increase in exercise intensity results in an immediate increase in energy demand. However, aerobic metabolism is unable to adjust instantly to the energy requirements of an increased external work rate. Whereas the AO$_2$ deficit quantifies the ATP produced through the anaerobic energy systems, modeling the time course of $\dot{V}O_2$ ($\dot{V}O_2$ kinetics) provides information about the adjustment of the aerobic energy system at the onset of exercise. The $\dot{V}O_2$ response to a step increase in exercise work rate has been previously considered in three phases.

*Phase I of oxygen uptake kinetics.* At the onset of constant-load exercise there is an immediate increase in $\dot{V}O_2$ lasting approximately 15-20 s (Xu and Rhodes, 1999) and changes in muscle metabolism are not immediately expressed at the mouth (Barstow et al., 1990). This instantaneous rise in $\dot{V}O_2$ has been termed phase I and represents the circulatory transit delay between the exercising muscle and the lungs. Phase I is attributed primarily to an increase in HR and SV that results in greater pulmonary blood flow with little or no change in the $(a - \overline{V})O_2$ difference (Whipp and Ward, 1990). During phase I, the oxygen gas tension of mixed venous blood perfusing the alveoli remains similar to the resting value. Phase I ends when the gas
contents of mixed venous blood perfusing the pulmonary capillaries begins to change as a result of increased skeletal muscle oxygen consumption (\( \dot{\text{QO}_2} \)).

**Phase II of oxygen uptake kinetics.** The phase II rise in \( \dot{\text{VO}_2} \) can be characterized by a slower increase in \( \dot{\text{VO}_2} \) after the phase I transit delay. Phase II kinetics reflect an increase in \( \dot{\text{QO}_2} \) and continued increases in \( \dot{\text{Q}} \) (Barstow et al., 1990). The increased \( \dot{\text{QO}_2} \) results in a widening of the \((a - \bar{v})\text{O}_2\) difference, as venous oxygen concentration decreases. The modeling of Barstow and Molé (1987) suggests that across a wide range of circulatory adjustments, the kinetics of \( \dot{\text{VO}_2} \) measured at the mouth during phase II are similar to those of \( \dot{\text{QO}_2} \). Thus, pulmonary \( \dot{\text{VO}_2} \) kinetics are a reliable indirect measure of skeletal muscle utilization at the onset of exercise. The phase II increase in \( \dot{\text{VO}_2} \) continues until a steady state or asymptote is reached. Phase II is commonly referred to as the primary component of the \( \dot{\text{VO}_2} \) response to a step increase in work rate.

**Phase III of oxygen uptake kinetics.** Phase III of \( \dot{\text{VO}_2} \) kinetics represents the new steady-state \( \dot{\text{VO}_2} \) for a particular exercise intensity. This asymptote in \( \dot{\text{VO}_2} \) is observed when an individual exercises at an intensity that is below their individual VT. If the exercise work rate is above the VT, \( \dot{\text{VO}_2} \) might not reach a phase III steady state and a slow upward drift or slow component of \( \dot{\text{VO}_2} \) (\( \dot{\text{VO}_{2SC}} \)) may be observed. The \( \dot{\text{VO}_{2SC}} \) has the potential to drive \( \dot{\text{VO}_2} \) to maximal values despite the work rate being classified as submaximal. The magnitude of the \( \dot{\text{VO}_{2SC}} \) has been recently quantified from the end of the phase II response until end-exercise, the final asymptote, or at exhaustion. It has been suggested that the \( \dot{\text{VO}_{2SC}} \) may be manifest some 90-150 s after the onset of exercise (Barstow and Molé, 1991; Paterson and Whipp, 1991).

**Oxygen uptake kinetics examined at selected work rates.** Three discreet domains of exercise intensity have been classified based on distinct \( \dot{\text{VO}_2} \) and blood La\(^-\) responses (Gaesser and Poole, 1996). Moderate-intensity exercise is performed at or below the VT and is accomplished without an elevation of arterial blood La\(^-\) concentration ([La\(^-\)]) above resting levels. Exercise performed within the moderate-intensity domain leads to an increase in \( \dot{\text{VO}_2} \) that reaches a steady state within about
3 min. The heavy exercise domain can be defined as the lowest work rate at which blood La\textsuperscript{-} production exceeds its rate of removal. Heavy-intensity exercise results in a sustained elevation of blood La\textsuperscript{-} for the duration of the exercise bout (Barstow, 1994). Heavy exercise is performed at an intensity above that achieved at individual’s VT. The emergence of a $\dot{V}O_2$ is an important characteristic of exercise performed in the heavy-intensity domain. Severe exercise constitutes the third exercise domain. During severe exercise that elicits a continuous rise in arterial blood La\textsuperscript{-}, $\dot{V}O_2$ is driven to maximal values (Xu and Rhodes, 1999). Recently, Hill et al. (2002) have described a fourth exercise domain that is characterized by the development of fatigue before $V0_2$ peak can be achieved. This fourth exercise domain has been classed in the extreme-intensity domain.

Figure 2. Oxygen uptake kinetics during the transition from rest to constant-load exercise in two individuals. The exercise work rate was applied at time 0 (dashed line). The work rate applied is below the VT for Subject 1 (black line) and the $\dot{V}O_2$ achieved remains steady throughout the exercise period. In contrast, the work rate is above the VT for Subject 2 (gray line) and $\dot{V}O_2$ continues to rise.
Modeling oxygen uptake kinetics at selected work rates. The response to moderate dynamic exercise may be considered as the basic gas-exchange response to constant-load exercise. The \( \dot{V}O_2 \) response to moderate-intensity exercise shows a monoexponential increase in \( \dot{V}O_2 \), after a short delay (phase I), that reaches a new steady state. The rate of increase can be described with a time constant; tau (\( \tau \)) that represents the time taken for \( \dot{V}O_2 \) to achieve 63% of the change in \( \dot{V}O_2 \) from baseline to steady state during constant-load exercise. In general, the phase II and III \( \dot{V}O_2 \) response in the moderate-intensity domain can be described mathematically by the single-term exponential equation (1):

\[
\Delta \dot{V}O_2(t) = A_2 \cdot (1 - e^{-t/\tau_{TD}}) \tag{1}
\]

However, when the exercise intensity is above the VT (heavy-intensity), the \( \dot{V}O_2 \) response becomes more complex as \( \dot{V}O_2 \) may not reach a steady state and an upward drift in \( \dot{V}O_2 \) may be observed. In this instance a two-term exponential equation (2) has been used to model \( \dot{V}O_2 \) kinetics after phase I:

\[
\Delta \dot{V}O_2(t) = A_2 \cdot (1 - e^{-t/\tau_{TD}}) + A_3 \cdot (1 - e^{-t/\tau_{TD}}) \tag{2}
\]

where \( A_2 \) and \( A_3 \) denote phase II and phase III (\( \dot{V}O_{2SC} \)), respectively and \( \tau \), TD and A are the associated time constant, delay and amplitude (i.e., \( \Delta \dot{V}O_2 \)) terms.

Modeling \( \dot{V}O_2 \) kinetics at the onset of exercise during supramaximal work rates presents several problems. It is unclear whether a \( \dot{V}O_{2SC} \) is present during exercise performed at an intensity at which the oxygen demand is greater than the maximal rate of \( \dot{V}O_2 \) that can be achieved through aerobic metabolism. Some researchers have suggested that the \( \dot{V}O_{2SC} \) is detectable during supramaximal cycling and have included an additional term to describe the phase III \( \dot{V}O_2 \) response (Hughson et al., 2000). In contrast, other researchers have suggested that the \( \dot{V}O_2 \) response (excluding phase I) to supramaximal exercise is well described by a single-term exponential equation (Özyener et al., 2001). There has been no previous discussion that specifically addresses the presence of a \( \dot{V}O_{2SC} \) during supramaximal exercise.
The modeling technique used in experiment three of this thesis is presented in the methods section of Chapter 4.

1.4 GENDER COMPARISON OF THE MAXIMAL ACCUMULATED OXYGEN DEFICIT

General findings. Few studies have investigated MAOD in both male and female subjects. In an earlier experiment conducted in this laboratory, gender differences in absolute MAOD of 46% were determined (Weber and Schneider, 2000). When MAOD was expressed relative to the active muscle mass (AMM) for cycling, men retained a 17% greater MAOD (mL·kgAMM\(^{-1}\)) than women. Moreover, Weyand et al. (1993) found that gender differences were not eliminated when MAOD was expressed relative to leg lean volume. Weyand et al. (1993) reported that a gender difference of approximately 23% for cycling remained even when the difference in the estimated AMM was taken into account. The higher MAOD (mL·kgAMM\(^{-1}\)) found in men than in women is suggested to be physiologically important to human performance.

The physiological significance of this gender difference can be highlighted when MAOD values are related to performance in sprint-type events. For example, an athlete required to sustain a high rate of ATP production, beyond the maximal rate of the aerobic energy systems, would benefit from a 17-23% increase in his/her capacity to produce ATP anaerobically. The importance of this gender difference in MAOD (mL·kgAMM\(^{-1}\)) found in previous studies (Weyand et al., 1993; Weber and Schneider, 2000) is supported by the significant relationship observed between sprint and middle-distance track performance (100-m, 200-m, 400-m, 800-m and 1500-m) and MAOD (L) values. Athletes with the highest MAOD values demonstrated the best run performances for each track event (Weyand et al., 1994).

The greater MAOD values observed in men compared to women could be due to gender differences in the ability to generate ATP anaerobically and/or the ability to counteract inhibiting by-products of anaerobic metabolism. This suggests that there
are gender differences in the physiological, biochemical and structural properties of skeletal muscle that augment or limit anaerobic ATP production. A brief overview of intracellular phosphagens, anaerobic glycolysis, and the buffering capacity (BC) of the skeletal muscle will help to explain the gender-specific differences that may exist in anaerobic metabolism during high-intensity exercise.

**Skeletal muscle characteristics.** Gender differences in the relative percent of muscle fiber types have been reported, suggesting a greater percent of type I fibers in women compared to men (Simoneau and Bouchard, 1989; Miller et al., 1993). In contrast, other studies have not found a gender difference in the percent distribution of muscle fiber type (Esbjörnsson et al., 1993; Froese and Houston, 1985; Houston et al., 1988; Prince et al., 1977; Ryushi et al., 1988; Schantz et al., 1983; Staron et al., 1994). Furthermore, Esbjörnsson et al. (1994) as well as Kent-Braun and Ng (2000) reported that the energy provided from ATP and CrP degradation was not different between male and female subjects during a 30-s maximal cycling sprint or during 15 s of plantar flexion, respectively.

It has been suggested that higher MAOD values found in male compared to female subjects may be due to their greater capacity to produce ATP via anaerobic glycolysis. Weber and Schneider (2000) demonstrated a significantly higher peak [La] in male than in female subjects after exhaustive cycling performed at 120% of VO₂ peak. These results are consistent with the findings of Naughton et al. (1997). These researchers suggested that the lower levels of plasma La reflected a smaller capacity for the regulation of anaerobic glycolysis in female subjects compared to male subjects. It is possible that the greater capacity to generate ATP via anaerobic glycolysis could account in part for the higher MAOD of men than women.

Anaerobic glycolysis during supramaximal exercise may be limited by the inhibition of glycogenolytic/glycolytic enzymes. A lower activity of PHOS might reduce the rate of glycogenolysis and limit the rate of ATP produced from anaerobic glycolysis (Sale and Spriet, 1996). Komi and Karlsson (1978) reported a 50% higher PHOS activity in untrained male than in untrained female subjects. However, data reported by Costill...
et al. (1976) and more recently by Cadefau et al. (1990) showed similar activities of PHOS in untrained male and female subjects. Furthermore, higher activities of PFK in male subjects may facilitate a greater ability to stimulate glycolysis. Phosphofructokinase activity in active and trained men has been reported to be 20-33% higher than in active and trained women (Cadefau et al., 1990; Green et al., 1996). Additionally, PFK activities have been reported to be 26% (Simoneau et al., 1985) and 75% (Cadefau et al., 1990) higher in untrained men than in untrained women. In contrast, Esbjörnsson et al. (1993) reported no significant difference in PFK activity between recreationally active male and female subjects. In the same study, men demonstrated significantly higher total LDH activity than women (Esbjörnsson et al., 1993). Higher PHOS, PFK and/or LDH activity in men may help to increase the glycolytic flux rate during supramaximal exercise and contribute to the greater capacity of men to perform supramaximal exercise compared to women.

Maintenance of ionic homeostasis. Anaerobic energy systems facilitate the rapid production of ATP during supramaximal exercise. However, anaerobic glycolysis has adverse consequences that may establish the upper limits of performance by inhibiting both contractile and energy producing processes. Anaerobic glycolysis results in a net production of $H^+$ that causes a lowered muscle and blood pH and may depress the activities of key enzymes of the glycogenolytic/glycolytic pathways (Trivedi and Danforth, 1966; Spriet et al., 1987; Stainsby, 1986). Furthermore, reduced pH indicates the direct effect of $H^+$ on the contractile properties of skeletal muscle resulting in decreased force capacity (Chasoliths et al., 1983). However, for a high level of performance it is necessary to expend as much ATP, at as high a rate as possible. Therefore, the ability to stimulate anaerobic glycolysis, while at the same time limiting the effects of $H^+$ production, will result in optimal performance during supramaximal exercise. The higher MAOD found in male subjects than in female subjects may in part result from the greater capacity of male subjects to buffer and/or remove the inhibiting end products of anaerobic metabolism. However, few investigations have examined the capacity of muscle intracellular fluids to buffer the acidic products of anaerobic glycolysis. In fact, I am unaware of any study that has directly compared the BC of men and women during supramaximal exercise.
In addition to an increased production of H\(^+\), the marked disturbances of Na\(^+\) and K\(^+\) that occur in contracting skeletal muscle have been well described (McKenna, 1999). The most important function of the Na\(^+\), K\(^+\)-pump in skeletal muscle is to regulate Na\(^+\) and K\(^+\) fluxes across muscle membranes. The intracellular-to-extracellular K\(^+\) concentration gradient is crucial in the maintenance of membrane potential and excitability. A diminution of this gradient during exercise has been linked with muscular fatigue during intense contractions (Sjøgaard et al., 1985; Kowalchuk et al., 1988). In contrast, it has been suggested that the maximum power of the Na\(^+\), K\(^+\)-pump may never be reached during exercise (Everts et al., 1997; Medbø and Sejersted, 1990) and thus, extracellular potassium may have little to do with skeletal muscle fatigue (Medbø et al., 2001). Nevertheless, Nørgaard (1986) indicated no gender differences in skeletal muscle Na\(^+\), K\(^+\)-pump concentration. However, in a more recent study, Everts et al. (1997) demonstrated greater Na\(^+\), K\(^+\)-pump concentration in men compared to women. Enhanced Na\(^+\), K\(^+\)-pump concentration in men may act to improve K\(^+\) regulation during supramaximal exercise, thereby facilitating enhanced muscle performance under conditions of severe contractile demand.

Despite extensive investigation, the control of blood flow during dynamic exercise is not fully understood. There is some evidence suggesting that sympathetic β-adrenergic receptors play a major role to increase active muscle blood flow (Buckwalter et al., 1998). Thus, it is possible that male subjects with an increased secretion of epinephrine are able to increase muscle blood flow to a greater extent than female subjects. Increased muscle blood flow could improve muscle function by an oxygen-independent mechanism. This is thought to involve the removal of metabolites such as H\(^+\) and delay the onset of muscle fatigue. Furthermore, circulating catecholamines have been associated with Na\(^+\), K\(^+\)-pump regulation in exercising humans (Katz et al., 1985). Catecholamine activation of the Na\(^+\), K\(^+\)-pump would minimize muscle interstitial K\(^+\) accumulation during exercise via increased K\(^+\) uptake by inactive muscles, thereby maintaining an interstitial-to-plasma K\(^+\) concentration gradient and facilitating K\(^+\) removal from muscle.
Reliability of MAOD in men and women. Additional studies are required to answer the questions that remain regarding the structural, physiological and biochemical differences that exist between male and female human skeletal muscle. Nevertheless, direct measures of the potential mechanisms that limit and/or facilitate anaerobic ATP regeneration might only be warranted if real differences in performance are established between men and women.

When discussing the validity of an experimental procedure, it is appropriate to consider reliability. Reliability refers to the consistency of the measurement and is dependent on the potential for subsequent researchers to reconstruct original methodological procedures. To enhance the validity of scientific research, reliable methodology must be applied. Bar-Or (1987) presented the methodology, reliability and validity of the 30-s Wingate Anaerobic Test as a measure of anaerobic performance capacity. However, despite recent interest in the use of MAOD to quantify an individual’s AC, inadequate data are available on the reliability of MAOD in male and female subjects. In an early study by Lawson and Golding (1981), MAOD was suggested to be a reliable indicator of AC and was associated with a reliability coefficient of 0.98. However, there was no evidence to suggest that maximal AO₂ deficit values had been reached during the 1-min cycling bouts. Medbø et al. (1988) have since demonstrated that at least 2 min of cycling is required to achieve a MAOD during constant-load supramaximal exercise. In addition, Lawson and Golding (1981) did not present individual subject values and the type of correlation coefficient used to assess reliability was not described. Thus, we cannot be sure of the intra-individual variation associated with MAOD measurements.

We must be certain that MAOD values can be interpreted with confidence and that the findings can be generalized to other sample groups. It should not be assumed that what is true of men is also true for women. Indeed, Esbjörsson-Liljedahl et al. (1996) has suggested that untrained men may have greater participation levels in recreational activities involving high-intensity exercise when compared to untrained females. Thus, female subjects may not achieve a true MAOD due simply to their unfamiliarity with this type of exercise. Therefore, an increase in the AO₂ deficit may occur across trials as female subjects become more familiar with the exercise. A
comprehensive study examining the reliability of the MAOD method in untrained male and female subjects is required and has been subsequently presented in Chapter 2 of this thesis.

**Gender specific changes in MAOD with high-intensity interval training.** It is commonly accepted that short-term, high-intensity training will result in improvements in the ability to perform supramaximal exercise. Indeed, Campbell et al. (1979) and Esbjörnsson-Liljedahl et al. (1996) have previously demonstrated significant increases in peak power output during short-duration sprint cycling in men and women. However, Medbø and Burgers (1990) demonstrated that the MAOD was unchanged in women after 6 wk of short-duration high-intensity training, whereas MAOD improved by 16% in men after the same period. No other previous studies have reported gender-specific changes in MAOD with training. It is unknown why the female participants in the study by Medbø and Burgers (1990) failed to demonstrate improvements in MAOD after training. In addition, it is not clear which mechanisms augment the adaptation of anaerobic metabolism in male but not in female subjects when exposed to similar training programs. Nonetheless, several factors that may contribute to the improvements in anaerobic ATP production after high-intensity interval training (HIT) are introduced here, with respect to gender.

Esbjörnsson-Liljedahl et al. (1996) found an increase in the type II fiber area in women, but not in men following 4 wk of sprint-cycle training. They suggested that male subjects may be more familiar with high-intensity exercise and that 4 wk of training was too short to stimulate any additional hypertrophy in men. Some earlier sprint-training studies in male subjects also failed to demonstrate any increase of fiber area (Thorstensson et al., 1975; Jacobs et al., 1987; Linossier et al., 1993). Conversely, based on the findings of Alway et al. (1989) and Bell and Jacobs (1990), Chorneyko and Bourgeois (1999) suggested that male subjects are able to hypertrophy both fiber types more readily than female subjects. It was stated that men increase the cross-sectional area of the type II fibers more dramatically, whereas women tend to hypertrophy both fibers more equally. It is difficult to make
conclusions describing the muscle fiber type changes in men and women after HIT based on previous literature.

It has been suggested that one explanation for the increase in sprint performance could be an increase in the muscle stores of ATP and CrP. However, no consistent increase in the storage of ATP or CrP following sprint training is apparent in the literature (Houston and Thomson, 1977; Nevill et al., 1989; Sharp et al., 1986). Esbjörnsson-Liljedahl et al. (1996) found a significantly greater training-induced increase in total creatine content in male subjects than in female subjects. Balsom et al. (1993) suggested that this might favor improved performance, whereas Esbjörnsson-Liljedahl et al. (1996) found no relationship between changes in performance and total creatine content of muscle.

The question as to whether HIT can result in an increase in the activity of glycolytic enzymes is also controversial. Whereas the majority of investigators have noted increases in glycolytic enzyme activity after sprint training (MacDougall et al., 1998; Cadefau et al., 1990; Jacobs et al., 1987; Roberts et al., 1982; Sharp et al., 1986; Simoneau et al., 1985), others have not (Henriksson and Reitman, 1976; Hickson et al., 1978). Following 6 wk of sprint training, a 17% increase in PFK activity was found with corresponding evidence of an enhanced potential to derive energy from glycolysis (Jacobs et al., 1987). In addition, Esbjörnsson-Liljedahl et al. (1996) reported that total LDH activity increased by the same relative amount in male and female subjects after 4 wk of HIT. Further research is required to examine the gender-specific changes in glycolytic enzyme activity with short-term sprint training.

Skeletal muscle BC may improve in response to HIT (Nevill et al., 1989; Sharp et al., 1986). It has been demonstrated that the BC of muscle is 18% higher in anaerobically trained individuals than untrained subjects (Sahlin and Henriksson, 1984). Bell and Wenger (1988) demonstrated that 7 wk of sprint-training increased muscle BC by 11% in male subjects, while it was reported that 8 wk of sprint-training increased muscle BC by 38% (Sharp et al., 1986). In contrast, Mannion et al. (1993) reported no change in muscle BC after sprint training. It is unclear if changes in the muscle BC following sprint training are similar in male and female subjects.
McKenna et al. (1993) demonstrated that the $^3$H-ouabain binding site concentration is increased after sprint training, thereby suggesting an up regulation of the Na$^+$, K$^+$-pump during exercise. Nevertheless, gender-specific changes in Na$^+$, K$^+$-pump concentrations after training and the importance of an up regulation in Na$^+$, K$^+$-pump concentrations on muscle fatigue has yet to be determined.

Existing studies clearly demonstrate the need for additional research regarding the effect of gender on skeletal muscle structure, physiology and biochemistry. Currently, no conclusions can be made about the skeletal muscle adaptations that occur in men and women in response to sprint-type training. However, it is important to first determine and directly compare the effects of training on anaerobic metabolism and AC in men and women in the same study. If there are true gender differences in MAOD, and if changes in MAOD are different in men and women after a period of HIT, the skeletal muscle mechanisms responsible for these gender differences can then be elucidated.

It is possible that AC is more “trainable” in men than in women (Medbø and Burgers, 1990). However, it is recognized here and later by Medbø (1991) that there is no strong evidence to suggest that MAOD can be improved in men, but not women after training. In order to confirm or reject the notion that increases in MAOD after HIT are gender-dependent, differences in MAOD have been examined before and after 4 and 8 wk of HIT in Chapter 3 of this thesis.

1.5 GENDER COMPARISON OF oxygen uptake KINETICS DURING EXERCISE

General findings. Few studies have compared VO$_2$ kinetics between men and women. Chilibeck et al. (1996a) examined the VO$_2$ kinetics of seven female and nine male subjects during 6 min of cycling at an intensity corresponding to 90% of the VT. Multiple linear regression indicated that VO$_2$peak (mL·kg$^{-1}$·min$^{-1}$), gender and age were significant explanatory variables of the variance in the phase II time constant. It was highlighted that the influence of gender on VO$_2$ kinetics was
independent of the gender differences in relative VO₂ peak values. Nevertheless, no discussion was presented on the implications of this finding. In a subsequent study published in the same year, Chilibeck et al. (1996b) reported that the phase II time constant determined during 6 min of cycling at 90% of VT was not significantly different between male and female subjects. It was acknowledged in the discussion of the later study that unequal numbers of male and females subjects might have been a confounding factor influencing the results of both studies. In addition, these researchers suggested based on the findings of both studies that the effect of gender on VO₂ kinetics might be at best, minimal. The primary aim of the studies presented by Chilibeck et al. (1996a, 1996b) was not to investigate gender-related differences in VO₂ kinetics and consequently, no gender-specific data were provided. In a more recent study, Fawkner et al. (2002) examined the transition from unloaded pedaling to a step increase in work rate designed to elicit 80% of the VT in thirteen inactive male and twelve inactive female subjects. They reported a mean time constant of 27.9±8.6 s for men which was not significantly different to the mean time constant of 26.0±4.5 s for women.

Few studies have examined the rise in pulmonary VO₂ at the transition between baseline exercise and a step increase in work rate to supramaximal intensities (Billat et al., 2000; Craig et al., 1995; Hebestreit et al., 1998; Hughson et al., 2000; Özyener et al., 2001). The intensity of the exercise bouts used by these investigators varied between 100% and 140% of VO₂ peak with exercise durations of between 45 s and 3 to 4 min. Among the studies that have examined the time constant of the phase II VO₂ response to supramaximal exercise, young and older subjects (Hebestreit et al., 1998) as well as sprint and endurance-trained athletes (Craig et al., 1995) have been compared. I am unaware of any study that has compared the phase II time constant during supramaximal exercise between male and female subjects. Hill and Stevens (2001) investigated the VO₂ response at the onset of exercise predicted to elicit exhaustion between 3 and 4 min in seven men and seven women. However, no gender-specific results were provided and no discussion of gender was presented. Cempla (1994) examined VO₂ in eight boys and nine girls during 2 min of treadmill running at a speed between 116% and 120% of the velocity achieved at VO₂ peak. Based on the mean VO₂ value achieved during the last 30 s of the 2-min exercise
bout, it was suggested by Cempla that the kinetics of oxygen uptake during supramaximal running are not different between boys and girls. However, no attempt to model the $\dot{V}O_2$ response was apparent. Therefore, no accurate evaluation of the $\dot{V}O_2$ kinetics could be determined from that study. Finally, the time course of $\dot{V}O_2$ during the supramaximal run was illustrated graphically. Although it is difficult to make accurate comments about the $\dot{V}O_2$ response based on a graphical illustration of the data, there is a clear deviation of the two responses suggesting faster kinetics in male than in female subjects.

A faster adjustment of skeletal muscle oxidative metabolism during the transition from unloaded cycling to a supramaximal work rate would reduce the reliance on intramuscular phosphagen stores and anaerobic glycolysis at the onset of exercise. Reduced metabolic and ionic perturbations in the muscle and blood at the beginning of supramaximal exercise could improve the ability of an individual to sustain the required work rate and to delay the onset of fatigue. Examination of the time course of $\dot{V}O_2$ during moderate-intensity and supramaximal cycling may provide valuable information about the way in which men and women adjust to meet new metabolic demands.

Absolute and relative measures of $\dot{V}O_2\text{peak}$ are lower in women than in men. After normalizing for lean body mass (LBM), some researchers demonstrate that $\dot{V}O_2\text{peak}$ remains higher in male than in female subjects (Sparling, 1980; Cureton and Sparling, 1980). This suggests that there may be differences in the ability of men and women to supply and/or utilize oxygen. The associated mechanisms are a matter of considerable debate and may be influenced by work rate, training status or exercise mode. In the following sections, the key factors suggested to limit the rate response of pulmonary $\dot{V}O_2$ at the onset of exercise have been addressed with particular reference to gender.

**Oxygen extraction by the active skeletal muscle.** At the onset of exercise performed at an intensity below the VT, oxygen supply to the active skeletal muscle is probably not a limiting factor of $\dot{V}O_2$ kinetics (Grassi et al., 1996, 1998; Hughson et
al., 1996; MacDonald et al., 1997; Barclay et al., 1995). Rather, adequate oxygen is delivered to the muscle at the onset of moderate-intensity exercise and skeletal muscle limitations establish the rate at which $\dot{V}O_2$ increases. The extent of peripheral oxygen extraction is dependent upon both the arterial oxygen content and the ability of the skeletal muscle to utilize the delivered oxygen.

The ability of the muscle to utilize oxygen may be dependent on the provision of acetyl CoA into the TCA cycle. At the beginning of exercise, PDH is transformed into the active form primarily by increase in intracellular Ca$^{2+}$ and pyruvate concentrations (Howlett et al., 1999). The PDH enzyme must be activated rapidly in order to increase acetyl CoA flux into the TCA cycle. Using pharmacological intervention, Howlett et al. (1999) increased the activation of PDH by inhibiting PDH kinase with dichloroacetate. During the initial stages of submaximal leg cycle exercise, these researchers showed attenuated lactate, ADP, AMP, and P$i$ accumulation as well as a reduced degradation of intramuscular CrP (Howlett et al., 1999). The increased production of acetyl CoA from pyruvate for subsequent flux into the TCA cycle reduced lactate production and speeded phase II $\dot{V}O_2$ kinetics. This study provides some evidence to suggest that rate-limiting enzymes may play a role in the ability of the muscle to extract oxygen at the onset of exercise. Several other researchers have suggested that other oxidative enzymes may affect the time course of $\dot{V}O_2$ at the onset of exercise. Many of the studies that have compared the maximal oxidative activity of CS, succinate dehydrogenase (SDH) and oxoglutarate dehydrogenase in untrained men and women failed to demonstrate gender differences (Simoneau et al., 1985; Cadefau et al., 1990; Bass et al., 1975; Costill et al., 1976). However, Green et al. (1984) and others (Saltin et al., 1977; Nygaard, 1981) have reported that the maximal activity of SDH was lower in female than in male subjects. These researchers suggested that women have a significantly lower overall capacity for aerobic oxidation than men.

At the ultrastructure level, little information is available about differences in male and female skeletal muscle in untrained individuals. Whereas, men and women have been reported to have similar capillary concentrations per square unit of muscle (Bell and Jacobs, 1990; Porter et al., 2002), other researchers (Coggan et al., 1992a,
(1992b; Sjøgaard, 1982) have demonstrated denser muscle capillarization in men compared to women. In addition, Hoppeler et al. (1973) found a higher volume density of mitochondria in nine untrained male subjects compared to three female subjects. Increased capillary density and mitochondrial volume in men would decrease the diffusive resistance to oxygen and improve the skeletal muscle oxygen extraction in male compared to female subjects. Additional research is required to examine the effect of gender on peripheral diffusion gradients and metabolic pathway regulatory processes. Nevertheless, reduced skeletal muscle oxygen extraction in female compared to male subjects could result in slower \( \dot{V}O_2 \) kinetics at the onset of moderate-intensity and supramaximal exercise in women compared to men.

**Oxygen supply to the active musculature.** In addition to skeletal muscle oxygen extraction, oxygen supply to the active muscle might also influence the time course of change in \( \dot{V}O_2 \) when the step increase in work rate is above the VT. MacDonald et al. (1997) clearly demonstrates that \( \dot{V}O_2 \) kinetics are not accelerated during moderate-intensity exercise in hyperoxia (\( F_{O_2} = 0.70 \)), whereas hyperoxia gas breathing resulted in a faster adjustment to steady-state \( \dot{V}O_2 \) for exercise work rates above VT. Linnarsson (1974) and Engelen et al. (1996) also concluded that reducing oxygen delivery to the muscle in hypoxia slows the phase II \( \dot{V}O_2 \) response kinetics at the onset of constant-load exercise. Furthermore, Koga et al. (1999) observed that HR and \( \dot{V}O_2 \) displayed slower kinetics when leg cycling was performed in the supine position compared to the upright position. MacDonald et al. (1998) supported these findings by demonstrating that leg blood flow and \( \dot{V}O_2 \) increased at a slower rate when knee extension/flexion was performed in the supine compared to the upright position.

Inherent physiological differences might exist between men and women in one or more of the components of the oxygen supply system. For instance, ventricular filling capacity may be reduced because of a smaller left ventricle in female subjects. Heart size is proportional to body size (Feigenbaum, 1986), so it follows that women would exhibit smaller left ventricular dimensions. Moreover, results from postmortem studies have documented smaller weight-adjusted relative myocardial mass in
women compared to men (Grande and Taylor, 1965). Nuclear magnetic resonance imaging in female endurance athletes confirm the earlier postmortem findings and suggest that the lower left ventricle mass of women persists when corrected for LBM (Riley-Hagan et al., 1992).

Furthermore, increases in $\dot{Q}$ are a major determinant of oxygen supply during a step increase in work rate. Under resting conditions, $\dot{Q}$ is similar between male and female subjects (Younis et al., 1990). In addition, Bar-Or (1983) and others (Proctor et al., 1998) have demonstrated similar patterns of increase in $\dot{Q}$ in response to continuous incremental exercise. When male and female subjects are matched for $\dot{VO}_2\text{peak}$ expressed relative to LBM, no gender-related differences in $\dot{Q}$ exist during leg cycling at 40-90% of $\dot{VO}_2\text{peak}$ (Zwieren et al., 1983; Proctor et al., 1998). However, it has been repeatedly demonstrated that higher maximal values for $\dot{Q}$ are recorded in male subjects (Åstrand et al., 1964; Åstrand and Rodahl, 1986; Bar-Or, 1983; Cumming, 1978; Sullivan et al., 1991; Younis et al., 1990). There is evidence to suggest that maximal $\dot{Q}$ is higher in men than in women even when normalized for body size (Wiebe et al., 1998). Perrault (1996) suggests that the reduced maximal $\dot{Q}$ in female subjects might serve as a potential limitation to oxygen supply during exercise at heavy work rates.

In three previous studies conducted in this laboratory (Weber and Schneider, 2000, 2001, 2002), peak HR measured during incremental cycling or during exhaustive, supramaximal work rates was not significantly different between male and female subjects. The effects of gender on the exercise-induced increases in SV are less clear. There remain several unanswered questions regarding the relative contribution of the Frank-Starling mechanism to increases in SV in men and women. While there have been a number of studies that have addressed the affect of gender on end-diastolic volume (Sullivan et al., 1991; Spina et al., 1993), diastolic filling time (Younis et al., 1990) and ventricular distensibility (Iwasaka et al., 1994), the findings of these investigations are at best controversial. Perrault (1996) suggested that caution should be observed in any conclusion about the Frank-Starling mechanism made between male and females subjects. Moreover, gender-related differences in the relative contribution of ventricular contractility have been investigated to explain
differences in the exercise-induced response in SV. Changes in ejection fraction induced by maximal dynamic exercise appear to be smaller in women than in men (Adams et al., 1987; Younis et al., 1990). Using regression analysis, Adams et al. (1987) demonstrated that gender was a significant predictor of changes in ejection fraction with exercise.

By increasing an individual's volume of total red blood cells using blood doping methods, $\dot{V}O_2^{\text{peak}}$ has been shown to increase by 4-9% (Gledhill, 1982). Cureton et al. (1986) and others (Green et al., 1999) concluded that the higher concentration of hemoglobin in male subjects accounted for some of the gender-difference observed in $\dot{V}O_2^{\text{peak}}$. Women typically exhibit hemoglobin concentrations between 120 and 160 g\text{L}^{-1}, whereas corresponding values for men are between 140 and 180 g\text{L}^{-1} (Freedson, 1981). Considering average hemoglobin concentrations in men and women, Perrault (1996) calculated a 13% lower arterial oxygen content for female subjects when compared to male subjects. As mentioned earlier, peripheral oxygen extraction potential might be dependent in part upon arterial oxygen content. A lower relative oxygen-carrying capacity and maximal $\dot{Q}$ demonstrated in female subjects could have the potential to slow the rate response of $\dot{V}O_2$ at the onset of exercise.

**System response to feedback and feed-forward signals.** Regulation of the cardiovascular system to match increases in oxygen demand with increases in oxygen supply and extraction are achieved by a control system that integrates various feed-forward and feedback signals. At the onset of an increase in work rate, error signals are established in proportion to the difference between the current $\dot{V}O_2$ and the required $\dot{V}O_2$ (Whipp and Wasserman, 1972). Astrand and Saltin (1961) showed that a $\dot{V}O_2$ of 4.1 L\text{min}^{-1} can be achieved in 1 min at an exercise intensity of 150% of $\dot{V}O_2^{\text{peak}}$ whereas it may take 5 min to achieve 4.1 L\text{min}^{-1} at an intensity of 100% of $\dot{V}O_2^{\text{peak}}$. This occurrence has also been demonstrated more recently by Billat et al. (2000) by showing that $\dot{V}O_2^{\text{max}}$ is attained in a time that is shorter when the exercise work rate is higher. Thus, the rate of oxygen supply and oxygen extraction at the onset of exercise may not be as rapid during exercise performed at 110% of $\dot{V}O_2^{\text{peak}}$ compared to exercise at a higher work rate (i.e., 150% of
Therefore, it could be hypothesized that faster \( \text{VO}_2 \) kinetics may be due to improved regulatory feedback and feed-forward systems rather than specific oxygen delivery and/or oxygen extraction mechanisms.

Preliminary studies offer insights into the potential gender-related differences in regulatory feedback and feed-forward systems such as muscle sympathetic nerve activity and cardiovagal baroreflex gain (Evans et al., 2001; Beske et al., 2001). Beske et al. (2001) examined the cardiovagal baroreflex gain in eleven sedentary men and fourteen women by manipulating arterial blood pressure above and below baseline levels using bolus injections of phenylephrine HCl and sodium nitroprusside, respectively. Cardiovagal baroreflex was described by the sigmoidal relationship between R-R interval length and systolic blood pressure. The linear portion of this slope was used to derive the gain of the cardiovagal baroreflex. These researchers demonstrated that the cardiovagal baroreflex gain was about 35% lower in female subjects compared with male subjects. In another study, Abdel-Rahamn et al. (1994) demonstrated that women exhibited lesser bradycardia than did men in response to a bolus administration of phenylephrine that produced an abrupt rise in blood pressure. These two studies suggest lower baroreflex sensitivity in female subjects and a lesser involvement of the cardiac vagal component in the baroreflex-mediated bradycardia. Considering that arterial and cardiopulmonary baroreflexes may be involved in the control of autonomic adjustments to exercise, gender-related differences in baroreflex sensitivity could potentially affect sympathetic nerve activity during exercise and contribute to the explanation of the reported gender-related differences in cardiovascular responses to exercise. Furthermore, Mazzeo et al. (2001) demonstrated an increased sympathoadrenal response to hypoxia in male subjects suggesting that increased circulating epinephrine might affect active muscle blood flow. In addition, Kowalchuk and Hughson (1990) found slower \( \text{VO}_2 \) kinetics with \( \beta \)-adrenergic blockade, supporting the suggestion that increased sympathetic outflow might affect oxygen supply to the working musculature. In a previous study, Weber and Schneider (2000) found significantly higher plasma epinephrine concentrations following cycling at 120% of \( \text{VO}_2 \) peak in ten untrained men compared to ten untrained women. It could be suggested that higher circulating epinephrine in
male subjects might enhance active muscle blood flow at the onset of exercise to a greater extent in men than in women.

**Gender-specific changes in VO₂ kinetics with high-intensity interval training.**

The limiting factors of VO₂ kinetics at the onset of exercise remain debatable. However, gender differences reported in maximal Q, hemoglobin concentration, mitochondrial number, oxidative enzyme activity and feedback and feed-forward signals continue to lend support to the idea that VO₂ kinetics may be different in men and women. In addition, it appears that VO₂ kinetics during intense exercise are not limited by any single step in the oxygen supply pathway or mitochondrial respiration.

Improvements in oxygen supply and/or oxygen extraction after training have been associated with the acceleration of VO₂ kinetics at the onset of exercise. However, some researchers have reported that phase II of the VO₂ response to submaximal exercise may be modified after a short-term endurance training program (Hagberg et al., 1980; Casaburi et al., 1987), whereas other researchers have reported no change (Brandenburg et al., 1999; Carter et al., 2000). No study has previously examined changes in VO₂ kinetics during exercise before and after a period of HIT. Harmer et al. (2000) demonstrated the importance of aerobic adaptations to intense exercise after sprint-type training in male subjects. These researchers provide strong evidence to suggest enhanced muscle oxidative metabolism after sprint-type training and proposed that increases in mitochondrial density and oxidative enzymes occur with training. Thus, HIT may enhance mitochondrial metabolic capacity and/or oxygen supply that may speed the VO₂ response at the onset of exercise. Several factors that might contribute to the speeding of VO₂ kinetics after HIT have been examined here and are related to the potential effect of gender.

After 6-8 wk of HIT, marked increases in the activity of oxidative enzymes have been previously reported in male subjects (Jacobs et al., 1987; Parra et al., 2000; Rodas et al., 2000). Significant increases in CS and 3-hydroxyacyl-CoA dehydrogenase (HAD) activity suggest the potential for improved aerobic metabolism after a period of HIT. In contrast, Esbjörnsson-Liljedahl et al. (1996) reported no change in CS or
HAD after 4 wk of HIT in either male or female subjects. It is possible that a 4 wk training period was too short to induce any changes in oxidative enzyme concentrations. Nevertheless, based on the current literature, no definitive conclusions can be made about changes in oxidative enzyme concentrations after a period of sprint-type training in male and female subjects.

The number of capillaries per mm$^2$ has been reported to increase in men (Klausen et al., 1981) and in women (Mandroukas et al., 1984) after endurance training. I am unaware of any study that has demonstrated the effect of HIT on skeletal muscle capillary density in male and female subjects. However, studies that have examined the changes in muscle fiber capillarization after resistance training in men and women have reported no change in capillary density in female (Wang et al., 1993; Hostler et al., 2001), or in male subjects (Hostler et al., 2001). It is clear that the paucity of information addressing oxygen extraction after sprint-type training makes it difficult to reach any conclusion about gender-specific adaptations to the mechanisms that facilitate improved mitochondrial metabolic capacity.

Previous studies demonstrate increases in maximal $\dot{Q}$ with endurance training in both male (Ekblom et al., 1968) and in female subjects (Saltin, 1990). Indeed, $\dot{Q}$ changes in female subjects appear to be qualitatively similar to those of male subjects after a period of endurance training (Perrault, 1996). However, the absolute maximal $\dot{Q}$ is lower in women than in men both in the untrained and trained state (Senitko et al., 2002). This can be attributed to a higher SV demonstrated in male compared to female subjects (Senitko et al., 2002). Nevertheless, it has not been established if maximal $\dot{Q}$ is improved in either men or women after HIT. Also, additional evidence is required to determine whether increases in maximal $\dot{Q}$ would contribute to accelerated $\dot{VO}_2$ kinetics during conditions of severe oxygen demand.

Plasma volume is not different in female endurance trained runners and cyclists when compared to untrained controls (Green et al., 1999). Conversely, Green et al. (1999) demonstrated that male runners and cyclists show marked (28%) differences in plasma volume compared to untrained men. The higher total blood volume observed in endurance-trained athletes compared to untrained controls is believed to
be a primary determinant of the higher maximal \( \dot{Q} \) observed in the trained state (Wagner, 1991). Changes in plasma volume have yet to be investigated in response to HIT. Based on the findings reported by Green et al. (1999) after endurance training, it could be suggested that HIT may produce an increase in plasma volume in male, but not female subjects thereby increasing maximal \( \dot{Q} \) and/or improving oxygen supply at the onset of exercise in men.

After 12 wk of endurance training, Proctor et al. (2001) found altered skeletal muscle blood flow during submaximal exercise in male subjects, whereas female subjects demonstrated no changes blood flow with training. It could be hypothesized that the ability of men to improve blood flow regulation after training would enhance the distribution of the \( \dot{Q} \) and augment peak skeletal muscle blood flow capacity during supramaximal exercise. Improved oxygen supply at the onset of supramaximal exercise could speed the time course of \( \dot{VO}_2 \) kinetics. Furthermore, male sprinters demonstrate greater concentrations of plasma epinephrine compared to endurance trained and untrained controls (Zouhal et al., 2001). It is not known if female subjects demonstrate increased epinephrine secretion in response to severe-intensity exercise after sprint-type training. Greater sympathetic autonomic activity after training may increase splanchnic and renal vasoconstriction and augment vasodilation in the active muscle vasculature during exercise.

Further research is required to answer the questions that remain about oxygen supply and oxygen utilization limitations at the onset of submaximal and supramaximal exercise bouts. However, it is evident that there are several gender differences in the mechanisms that have the potential to limit and/or facilitate oxygen supply and oxygen utilization. Thus, it seems reasonable to suggest that the \( \dot{VO}_2 \) response (\( \dot{VO}_2 \) kinetics) to moderate-intensity and supramaximal cycling may be different in male and female subjects both before and after training. In accordance with this hypothesis, Chapter 4 of this thesis examined the \( \dot{VO}_2 \) kinetics of untrained male and female subjects during moderate-intensity and supramaximal exercise. Furthermore, \( \dot{VO}_2 \) kinetics were examined in men and women after 8 wk of HIT in order to confirm or reject the notion that \( \dot{VO}_2 \) kinetics are speeded after HIT and that the changes in \( \dot{VO}_2 \) kinetics are gender-dependent.
1.6 GENERAL AIMS OF THE THESIS

It appears that the acute response to exercise and the adaptations that occur with training are not precisely the same in men and women. Thus, it is surprising that few studies have compared the physiological responses and performance capabilities of male and female subjects. Consequently, recommendations made by exercise specialists and coaches are largely based on studies completed with men. It is clear that little is known about the gender-specific responses to supramaximal exercise. Aside from aerobic power, AC is important to many athletic activities. Although MAOD has been accepted by many researchers as a valid measure of an individual’s AC, inadequate data are available on the reliability of this well-established technique in male and female subjects. Furthermore, it is generally accepted that sprint-type training results in improvements in anaerobic ATP production in male subjects. However, it remains unclear if female subjects can increase MAOD after a period of HIT. In addition, the contribution of oxidative metabolism may be important in determining supramaximal exercise performance. The $\dot{V}O_2$ response at the onset of moderate-intensity and supramaximal exercise may provide some insight into the gender-specific responses of aerobic metabolism and the adaptations that occur with HIT.

Aim one: To determine the reliability of measuring MAOD in untrained male and female subjects using well-established experimental techniques.

Aim two: To examine the gender-specific adaptations of the anaerobic and aerobic energy systems during supramaximal cycling after 8 wk of HIT.

Aim three: To model the time course of $\dot{V}O_2$ changes at the onset of cycling at 50% and 110% of $\dot{V}O_2$ peak in men and women before and after 8 wk of HIT.
Maximal accumulated oxygen deficit (MAOD) has been proposed to be a valid (Medbø et al., 1988; Scott et al., 1991) and reliable (Graham and McLellan, 1989; Jacobs et al., 1997) measure of anaerobic capacity (AC). The method of measuring MAOD described by Medbø and colleagues (Medbø and Burgers, 1990; Medbø et al., 1988; Medbø and Tabata, 1993) involves the establishment of a linear relationship between oxygen uptake ($\dot{V}O_2$) and power output from several submaximal exercise intensities. The linear regression determined from the $\dot{V}O_2$-power relationship is used to estimate the oxygen demand for supramaximal exercise intensities (usually 110-125% of peak oxygen uptake; $\dot{V}O_2$-peak). MAOD is then determined as the difference between the calculated oxygen demand and the measured $\dot{V}O_2$ during the MAOD test (Medbø et al., 1988).
Aside from the slope of the regression line calculated from the submaximal exercise bouts, the duration of the supramaximal exercise bout is the most important source of methodological error (Medbø et al., 1988). Based on previous findings (Medbø et al., 1988; Medbø and Tabata, 1989), subsequent investigations (Graham and McLellan, 1989; Jacobs et al., 1997; Weber and Schneider, 2000) have used test duration's of 2 to 3 min for the determination of MAOD during exhaustive, constant-load cycling. Tests of 2 and 3 min in duration are needed to achieve a maximum contribution from anaerobic energy systems and to minimize the methodological error associated with the MAOD technique (Medbø et al., 1988; Medbø and Tabata, 1989). The accumulated oxygen (AO2) deficit for supramaximal cycling might not be maximal if the duration of the exercise test is less than 2 min (Medbø et al., 1988). In the present study, the test-retest reliability of MAOD was examined at 110% and 120% of VO2peak because these exercise intensities have been shown to produce exhaustion in about 2 to 4 min (Medbø et al., 1988; Weber and Schneider, 2000).

Several researchers (Graham and McLellan, 1989; Jacobs et al., 1997; Scott et al., 1991; Weyand et al., 1993; Withers et al., 1991) have simplified the procedures proposed by Medbø et al. (1988) by altering the number and/or the duration of the submaximal exercise bouts used to determine the VO2-power relationship. At present, inadequate data are available on the reliability of MAOD (Graham and McLellan, 1989; Jacobs et al., 1997) using well-established testing procedures in male and female subjects. The determination of MAOD must be reliable enough to be used in individual subjects to quantify AC and to monitor changes in anaerobic performance that occur with training. Researchers, coaches and athletes need to be confident that changes in MAOD over time reflect real differences in performance and are not artifacts of test procedures. The purpose of the present study was to examine the test-retest reliability of MAOD measured at 110% and 120% of peak VO2 for cycling in untrained male and female subjects.
2.1 METHODS

Subjects and experimental design. Seven untrained male subjects and seven untrained female subjects volunteered to participate in the present study. Subjects were considered untrained if they were not training and had not participated or competed in a sport for 24 mo. The procedures used in this investigation were reviewed and approved by the Griffith University Ethics Review Committee for Human Experimentation. Written informed consent was obtained from each subject. All subjects performed a total of eighteen exercise tests during ten testing sessions conducted over a 6 wk period. During this time, subjects were not involved in any other form of physical activity. Subjects were familiarized with all testing equipment and experimental procedures before any exercise testing.

The first testing session involved the determination of the subject’s $\dot{V}O_2$peak for cycling. Steady-state $\dot{V}O_2$ was measured at three submaximal power outputs during session two and at an additional three submaximal power outputs in session three. The six submaximal exercise bouts were then repeated over the next two testing sessions. The sixth testing session was used to familiarize subjects with the MAOD test used to measure AC for cycling. Subjects performed a practice trial of the MAOD test, randomly selected at a power output predicted to elicit either 110% or 120% of $\dot{V}O_2$peak for cycling. Each subject was then required to perform a series of four MAOD tests separated by at least 48 hr. All subjects performed two MAOD tests at a power output corresponding to 110% and two tests at 120% of $\dot{V}O_2$peak. The four MAOD tests were conducted in random order.

Determination of peak $\dot{V}O_2$ for cycling. Peak $\dot{V}O_2$ for cycling was measured using a Lode (Excalibur Sport, Groningen, The Netherlands) cycle ergometer. After 3 min of unloaded cycling at 70 rev-min$^{-1}$, the power output was increased by 25 W-min$^{-1}$ for males and 20 W-min$^{-1}$ for females until the subject reached exhaustion. Oxygen uptake was measured over 30-s intervals throughout exercise using open-circuit spirometry (MedGraphics CardiO$^2$, Cardiopulmonary Diagnostic Systems, St. Paul, MN, USA). The two highest 30-s values achieved during exercise were averaged.
and used as $\dot{V}O_2$peak. The oxygen and carbon dioxide analyzers were calibrated before and after each test using precision reference gases, while volume was calibrated using a Hans Rudolph 3-L syringe. Heart rate and rhythm were monitored continuously during exercise using a CM5 electrode configuration and a Lohmeier (M607, Munchen, Germany) electrocardiograph.

**Submaximal exercise tests.** Subjects performed a series of six submaximal cycling bouts over two testing sessions, which were repeated during two additional testing sessions. The six submaximal power outputs varied between 20 and 75% of $\dot{V}O_2$peak. Subjects performed 2 min of unloaded cycling at 70 rev-min$^{-1}$ and then the predetermined power output was applied immediately for 10 min. Oxygen uptake was measured throughout each 10-min exercise bout as described previously. The $\dot{V}O_2$ values measured during minutes nine and ten were averaged and used as the steady-state $\dot{V}O_2$ for the corresponding power output. The linear regression of $\dot{V}O_2$ and power output for the six submaximal exercise bouts was then determined for the two separate trials.

**Determination of the maximal accumulated oxygen deficit.** After one familiarization trial, each of the subjects performed two MAOD tests at a power output corresponding to 110% and two tests at 120% of $\dot{V}O_2$peak. The subjects warmed-up by cycling at 70 rev-min$^{-1}$ for 5 min between 25-50 W on a Lode (Excalibur Sport, Groningen, The Netherlands) cycle ergometer followed by 2 min of unloaded cycling. At the end of the 2-min period of unloaded cycling, the predetermined power output was applied immediately. The subject cycled at this power output until they could no longer maintain the pedal frequency above 60 rev-min$^{-1}$ despite verbal encouragement. The $\dot{V}O_2$ was measured continuously throughout the exercise bout and the duration of exercise was recorded to the nearest second. MAOD was calculated using the method previously described by Medbø et al. (1988) and subsequently reduced by 9% to correct for reductions in the oxygen stores of the body. The MAOD for each subject was calculated in oxygen consumption
equivalents as the difference between the oxygen demand of exercise and the measured \( \dot{V}O_2 \).

**Statistical analysis.** Results are presented as means±standard error of the mean. Independent t-tests were used to test for gender differences in physical characteristics and in peak exercise values obtained during incremental cycling. Paired-sample t-tests were used to examine differences between repeated exercise trials for submaximal and supramaximal (MAOD) tests. Linear regression analysis was used to determine the \( \dot{V}O_2 \)-power relationship for six submaximal exercise bouts. Test-retest reliability coefficients were determined using an intra-class correlation coefficient. The difference between trials was calculated as the absolute difference between trial one and trial two expressed as a percentage of the initial trial (% difference).

### 2.2 RESULTS

**Subject characteristics and incremental cycling.** Physical characteristics of the subjects and peak exercise values obtained during incremental cycling are presented in Table 1. The mean values for age and body mass were not significantly different between genders, whereas height was significantly greater for male than for female subjects. Peak power and \( \dot{V}O_2 \)peak values achieved during incremental cycling were significantly greater for male than for female subjects. There was no significant difference between the two groups in either peak respiratory exchange ratio (RER) or peak exercise HR values.

**Reliability of the \( \dot{V}O_2 \)-power relationship.** In order to examine the reproducibility of the \( \dot{V}O_2 \)-power relationship, each subject performed all six submaximal cycling bouts on two separate occasions. Gender differences in the slope or y-intercept of the \( \dot{V}O_2 \)-power relationship were not statistically significant. When the two groups
were combined (n=14), the mean values for the slope (trial 1 0.010±0.000; trial 2 0.010±0.000 L·min⁻¹·W⁻¹) and y-intercept (trial 1 0.46±0.02; trial 2 0.45±0.03 L·min⁻¹) did not differ significantly between the first and the second \(\dot{\text{V}}\text{O}_2\)-power relationships.

Table 1. Physical characteristics of the subjects and peak exercise values obtained during incremental cycling in untrained men and women.

<table>
<thead>
<tr>
<th></th>
<th>Men (n=7)</th>
<th>Women (n=7)</th>
<th>Total (n=14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>23.6±1.3</td>
<td>25.3±2.0</td>
<td>24.4±1.2</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>71.7±1.6</td>
<td>65.2±3.9</td>
<td>68.5±2.2</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>174±1</td>
<td>168±1*</td>
<td>171±1</td>
</tr>
<tr>
<td>(\dot{\text{V}}\text{O}_2) peak (L·min⁻¹)</td>
<td>3.18±0.12</td>
<td>2.33±0.21*</td>
<td>2.76±0.16</td>
</tr>
<tr>
<td>(\dot{\text{V}}\text{O}_2) peak (mL·kg⁻¹·min⁻¹)</td>
<td>44.6±1.9</td>
<td>35.8±2.8*</td>
<td>40.2±2.0</td>
</tr>
<tr>
<td>Peak HR (beats·min⁻¹)</td>
<td>192±2</td>
<td>191±2</td>
<td>191±1</td>
</tr>
<tr>
<td>Peak RER</td>
<td>1.30±0.02</td>
<td>1.30±0.04</td>
<td>1.30±0.02</td>
</tr>
<tr>
<td>Peak Power (W)</td>
<td>320±14</td>
<td>234±28*</td>
<td>280±19</td>
</tr>
</tbody>
</table>

Values are means±standard error of the mean. *Significant difference between male and female subjects, p < 0.05. Peak power was determined as the average power achieved during the last two 30-s intervals of the incremental exercise test. \(\dot{\text{V}}\text{O}_2\) peak = peak oxygen uptake; HR = Heart rate; RER = respiratory exchange ratio.

Reliability of the MAOD tests. After one familiarization trial, all subjects performed two MAOD tests at 110% and two tests at 120% of \(\dot{\text{V}}\text{O}_2\) peak in random order. There were no significant differences between male and female subjects in the time to exhaustion (TE) achieved for either the 110% or the 120% tests. The TE for the combined group was not significantly different between trial one (226±13 s) and trial two (223±14 s) of the 110% test. Likewise, no significant difference was found in the mean TE between trial one (158±11 s) and trial two (159±10 s) for the 120% test. Also, the mean % difference between trials in the TE was not significantly different between the 110% test (5.5±1.1%) and the 120% test (3.9±1.3%). Reproducibility of
the TE during the MAOD tests performed at 110% and 120% of \( \dot{V}O_2 \text{peak} \) is illustrated in Figure 1. The intra-class correlation coefficients for the TE were 0.95 and 0.98 for the 110% and the 120% tests, respectively (\( p < 0.001 \) for both tests).

The mean MAOD was significantly higher in male than in female subjects for both the 110% (men 3.01±0.11; women 2.15±0.16 L, mean of two trials) and 120% (men 3.11±0.12; women 2.16±0.17 L, mean of two trials) tests. However, none of the between trial differences in MAOD were statistically significant for either male or female subjects. When the male and female groups were combined (\( n=14 \)), the mean MAOD value obtained in trial one (2.62±0.17 L) was not significantly different from the mean value obtained in trial two (2.54±0.19 L) for the 110% test. Likewise, the difference in MAOD between trial one (2.64±0.21 L) and trial two (2.63±0.19 L) was not significant for the 120% test. Additionally, the mean percent difference in MAOD between trial one and trial two for the 110% test (7.0±1.3%) was not significantly different from the mean percent difference between trials obtained in the 120% test (6.3±1.0%).

![Figure 1. Test-retest reliability for the time to exhaustion determined during the maximal accumulated oxygen deficit tests performed at 110% (left panel) and 120% (right panel) of \( \dot{V}O_2 \text{peak} \) for cycling in men (open circles) and women (closed circles). The diagonal line is the line of identity.](image-url)
Reproducibility of MAOD measured at 110% and 120% of \( \dot{V}O_2 \) peak is illustrated in Figure 2. The intra-class correlation coefficients for MAOD were 0.95 and 0.97 for the 110% and 120% tests, respectively (p < 0.001 for both tests). The mean MAOD obtained for the two 110% trials (2.58±0.18 L, n=28) was not significantly different from the mean MAOD value obtained for the two 120% trials (2.64±0.20 L, n=28).

![Figure 2](image_url)

**Figure 2.** Test-retest reliability of the maximal accumulated oxygen deficit measured at 110% (left panel) and 120% (right panel) of \( \dot{V}O_2 \) peak for cycling in men (open circles) and women (closed circles). The diagonal line is the line of identity.

### 2.3 DISCUSSION

The major finding of the present study was that the determination of MAOD for cycling was highly repeatable at both 110% and 120% of \( \dot{V}O_2 \) peak in untrained male and female subjects. In the present study, the average TE for the 110% test was 225±9 s (mean of the two trials, n=28), whereas the duration of the 120% test was 158±7 s (mean of the two trials, n=28). Although the 110% test was significantly longer than the 120% test, the mean MAOD obtained for the two 110% trials was not
significantly different from the mean MAOD value obtained for the two 120% trials. These findings are in agreement with those of Medbø et al. (1988) who found that the MAOD for running did not increase as long as the MAOD test was at least 2 min in duration.

There was a similar error in the TE for the 110% (5.5±1.1%) and the 120% (3.9±1.3%) test when the absolute difference between trial one and trial two was expressed as a percentage of the total exercise time. Moreover, as the TE increased, there was no significant increase in the error of the MAOD measurement (110% test 7.0±1.3%; 120% test 6.3±1.0%, p > 0.05). This suggests that using the methods described in the present study, MAOD can be measured reliably at either exercise intensity. In contrast to the present findings, it has been suggested by Medbø et al. (1988) that the imprecision of measuring MAOD is proportional to the duration of the test. These investigators suggest that to minimize methodological error, the MAOD test should not last more than 3 min.

Jacobs et al. (1997) demonstrated the reproducibility of MAOD measured for cycling at 125% $\dot{V}O_2$peak in twenty-six subjects. In that study, the mean for the two trials did not differ significantly for either MAOD or the TE. Intra-class correlation coefficients were not reported. However, eight of the twenty-six subjects (31%) were unable to cycle for 2 min at 125% of $\dot{V}O_2$peak and therefore MAOD may not have been measured in those individuals. These findings suggest that 125% of $\dot{V}O_2$peak for cycling is too high for the determination of MAOD in many subjects.

Atkinson and Nevill (1998) suggest that the effects of learning and fatigue on successive trials can be a considerable source of test-retest measurement error. The present study did not demonstrate a systematic bias between trials for either the 110% or the 120% test. Performance was not observed to improve or decrease over the two trials for either exercise intensity. A comprehensive familiarization process and highly controlled testing conditions ensured that learning effects and/or a higher level of motivation did not influence trial two. In addition, no systematic decrement in performance across trials indicates sufficient recovery time and the absence of fatigue prior to the second trial. These precautions and controlled testing conditions
are important to prevent systematic bias in successive trials in the measurement of MAOD.

Untrained subjects were studied in order to examine the repeatability of MAOD in subjects who were not familiar with high-intensity exercise performed to volitional fatigue. Moreover, it was important to minimize training as a confounding factor since high-intensity interval training has been shown to increase MAOD by about 10% after only 6 wk of training (Medbø and Burgers, 1990). Additionally, Esbjörsson-Liljedahl et al. (1996) suggested that untrained females might be less familiar with high-intensity exercise than untrained males. Therefore, it was hypothesized that the measurement of MAOD would be less reliable in females than in males. However, there were no gender differences in the reliability of MAOD. While the present study demonstrated that MAOD is highly repeatable in male and female subjects at both 110% and 120% of VO$_2$peak, it is recommended that the preparation and execution of the MAOD test be carefully standardized.
CHAPTER 3: Increases in maximal accumulated oxygen deficit after high-intensity interval training are not gender dependent

The maximal amount of adenosine triphosphate (ATP) that can be produced through anaerobic metabolism during a supramaximal exercise bout has been defined as a person's anaerobic capacity (AC) (Green and Dawson, 1993). Several researchers have suggested that the maximal accumulated oxygen deficit (MAOD), measured during 2-3 min of exhaustive exercise, is an accurate method of quantifying an individual's AC (Miller et al., 1993; Naughton et al., 1997; Tabata et al., 1996). Medbø and Burgers (1990) reported a 16% increase in MAOD for males following 6 wk of high-intensity interval training (HIT), whereas females did not significantly improve their MAOD. Medbø and Burgers (1990) speculated that AC might be more "trainable" in males than in females.

Exercise tests that are shorter in duration (e.g., 20-30 s) have also been used to examine anaerobic metabolism before and after intense interval training. Campbell
et al. (1979) demonstrated significant improvements in the peak power output achieved during a 20-s sprint cycling test for female subjects after 6 wk of training. Also, a significant increase in the peak power attained during a Wingate Anaerobic Test was demonstrated in females, but not in males following 4 wk of HIT (Esbjörnsson-Liljedahl et al., 2000). While all-out sprint exercise tests of less than 1 min in duration do not provide direct information about AC (Green and Dawson, 1993; Withers et al., 1993), these studies suggest that female subjects are able to increase the rate of anaerobic energy release during short-term exercise after HIT. Although it has been suggested that there is a close relationship between the rate of anaerobic energy release and MAOD (Medbø and Burgers, 1990), the lack of improvement in MAOD by female subjects is inconsistent with the significant increases in peak power obtained during sprint exercise reported after training in other studies.

There is no strong evidence to suggest that changes in anaerobic ATP production are different in men and women following a period of HIT. Furthermore, it is unclear if changes in oxidative metabolism after intense interval training are gender dependent. While several investigators have demonstrated an increase in oxidative enzyme activity and/or peak oxygen uptake (VO$_2$) in male subjects after sprint-type training (MacDougall et al., 1998; Parra et al., 2000; Rodas et al., 2000; Sharp et al., 1986), others have reported no change in aerobic energy production following HIT (Esbjörnsson-Liljedahl et al., 1996; Nevill et al., 1989). Less is known about changes in oxidative metabolism in female subjects following short-term HIT. Ready et al. (Ready et al., 1981) demonstrated an 8% increase in maximal VO$_2$, whereas Campbell et al. (1979) found no change in VO$_2$peak after short-term sprint cycle training in female subjects. Furthermore, I am unaware of any study that has compared the aerobic contribution to a bout of intense cycling between men and women both before and after sprint-type training. Increased aerobic metabolism during high-intensity cycling could reduce the reliance on anaerobic energy production and consequently delay fatigue.

The purpose of the present study was to examine the MAOD in male and female subjects before and after 4 and 8 wk of HIT. Furthermore, this study investigated the
effects of intense interval training on peak \( \text{VO}_2 \) and aerobic energy production during 2-3 min of exhaustive cycling performed at 120% of \( \text{VO}_2 \) peak in men and women. It was hypothesized that the changes in MAOD after 4 and 8 wk of HIT are not gender dependant and that oxidative metabolism would increase following intense interval training in both groups.

### 3.1 METHODS

**Subjects and experimental design.** Seven untrained male and seven untrained female subjects volunteered to participate in the present study. Subjects were considered untrained if they were not training and had not regularly participated or competed in a sport for 24 mo. Additionally, no subject had a history of highly competitive sport. Subjects were familiarized with the experimental procedures and provided written informed consent before testing. The Griffith University Ethics Committee for Human Experimentation approved the testing procedures used in this study. All female subjects had regular menstrual cycles and performed the pre-, mid- and post-training cycling tests in the follicular phase of their menstrual cycle. It is important to control for the potential effect of menstrual cycle status on exercise performance (Tarnpolsky, 1999) and it has been demonstrated previously that the peak power output obtained during sprint cycling may be lower during the luteal phase compared to the follicular phase (Parish and Jakeman, 1987).

The present study involved 8 wk of HIT. Subjects performed six submaximal cycling tests 2 wk before the initiation of training in order to determine their \( \text{VO}_2 \)-power relationship. In the week preceding the commencement of training (pre-training), the subject’s \( \text{VO}_2 \) peak was measured and the MAOD for cycling was determined at least 2 d later. MAOD was also determined after 4 wk of training (mid-training). Following 8 wk of HIT (post-training), subjects completed an additional cycling test (post-training timed test) 48 h after the final training session. The post-training timed test was performed at the same power output used in the MAOD tests, but the test was stopped at the time to exhaustion (TE) achieved during the pre-training MAOD test.
Subjects rested for 2 d before the post-training MAOD test was conducted. After an additional 48 h of rest, the \( \dot{V}O_2 \)\text{peak} was measured. The active muscle mass (AMM) for cycling was measured pre- and post-training.

**Determination of peak \( \dot{V}O_2 \) for cycling.** The \( \dot{V}O_2 \)\text{peak} for cycling was measured using a continuous ramp protocol conducted on a Lode electronically-braked cycle ergometer (Excalibur Sport V2.0, Groningen, The Netherlands). Pedal rate was maintained at 70 rev\( \cdot \)min\(^{-1}\) and the power output was increased by 20 W\( \cdot \)min\(^{-1}\) for females and by 25 W\( \cdot \)min\(^{-1}\) for males until exhaustion. Heart rate (HR) was monitored continuously during exercise using an electrocardiograph (Lohmeier M 607, Munich, Germany) and \( \dot{V}O_2 \) was measured breath-by-breath (MedGraphics Cardiorespiratory Diagnostic Systems, St. Paul, MN, USA) and averaged over 30-s intervals. The two highest 30-s values for \( \dot{V}O_2 \) were averaged and reported as the \( \dot{V}O_2 \)\text{peak} for cycling.

**Submaximal exercise tests.** Steady-state \( \dot{V}O_2 \) was measured at six, submaximal power outputs between 20\% and 75\% of \( \dot{V}O_2 \)\text{peak} prior to training. Subjects cycled at 70 rev\( \cdot \)min\(^{-1}\) for 10 min and the \( \dot{V}O_2 \) values measured at minutes 9 and 10 were averaged and reported as the steady-state \( \dot{V}O_2 \) for the corresponding power output. Data collected from the six submaximal bouts were used to establish the \( \dot{V}O_2 \)-power relationship for cycling. The linear regression of the \( \dot{V}O_2 \)-power relationship was used to calculate the power output that corresponded to 120\% of \( \dot{V}O_2 \)\text{peak}. This power output was then used in all subsequent MAOD tests (pre-, mid- and post-training) and in the timed cycling test conducted after training.

**The MAOD test and post-training timed cycling test.** Subjects warmed up by cycling on a Lode cycle ergometer for 5 min at 50 W for males and at 35 W for females. Subjects were then asked to rest quietly on the cycle ergometer for 5 min. Immediately before the MAOD test, the subject’s hyperemic earlobe was sterilized with 70\% ethanol and punctured with a 1.5 mm lancet (Microlancet, Becton Dickson,
Sandy, UT, USA). The first drop of blood was wiped away and 50 µL of free-flowing blood was collected in a capillary tube and immediately dispensed into a pre-chilled Eppendorf tube for subsequent analysis of blood lactate concentration ([La\(^{-}\)]) (Yellow Spring Instruments, 2700 SELECT, OH, USA). Following 2 min of unloaded cycling at 70 rev·min\(^{-1}\), the pre-determined power output of 120% VO\(_2\)peak was applied immediately. HR was monitored continuously while VO\(_2\) and minute ventilation (V\(_{E}\)) were measured breath-by-breath throughout the exercise bout. Subjects were required to maintain pedal cadence at 70 rev·min\(^{-1}\) throughout the MAOD tests and the test was terminated when the subject could no longer maintain a pedal cadence of 60 rev·min\(^{-1}\) despite verbal encouragement. Blood samples were obtained for subsequent lactate analysis 3 min after ([La\(^{-}\)]3 min) the MAOD test while the subject cycled at 50 W for males and at 35 W for females.

The accumulated oxygen (AO\(_2\)) deficit was calculated as the difference between the AO\(_2\) demand and the AO\(_2\) uptake measured during the MAOD test (Medbø et al., 1988). The AO\(_2\) deficit calculated for the MAOD test was reported as the maximal AO\(_2\) deficit (MAOD) for cycling, whereas the AO\(_2\) deficit measured during the post-training timed test was not maximal as subjects did not cycle to exhaustion. Absolute MAOD values were decreased by 9% to correct for reductions in the O\(_2\) stores of the body (Medbø et al., 1988). Weber and Schneider (2000) have demonstrated that this method of determining MAOD for cycling is highly repeatable in untrained male and female subjects (intra-class correlation coefficients of 0.983 for TE and 0.968 for MAOD values). Changes in MAOD determined after 4 and 8 wk of training were reported as the percent increase calculated from pre- to mid-training, mid- to post-training and pre- to post-training.

**Training protocol.** Training was performed on a basket-loading Monark cycle ergometer (Monark Ergomedic 824E, Varberg, Sweden) so the load could be applied immediately. Subjects trained 3 d·wk\(^{-1}\) for a total of 8 wk. The training sessions consisted of three, 2-min constant-load cycling intervals performed at 70 rev·min\(^{-1}\). Recovery between intervals was set at 6 min. All training parameters (recovery time, number of intervals and cadence) except cycling intensity, were kept constant.
throughout the 8-wk training period. The intensity of training began at 82.5% of the power output used in the MAOD tests for each subject and was increased by 2.5% of the initial work rate every week. Each subject was training at an intensity equal to 100% of the power output used in the MAOD test by week eight of training. Peak HR was measured during each 2-min cycling interval using a HR monitor (Polar Beat, Polar Electro Oy, Kempele, Finland). Blood \([\text{La}^-]\) was determined during the first training session in week one and during the third session in weeks four and eight for all subjects. Blood samples were collected at rest and 3 min after the third cycling repetition of the training session.

**Determination of active muscle mass.** Body composition was assessed pre- and post-training using dual-energy X-ray absorptiometry (DXA) (Norland XR36, Fort Atkinson, WI, USA). The total lean mass for both legs and the gluteal muscle group was measured and reported as the AMM for cycling. The gluteal muscle mass has been shown to be one of the major muscle groups involved in cycling (Raasch et al., 1997) and has been largely ignored when traditional methods of determining the AMM for cycling are used (Winter et al., 1991). AMM is reported independently of fat mass and bone mineral content. Body composition values obtained using DXA were used to express MAOD relative to the AMM for cycling in each subject.

**Statistical analysis.** MAOD test variables (MAOD, TE and HR) were examined using a 2 x 3 (gender – between group factor; pre-, mid-, and post-training – within group factors) ANOVA with repeated measures for training. Increases (%) in MAOD and TE measured for males and females after 4 wk and again after 8 wk of training were compared using a 2 x 2 ANOVA with repeated measures. In addition, a 2 x 2 ANOVA with repeated measures was used to examine gender differences in peak exercise values measured pre- and post-training. Post-hoc analyses were performed where appropriate using pairwise comparisons with Bonferroni adjustments. A linear regression analysis was used to determine the \(\text{VO}_2\)-power relationship for the six submaximal cycling bouts. Statistical significance was accepted at \(p < 0.05\).
3.2 RESULTS

Subject characteristics and incremental cycling. The physical characteristics of the subjects determined before and after 8 wk of training are presented in Table 1. Body mass (BM) did not change significantly in male or female subjects with training. In addition, the determination of body composition using DXA did not reveal any significant changes in the AMM for cycling in either group following 8 wk of HIT.

Table 1. Physical characteristics of the subjects determined before (pre) and after (post) 8 wk of training.

<table>
<thead>
<tr>
<th></th>
<th>Men (n=7)</th>
<th>Women (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-training</td>
<td>Post-training</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>23.7±1.6a</td>
<td>-</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>177.1±1.7b</td>
<td>-</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>80.8±2.3b</td>
<td>82.6±2.8</td>
</tr>
<tr>
<td>AMM (kg)</td>
<td>29.6±1.1b</td>
<td>28.9±1.6</td>
</tr>
</tbody>
</table>

Values are means±standard error of the mean. AMM = active muscle mass for cycling. Men significantly higher than women; a p < 0.01, b p < 0.001.

The peak exercise values obtained during incremental cycling pre- and post-training are presented in Table 2. Male subjects obtained a significantly higher absolute $\dot{V}O_2$peak (L·min$^{-1}$) than female subjects before training. The gender difference remained significant when $\dot{V}O_2$peak values were expressed relative to BM (men 44.4±2.4; women 39.6±0.9 mL·kg$^{-1}$·min$^{-1}$, p<0.05). Peak $\dot{V}O_2$ increased significantly in male subjects after 8 wk of training, whereas the change in $\dot{V}O_2$peak with training was not significant in female subjects. Both male and female subjects obtained significantly higher peak power outputs after training than before training. There were no significant gender differences in peak HR before training and the peak HR obtained during incremental cycling did not change with training in either group.
Table 2. Peak exercise values obtained during incremental cycling before (pre) and after (post) 8 wk of training.

<table>
<thead>
<tr>
<th></th>
<th>Men (n=7)</th>
<th></th>
<th>Women (n=7)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-training</td>
<td>Post-training</td>
<td>% increase</td>
<td>Pre-training</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \dot{V}O_2 ) peak (L min(^{-1}))</td>
<td>3.58±0.19(^a)</td>
<td>3.85±0.17(^ab)</td>
<td>7.9±2.0(^c)</td>
<td>2.55±0.11</td>
</tr>
<tr>
<td>Peak power (W)</td>
<td>379±19(^a)</td>
<td>419±23(^ab)</td>
<td>10.7±2.0</td>
<td>269±13</td>
</tr>
<tr>
<td>Peak HR (beats min(^{-1}))</td>
<td>192±3</td>
<td>193±3</td>
<td>0.9±1.4</td>
<td>193±2</td>
</tr>
</tbody>
</table>

Values are means±standard error of the mean. \( \dot{V}O_2 \) peak = peak oxygen uptake for cycling. HR = heart rate. Men significantly higher than women; \(^a\)p < 0.001. Post-training significantly higher than pre-training; \(^b\)p < 0.01. Significant difference between men and women in the percent increase from pre- to post-training; \(^c\)p < 0.05.

**MAOD test results.** Table 3 presents the MAOD and TE values determined pre-, mid- and post-training in male and female subjects. Males obtained a greater absolute MAOD for cycling than females pre-, mid- and post-training. In addition, when MAOD was expressed relative to the AMM for cycling, males maintained a higher MAOD than females pre- (men 132.2±4.5; women 116.5±6.6 mL·kg AMM\(^{-1}\), p < 0.05) and post-training (men 166.0±10.6; women 142.7±7.1 mL·kg AMM\(^{-1}\), p < 0.05). Both male and female subjects demonstrated a significant increase in MAOD after only 4 wk of training (pre- to mid-training). Following the final 4 wk of training (mid- to post-training), both male and female subjects demonstrated an additional increase in MAOD. There was no gender-dependent difference in the percent increase in MAOD at either 4 or 8 wk of training. The total increase in MAOD of 21.9±6.3% for males was not different from the 19.6±3.1% increase obtained for females after 8 wk of training. There was no difference between male and female subjects in the percent increase in TE after 4 or 8 wk of training.

Peak HR values attained during the pre-training MAOD test were not different between males (187±3 beats·min\(^{-1}\)) and females (188±3 beats·min\(^{-1}\)) and there was no change in peak HR for either group after 8 wk of training. Pre-training, blood
[La\(^-\)]\(_3\)\(_{\text{min}}\) for males was significantly higher than for females (p < 0.001). After 8 wk of training, blood [La\(^-\)]\(_3\)\(_{\text{min}}\) increased to 19.9±0.9 mmol·L\(^{-1}\) in males (p < 0.01) and to 16.0±0.6 mmol·L\(^{-1}\) in females (p < 0.01), but [La\(^-\)]\(_3\)\(_{\text{min}}\) remained significantly higher in male than in female subjects. However, the percent increase in blood [La\(^-\)]\(_3\)\(_{\text{min}}\) from pre- to post-training was not different between the two groups.

### Table 3. Maximal accumulated oxygen deficit (MAOD) and time to exhaustion (TE) measured pre-, mid- and post-training in men and women.

<table>
<thead>
<tr>
<th></th>
<th>Men (n=7)</th>
<th>Women (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MAOD (L)</td>
<td>TE (s)</td>
</tr>
<tr>
<td>Pre-training</td>
<td>3.93±0.22(^a)</td>
<td>175±16</td>
</tr>
<tr>
<td>Mid-training</td>
<td>4.53±0.43(^ab)</td>
<td>262±35(^c)</td>
</tr>
<tr>
<td>Post-training</td>
<td>4.82±0.46(^ae)</td>
<td>303±42(^d)</td>
</tr>
<tr>
<td>Increase (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pre- to mid-training</td>
<td>14.3±5.2</td>
<td>30.6±3.4</td>
</tr>
<tr>
<td>mid- to post-training</td>
<td>6.6±1.9</td>
<td>12.4±3.7</td>
</tr>
<tr>
<td>MAOD (L)</td>
<td>2.75±0.17</td>
<td>166±14</td>
</tr>
<tr>
<td>TE (s)</td>
<td>3.13±0.19(^c)</td>
<td>235±21(^c)</td>
</tr>
<tr>
<td>Increase (%)</td>
<td>14.0±3.0</td>
<td>29.0±3.2</td>
</tr>
<tr>
<td>mid- to post-training</td>
<td>5.1±2.3</td>
<td>9.6±3.9</td>
</tr>
</tbody>
</table>

Values are means±standard error of the mean. Men significantly higher than women; \(^a\)p < 0.01. Mid-training significantly higher than pre-training; \(^b\)p < 0.05, \(^c\)p < 0.01. Post-training significantly higher than mid-training; \(^d\)p < 0.05, \(^e\)p < 0.01.

### Pre-training MAOD test and post-training timed cycling test.

The mean AO\(_2\) deficit determined during the post-training timed cycling test (see Table 4) was significantly lower than the AO\(_2\) deficit achieved during the pre-training MAOD test in male subjects. In contrast, the AO\(_2\) deficit was not different between the two tests in female subjects. The AO\(_2\) uptake measured during the timed cycling test after training was significantly higher when compared to the AO\(_2\) uptake obtained during the pre-training MAOD test in male subjects. There was no change in AO\(_2\) uptake in female subjects as a result of training. During the post-training timed cycling test,
mean $V_e$ was significantly ($p < 0.01$) lower when compared to the pre-training MAOD test in both male (post-training timed 87.6±4.0; pre-training 109.7±6.2 L-min⁻¹) and female (post-training timed 63.9±4.1; pre-training, 75.6±3.9 L-min⁻¹) subjects. The peak HR obtained for both male and female subjects was significantly lower during the timed cycling test after training compared to the pre-training MAOD test. Blood $[\text{La}^-]_3$ was observed to be 21.1±7.2% lower in males and 15.8±4.1% lower in females during the post-training timed cycling test compared to the pre-training MAOD test. The relative decrease in blood $[\text{La}^-]_3$ during the post-training timed test was not different between the two groups.

<table>
<thead>
<tr>
<th></th>
<th>Men (n=7)</th>
<th>Women (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-training</td>
<td>Timed cycling test</td>
</tr>
<tr>
<td>$\text{AO}_2$ deficit (L)</td>
<td>3.93±0.22b</td>
<td>3.71±0.28bd</td>
</tr>
<tr>
<td>$\text{AO}_2$ uptake (L)</td>
<td>8.75±0.99c</td>
<td>8.95±0.95cd</td>
</tr>
<tr>
<td>Peak HR (beats⋅min⁻¹)</td>
<td>187±3</td>
<td>182±2e</td>
</tr>
<tr>
<td>$[\text{La}^-]_3$ (mmol⋅L⁻¹)</td>
<td>16.9±0.4a</td>
<td>14.2±0.7bd</td>
</tr>
</tbody>
</table>

Values are means±standard error of the mean. HR = heart rate. $[\text{La}^-]_3$ is the blood lactate concentration measured 3 min post exercise. Men significantly higher than women; $^a p < 0.001$, $^b p < 0.01$, $^c p < 0.05$. Significant change between pre-training and timed tests; $^d p < 0.01$, $^e p < 0.05$. MAOD = maximal accumulated oxygen deficit.

**Blood lactate and heart rate responses to a training session.** Blood $[\text{La}^-]_3$ measured after the third cycling repetition of the three training sessions was significantly higher in males than in females pre- (men 14.3±0.5; women 11.5±0.6 mmol⋅L⁻¹, $p < 0.01$), mid- (men 14.6±0.6; women 12.5±0.4 mmol⋅L⁻¹, $p < 0.01$) and post-training (men 15.8±0.4; women 12.7±0.3 mmol⋅L⁻¹, $p < 0.001$). However, the
significant increase of 9.6±3.0% (p < 0.05) in blood [La]\(^{-3}\)\(_{\text{min}}\) reported for males was not different from the significant increase of 9.6±4.6% (p < 0.05) measured in females following 8 wk of training. The peak HR recorded during the third cycling interval of a training session in week one was not different between male (183±4 beats-min\(^{-1}\)) and female (185±4 beats-min\(^{-1}\)) subjects. There was no significant change in the peak HR obtained during a training session after 8 wk of HIT for either male (184±3 beats-min\(^{-1}\)) or female (185±3 beats-min\(^{-1}\)) subjects.

### 3.3 DISCUSSION

The primary finding of this study is that the increase in MAOD after 4 and 8 wk of intense interval training was not gender dependent. The total increase in MAOD of 21.9±6.3% for males and the 19.6±3.1% for females in the present study is comparable to the 16-28% increase in MAOD measured for male subjects in previous HIT studies (Harmer et al., 2000; Medbø and Burgers, 1990; Tabata et al., 1996). However, the present study is the first to demonstrate a significant increase in MAOD with training in untrained female subjects.

Before training, males demonstrated a significantly higher MAOD than females even when values were expressed relative to the AMM for cycling. This finding is consistent with an earlier study by Dr D. Schneider and I performed in untrained men and women (Weber and Schneider, 2000). Higher MAOD (mL-kgAMM\(^{-1}\)) values in men suggest differences between untrained men and women in morphological and/or biochemical skeletal muscle characteristics. While Esbjörnsson-Liljedahl et al. (1996) failed to show any gender-related difference in muscle phosphofructokinase (PFK) activity measured before 4 wk of HIT, PFK activity as well as the proportion of type II muscle fibers have previously been demonstrated to be higher in male than in female subjects (Cadefau et al., 1990; Miller et al., 1993; Sahlin et al., 1979). These factors may contribute to a greater ability to produce ATP anaerobically during sprint-type exercise in males than in females. Despite such gender differences in muscle enzyme activity and fiber type, the increases observed in MAOD and blood [La]\(^{-3}\)\(_{\text{min}}\)
in the present study, suggest that the ability to increase anaerobic ATP production in response to HIT is not different between men and women.

Both male and female subjects achieved large increases (~14%) in MAOD after 4 wk followed by a smaller increase (~5-7%) after the final 4 wk of training. This time-course of change is similar to that reported by Tabata et al. (1996) for male subjects after 4 wk (23%) and a further 2 wk (5%) of intense interval training. Similarly, Ready et al. (1981) reported that the greatest increment in peak blood \([La]^-\) in female subjects occurred in the first 2 wk of a 6 wk sprint-training program. The time-course of changes in MAOD found for men and women in the present study support the concept that the adaptive response to training becomes less with time (Bouchard, 1986).

It has been demonstrated that the resting muscle content of creatine phosphate and ATP as well as the degradation of these high-energy phosphates during sprinting are unchanged after 8 wk of HIT (Nevill et al., 1989). In addition, energy derived from anaerobic glycolysis has been reported to contribute about 70-80% of the MAOD (Medbø and Tabata, 1989; Medbø et al., 1988). Thus, an increase in anaerobic glycolysis is likely to be the main metabolic process accounting for the greater MAOD following training. The possible mechanisms that contribute to the increase in MAOD following HIT may include an increase in glycolytic flux rate due in part, to an increased activity of glycolytic enzymes such as PFK and lactate dehydrogenase (LDH). However, some researchers have reported increases in PFK activity after HIT (MacDougall et al., 1998; Parra et al., 2000; Sharp et al., 1986), whereas others have reported no change in PFK activity with sprint-type training (Esbjörnsson-Liljedahl et al., 1996). Nevertheless, PFK activity has been reported to be greater in trained compared to untrained men (Sahlin et al., 1979). In addition, Esbjörnsson-Liljedahl et al. (1996) reported that total LDH activity increased by the same relative amount in male and female subjects following 4 wk of HIT. This suggests an increased glycolytic rate and an enhanced ability to stimulate anaerobic ATP production in both men and women after intense interval training. Furthermore, McKenna et al. (1993) demonstrated improved skeletal muscle potassium regulation with intense sprint training. While the relationship between plasma potassium
concentration and work output requires further investigation, improved potassium regulation by skeletal muscle is consistent with reduced fatigability after sprint training. Alternatively, an improvement in MAOD following intense training may be explained in part by an increase in muscle strength. While the anthropometric measurements obtained from DXA in the present study did not indicate a significant change in muscle mass with training, an increase in muscle recruitment could contribute to an enhanced ability to sustain anaerobic energy production.

In contrast to the findings of the present study, Medbø and Burgers (1990) failed to demonstrate a significant increase in MAOD for women following 6 wk of HIT, whereas men achieved a 16% increase. Several limitations in the investigation by Medbø and Burgers (1990) could account for these conflicting observations. Medbø and Burgers (1990) divided five men and seven women into two different training groups. They did not state if subjects completed the same volume of training, or if the response to the different training protocols was gender dependent. In addition, male and female subjects achieved similar peak [La'] following the pre-training MAOD test. This suggests that the female subjects were more anaerobically trained than the male subjects given that women have been reported to have lower peak [La'] than men following high-intensity activity when equally trained (Esbjörnsson-Liljedahl et al., 1996; Naughton et al., 1997; Weber and Schneider, 2000). Therefore, any improvement in MAOD may have been attenuated in female subjects because the training response may be less in subjects closer to their upper limit of performance. Furthermore, Medbø and Burgers (1990) did not attempt to control for menstrual cycle phase as the pre- and post-MAOD tests were 6 wk apart. Parish and Jakeman (1987) demonstrated that women achieved a greater mean and peak power output for a sprint cycling test during the follicular phase of their menstrual cycle when compared to values recorded during the luteal phase. While the magnitude of the effect of menstrual cycle status on intense exercise remains unclear, Tarnopolsky (1999) has suggested that researches should consider the potential effects of menstrual cycle status on exercise performance when designing research studies. The present study tested female subjects in the follicular phase of their menstrual cycle and testing sessions were 4 wk apart to control for any effect of menstrual cycle phase on exercise performance.
Few other studies have examined the changes in anaerobic ATP production pre- and post-training in female subjects. Ready et al. (1981) demonstrated a 20% increase in maximal “oxygen debt” as well as a concomitant increase in peak [La'] following 6 wk of intense cycle training in females. In addition, some researchers have measured the total work performed during 20-30 s of sprint cycling to determine "anaerobic performance capacity" in female subjects before and after 6 wk of sprint cycle training (Campbell et al., 1979; Esbjörnsson-Liljedahl et al., 1996). These investigations report significant improvements in the ability of female subjects to produce ATP anaerobically with training. However, the interpretation of these results should be questioned as several researchers indicate that both maximal oxygen debt and total work achieved during short-term (< 30 s) cycling are not a valid measure of AC (Green and Dawson, 1993; Saltin, 1989).

Medbø and Tabata (1989) suggested that about 65% of the energy required for an exhaustive 2 min exercise bout is provided by aerobic energy systems. Thus, an increase in aerobic power found in response to HIT by some researchers is not surprising (MacDougall et al., 1998; McKenna et al., 1997; Rodas et al., 2000; Sharp et al., 1986). In the present study, a significant increase of 7.9±2.0% in peak $\dot{V}O_2$ was found in male subjects following 8 wk of training. However, the present study failed to demonstrate a significant improvement in $\dot{V}O_2$peak in female subjects in response to HIT. This finding is in agreement with the results reported by Campbell et al. (1979) who found no change in $\dot{V}O_2$max after 6 wk of sprint-cycle training in female subjects.

The increase in $\dot{V}O_2$peak demonstrated in men but not in women in the present study, suggests that male subjects increased maximal cardiac output and/or increased maximal oxygen extraction in response to training to a greater extent than female subjects. Improvements in $\dot{V}O_2$peak reported in men after HIT have been related to increases in oxidative enzyme activity (MacDougall et al., 1998; Parra et al., 2000; Rodas et al., 2000) and muscle blood flow (McKenna et al., 1997). However, Esbjörnsson-Liljedahl et al. (1996) reported no change in the activity of oxidative enzymes following 4 wk of HIT, for either men or women. Alternatively, it has been suggested that women may have a more rapid recovery between training
repetitions compared to men as indicated by the lower accumulation of inosine monophosphate and inosine following high-intensity exercise (Esbjörnsson-Liljedahl et al., 2000). This would allow each successive training repetition to be performed more anaerobically in females compared to males. Thus, female subjects in the present study may have placed less stress on the aerobic energy system during this type of training than male subjects. It is clear that further research is required to examine the mechanisms that control gender-specific changes in peak $\hat{V}O_2$ with intense interval training.

Nevertheless, the improvement of $\hat{V}O_2$peak in male subjects may be associated with the greater $AO_2$ uptake observed in men during the timed cycling test when compared to the pre-training MAOD test. Harmer et al. (2000) measured the $AO_2$ uptake in male subjects during exhaustive cycling at 130% of $VO_2$peak during a timed test conducted after 7 wk of HIT. In contrast to the present study, they reported no change in $AO_2$ uptake in male subjects with training. However, Harmer et al. (2000) used 30 s training intervals whereas 2 min training repetitions were used in the present study. Oxidative ATP generation would be greater with longer repetitions, perhaps explaining the resultant increase in $AO_2$ uptake observed in male subjects in the present study.

It has been suggested that a higher $\hat{V}_E$ obtained after HIT may contribute to a greater $\hat{V}O_2$ and improved acid-base regulation during sprinting (McKenna et al., 1997). However, it is unlikely that the greater $AO_2$ uptake seen in male subjects during the post-training timed test, compared to the pre-training MAOD test, could be accounted for by an increase in $\hat{V}_E$ since mean $\hat{V}_E$ was actually decreased in the timed test. Further evidence that an increase in $AO_2$ uptake in males was not secondary to changes in cardiorespiratory function relates to the fact that the relative decrease in mean $\hat{V}_E$ and peak HR during the timed test was not different between men and women. Nonetheless, no change in $AO_2$ uptake was found in female subjects with training. These findings suggest that adaptations in $\hat{V}_E$ and HR did not contribute to the gender-specific increase in $AO_2$ uptake observed in males during the post-training timed test. Alternatively, the increase in $AO_2$ uptake in male subjects could have been a result of enhanced skeletal muscle oxygen extraction.
after training. McKenna and colleagues (1997) suggested improved gas exchange in
the active musculature during sprinting following 7 wk of HIT in men. Thus, the
unchanged AO$_2$ uptake observed in female subjects in the present study suggests
that skeletal muscle oxygen extraction was not improved with training. In the
absence of cardiac output and arteriovenous oxygen difference measurements, it is
difficult to speculate about the possible mechanisms that account for gender-specific
training adaptations in aerobic metabolism.

Women demonstrated a similar decrease in blood [La$^-$]$_{3\text{min}}$ compared to males during
the post-training timed test. In light of these results, it is possible that female
subjects achieved an increase in cycling efficiency due in part to a decrease in the
energy expenditure of the respiratory and/or stabilizing musculature of the upper
body. This would allow an increased active muscle VO$_2$ without any change in whole
body VO$_2$ in female subjects. It is also possible that 8 wk of intense interval training
enhanced blood lactate removal during the post-training MAOD test and during the
first 3 min of recovery in both male and female subjects. This would account for a
decrease in blood [La$^-$]$_{3\text{min}}$ with no change in AO$_2$ deficit or AO$_2$ uptake in female
subjects.

In summary, the present study demonstrated that the increase in MAOD after 4 and 8
wk of intense interval training was not different between men and women. However,
an increase in VO$_2$ peak and a greater AO$_2$ uptake measured during the post-training
timed test in male subjects only, suggests that 8 wk of HIT improves oxidative
metabolism in men but not in women. These findings suggest that there are basic
gender differences that may predispose males and females to specific metabolic
adaptations following a period of intense interval training. Therefore, the findings of
the present investigation are important for the implementation of gender-specific
training programs where improvement in both anaerobic and aerobic metabolism is
required.
CHAPTER 4: Gender-specific differences in oxygen uptake kinetics determined before and after high-intensity interval training

Researchers have examined the contribution of the anaerobic energy systems to exhaustive cycling performed at a work rate above that achieved at peak oxygen uptake (\(\dot{V}O_2\text{peak}\)) in male and female subjects (Weber and Schneider, 2000; Weyand et al., 1993). Medbø and Tabata (1989) suggest that about 65% of the energy required for a 2 min exercise bout to exhaustion is provided by aerobic energy systems, whereas the remaining 35% of the total energy is derived from anaerobic metabolism. Thus, time to exhaustion (TE) during supramaximal exercise may be determined in part by the rate at which skeletal muscle oxygen consumption (\(\dot{Q}O_2\)) adjusts to a new metabolic requirement. A faster adjustment of \(\dot{Q}O_2\) during the transition from unloaded cycling to a supramaximal work rate would reduce the reliance on intramuscular phosphagen stores and anaerobic glycolysis at the onset of exercise. Reduced metabolic and ionic perturbations in the muscle and blood at the
beginning of supramaximal exercise could improve the ability of an individual to sustain the required work rate by delaying muscle fatigue.

Many researchers have modeled the exponential rise in pulmonary oxygen uptake (\(\dot{\text{VO}}_2\)) at the onset of exercise in order to examine the rate at which \(\dot{\text{Q}}\text{O}_2\) adjusts to a new work rate. The time constant that describes the \(\dot{\text{VO}}_2\) response is defined as the time required to attain 63% of the steady-state \(\dot{\text{VO}}_2\). Currently, there are conflicting results indicating the effect of gender on the \(\dot{\text{VO}}_2\) response to moderate-intensity exercise (below the ventilation threshold; VT). Chilibeck et al. (1996a) indicated that gender accounted for a significant proportion of the variability in the \(\dot{\text{VO}}_2\) time constant during moderate-intensity exercise. However, in a subsequent study, these researchers (Chilibeck et al., 1996b) reported that the \(\dot{\text{VO}}_2\) time constant was not significantly different between nine men and seven women during exercise performed below the VT. In support of the later findings (Chilibeck et al., 1996b), Fawkner et al. (2002) found no significant difference in the time constant between men and women during a work rate designed to elicit 80% of the \(\dot{\text{VO}}_2\) determined at the VT.

The \(\dot{\text{VO}}_2\) response at the onset of supramaximal exercise has also been examined in male subjects (Özyener et al., 2001; Hebestreit et al., 1998; Billat et al., 2000; Craig et al., 1995). However, no study has reported the parameters of \(\dot{\text{VO}}_2\) kinetics during heavy or supramaximal exercise for female subjects. It has been suggested that both oxygen supply and oxygen extraction by the skeletal muscle are rate limiting to \(\dot{\text{VO}}_2\) kinetics during exercise performed above the VT (heavy-intensity exercise). Gender differences have previously been reported in maximal cardiac output (\(\dot{\text{Q}}\)) (Wilmore and Costill, 1994; Hossack and Bruce, 1982) and there is evidence to suggest that maximal \(\dot{\text{Q}}\) is higher in men than in women even when normalized for body size (Wiebe et al., 1998; Reybrouck and Fagard, 1999). Furthermore, hemoglobin concentration is higher in male subjects (Freedson, 1981; Cureton et al., 1986) and men demonstrate a greater volume density of mitochondria than women (Coggan et al., 1992a, 1992b; Hoppeler et al., 1973). Therefore, women may have a reduced potential to supply and/or extract oxygen compared to men at work rates that demand a maximal rate of \(\dot{\text{VO}}_2\). Thus, it can be hypothesized that \(\dot{\text{VO}}_2\) kinetics
are faster in men than in women at the onset of supramaximal exercise. Previous studies have shown a speeding of the \( \dot{\text{VO}}_2 \) response at the onset of submaximal exercise after endurance training in male subjects (Hickson et al., 1978; Phillips et al., 1995; Berry and Moritani, 1985). It has been suggested that young adult women demonstrate similar improvements in cardiovascular function and skeletal muscle oxygen extraction after endurance training when compared to men (Wilmore et al., 2001; O'Toole, 1989). However, little is known about the potential speeding of the \( \dot{\text{VO}}_2 \) time constant after training in women. The only study to examine \( \dot{\text{VO}}_2 \) kinetics before and after endurance training for female subjects showed no significant improvement in the time constant during exercise performed above the lactate threshold (Brandenberg et al., 1999). Nevertheless, \( \dot{\text{VO}}_2 \) kinetics improved by 30% at a work rate below the lactate threshold. These researchers suggest that the exercise-training program employed may not have been of adequate intensity to facilitate improvements in cardiovascular function and/or skeletal muscle oxygen extraction in female subjects.

There is a lack of information regarding changes in \( \dot{\text{VO}}_2 \) kinetics after sprint-type training. Previous studies might lead us to suspect that men, but not women, will demonstrate faster \( \dot{\text{VO}}_2 \) kinetics during supramaximal exercise after high-intensity interval training (HIT). Findings from recent research conducted in our laboratory indicated that men achieved an increase in the accumulated oxygen (\( \text{AO}_2 \)) uptake during supramaximal cycling after 8 wk of HIT. However, no change in oxidative metabolism was observed in female subjects after training (Weber and Schneider, 2001). In accordance with the findings reported for male subjects in Chapter 3 of this thesis, Harmer et al. (2000) observed an increase in the contribution of the aerobic energy system during cycling at 130% of \( \dot{\text{VO}}_2 \) peak in men after 7 wk of sprint-cycle training. Thus, it can be suggested that there are significant improvements in oxygen supply to the active muscle and/or oxygen extraction at the onset of supramaximal cycling in men after training. In contrast, based on the findings of Weber and Schneider (2002), it appears that oxygen supply and utilization adaptations do not occur in women after short-term HIT. Consequently, it can be hypothesized that HIT will result in improved \( \dot{\text{VO}}_2 \) kinetics in male subjects at the onset of both moderate-intensity and supramaximal cycling, whereas female subjects will show no
improvement. Examination of the phase II $\dot{V}O_2$ kinetics during the transition states of both moderate-intensity and supramaximal work rates will provide further understanding of the oxidative adaptations that occur with HIT in men and women. The purpose of the present study was to compare the phase II $\dot{V}O_2$ time constant at the onset of cycling performed at 50% and 110% of $\dot{V}O_2$peak in untrained male and female subjects. In addition, phase II $\dot{V}O_2$ kinetics were re-assessed after 8 wk of HIT.

4.1 METHODS

**Subjects and experimental design.** Six untrained male and six untrained female subjects volunteered to participate in the present study. In order to minimize the influences of cardiorespiratory fitness on $\dot{V}O_2$ kinetics, male and female subjects were selected based on the assessment of training history and aerobic power expressed relative to the estimated active muscle mass (AMM) for cycling. Individuals were considered for the study if they had not regularly participated or competed in a sport for at least 2 yr and had no history of highly competitive sport. Following familiarization with all testing equipment and procedures, written informed consent was obtained from each subject. The Ethics Review Committee of Griffith University granted approval of this experiment.

Pre-testing began 2 wk before the initiation of training. Subjects performed six submaximal cycling tests between 25% and 75% of $\dot{V}O_2$peak in the first week of pre-testing in order to determine their $\dot{V}O_2$-power relationship. The $\dot{V}O_2$-power relationship was used to determine the work rates predicted to elicit 50% and 110% of $\dot{V}O_2$peak for cycling. Peak $\dot{V}O_2$ and VT determined during incremental cycling was also performed in the first week of pre-testing. In the week immediately prior to the commencement of training, $\dot{V}O_2$ kinetics were examined during 6 min of cycling at 50% of $\dot{V}O_2$peak (50% test) and during 3 min of cycling at 110% of $\dot{V}O_2$peak (110% test). After 8 wk of HIT, $\dot{V}O_2$peak and VT were re-measured and subjects repeated the 50% and 110% tests at the pre-training work rates.
CHAPTER 3: MAOD, TRAINING and GENDER

**Determination of peak \( \dot{V}O_2 \) and the ventilatory threshold.** The \( \dot{V}O_2 \)peak for cycling was measured before and after 8 wk of HIT using a continuous ramp protocol conducted on a Lode electronically-braked cycle ergometer (Excalibur Sport V2.0, Groningen, The Netherlands). Pedal rate was maintained at 70 rev-min\(^{-1}\) and the power output was increased by 20 W-min\(^{-1}\) for women and by 25 W-min\(^{-1}\) for men until exhaustion. Gas-exchange variables were measured breath-by-breath and averaged over 30-s intervals using a metabolic measurement system (MedGraphics CardiO\(_2\), Cardiopulmonary Diagnostic Systems, St.Paul, MN, USA). The oxygen and carbon dioxide analyzers and the pneumotachograph were calibrated before and after each test using precision reference gases and a syringe of known volume (3-L). Heart rate (HR) was monitored continuously during exercise using an electrocardiograph (Lohmeier M 607, Munich, Germany). The VT was determined using \( \dot{V}O_2 \) and carbon dioxide output (\( \dot{V}CO_2 \)) data obtained during the continuous ramp cycling test. VT was calculated using the simplified V-slope method previously described by Schneider et al. (1993) and is reported relative to \( \dot{V}O_2 \)peak.

**Submaximal exercise tests.** Before training commenced, steady-state \( \dot{V}O_2 \) was measured at six submaximal power outputs between 25% and 75% of \( \dot{V}O_2 \)peak over a 2-d period. Subjects cycled at 70 rev-min\(^{-1}\) for 10 min and the \( \dot{V}O_2 \) values measured at minutes 9 and 10 were averaged and reported as the steady-state \( \dot{V}O_2 \) for the corresponding power output. At least 30 min of recovery were allowed between each submaximal exercise bout. Data collected from the six submaximal bouts were used to establish the \( \dot{V}O_2 \)-power relationship for cycling in each subject. The linear regression of the \( \dot{V}O_2 \)-power relationship was used to calculate the work rate that corresponded to 50% and 110% of pre-training \( \dot{V}O_2 \)peak (Medbø et al. 1988). These work rates were then used in the pre- and post-training 50% and 110% tests.

**Cycling tests performed at 50% and 110% of pre-training \( \dot{V}O_2 \)peak.** Each subject completed two constant-load cycling tests at 50% and two additional tests at 110% of pre-training \( \dot{V}O_2 \)peak before and again after training. The four cycling tests
were completed over two testing sessions at least 48 h apart. Subjects completed one of the 50% tests during the first of the two testing sessions. At least 60 min of recovery was allowed before the subject performed the first of the 110% tests. Approximately 48 h later, subjects completed the second trial of the 50% and 110% tests under the same testing conditions. All female subjects reported having a regular menstrual cycle and performed the pre- and post-training 50% and 110% tests in the follicular phase of their menstrual cycle.

Subjects warmed-up by cycling on a Lode cycle ergometer for 5 min at 50 W for men and at 35 W for women. Subjects were then asked to rest quietly on the cycle ergometer for 5 min. Following 4 min of unloaded cycling at 70 rev-min$^{-1}$, the pre-determined work rate of either 50% or 110% of pre-training VO$_2$peak was applied instantaneously. Subjects were required to maintain a pedal cadence of 70 rev-min$^{-1}$. Subjects were unaware of exercise time and did not know the point at which the power output was applied or the elapsed time during exercise. VO$_2$ was measured breath-by-breath (MedGraphics CardiO$_2$, Cardiopulmonary Diagnostic Systems, St.Paul, MN, USA) throughout exercise. Heart rate was recorded by transferring the ECG signal into the metabolic measurement system. Exercise was terminated after 3 min of cycling for the 110% tests and after 6 min for the 50% tests. I have previously demonstrated that most untrained men and women are able to complete at least 3 min of cycling at a power output predicted to elicit 110% of VO$_2$peak (Weber and Schneider, 2000). All subjects in the present study completed 3 min of cycling during the 110% tests.

**VO$_2$ response during the 50% and 110% cycling tests.** The breath-by-breath VO$_2$ data obtained during each trial of the 50% and 110% cycling tests were smoothed using a middle five-of-seven breaths filtering process (see Chapter 6). Each data set was then linearly interpolated second-by-second. The VO$_2$ data from the two trials were time aligned for each exercise intensity and averaged thereby maximizing dynamic resolution. Non-linear regression techniques were used to model the VO$_2$ response. An iterative process ensured that the residual sum of squared error was minimized (SigmaPlot 4.0, SPSS Inc., Chicago, IL, USA).
Phase I was identified visually by two independent researchers and was considered from the application of the work rate to the sharp downward turn in respiratory exchange ratio (RER), end-tidal PO$_2$ and end-tidal PCO$_2$. Phase I data were excluded for the modeling process such that the first exponential term of the equation included a delay term that was constrained to start after the end of phase I (Fawkner et al., 2002). The single-term exponential equation (1) was used to model both the 50% and the 110% VO$_2$ test data pre- and post-training. In addition, the 110% test data were also fitted using a two-term exponential equation (2) to test for the possible presence of a higher order exponential component (i.e., VO$_2$ slow component; VO$_{2SC}$):

\[ \Delta \text{VO}_2(t) = A_2 \cdot (1 - e^{-\frac{t}{\tau_{TD}}}) \]  

(1)

\[ \Delta \text{VO}_2(t) = A_2 \cdot (1 - e^{-\frac{t}{\tau_{TD}}}) + A_3 \cdot (1 - e^{-\frac{t}{\tau_{TD}}}) \]  

(2)

where 2 and 3 denote phase II and phase III (VO$_{2SC}$), respectively and $\tau$, TD and A are the associated time constant, delay and amplitude (i.e., $\Delta$VO$_2$) terms. The amplitude of phase II ($A_2$) was characterized to end-exercise VO$_2$ ($\text{VO}_2\text{EE}$) calculated by averaging the last 10 s of the interpolated response. To compare $\text{VO}_2\text{EE}$ with the final VO$_2$ asymptote predicted by the model, $A_2$ and the $\text{VO}_2$ achieved at the end of the phase I response were summed and have been reported as $A_2^\prime$.

Three steps were taken for each 110% data set to determine the most appropriate model fit: 1) the parameters derived from the non-linear regression were examined to make sure they were scientifically reasonable. If any parameter made no sense (e.g., if a TD value was negative) the model was rejected. 2) In the case where both the single-term and the two-term model fit the data with sensible values, the residual sum of squares were plotted for both models and visually inspected for randomness and obvious trends. Where a trend was clearly noted in the residual plot, this model was rejected. 3) A Fisher test (F-test) was performed to compare the residual sums of squares between the two models, each corrected for the appropriate degrees of freedom (see Chapter 6).
Training protocol. Subjects trained 3 d-wk\(^{-1}\) for a total of 8 wk. The training sessions consisted of three, 2-min constant-load cycling intervals performed at 70 rev-min\(^{-1}\) on a basket-loading Monark cycle ergometer (Monark Ergomedic 824E, Varberg, Sweden). The basket-loading ergometer allowed the pre-determined work rate to be applied immediately during training bouts. Recovery between intervals was set at 6 min. All training parameters (recovery time, number of intervals and cadence) except cycling intensity were kept constant throughout the 8-wk training period. The training intensity began at 82.5% of a power output predicted to elicit 120% of VO\(_2\)peak for each subject and was increased by 2.5% of the initial work rate every week. The training protocol is consistent with the training protocol outlined in experiment two of this thesis (Weber and Schneider, 2002).

Determination of active muscle mass. Body composition was assessed pre- and post-training using dual-energy X-ray absorptiometry (DXA) (Norland XR36, Fort Atkinson, WI, USA). The total lean mass for both legs and the gluteal muscle group was measured and reported as the AMM for cycling. AMM is reported independently of fat mass and bone mineral content. Body composition values obtained using DXA were used to express VO\(_2\)peak relative to the AMM for cycling in each subject (mL.kgAMM\(^{-1}\).min\(^{-1}\)).

Statistical analysis. The parameters describing the VO\(_2\) response for each work rate were examined using a 2 x 2 (gender – between group factor; pre-, and post-training – within group factors) ANOVA with repeated measures for training (SPSS 10.1, SPSS Inc., Chicago, IL, USA). In addition, a 2 x 2 ANOVA with repeated measures was used to examine gender differences in physical characteristics and peak exercise values measured during incremental cycling pre-, and post-training. Differences in height, weight and age determined pre-training between male and female subjects were assessed using an independent t-test. Correlation coefficients were used to examine relationships between VO\(_2\)peak and the phase II time constants. Statistical significance was accepted at p < 0.05. Values are reported as means±standard error of the mean.
4.2 RESULTS

**Subject characteristics and incremental cycling.** The physical characteristics of the subjects and peak exercise values obtained during incremental cycling are presented in Table 1 for both groups. There was no significant difference in age, but male subjects were taller and heavier than female subjects (p < 0.05). Body mass did not change significantly from pre-to post-training in either male or female subjects. The AMM for cycling was significantly higher in male than in female subjects, but did not change with training in either group. Male subjects obtained a significantly higher absolute $\dot{V}O_2$peak ($L\cdot min^{-1}$) than female subjects before training. However, when $\dot{V}O_2$peak was expressed relative to AMM, there was no significant difference between men and women. Peak $\dot{V}O_2$ increased significantly in male subjects after 8 wk of training, whereas the change in $\dot{V}O_2$peak with training was not significant in female subjects. There were no significant gender differences in peak HR or respiratory exchange ratio (RER) obtained during incremental cycling before training and the peak HR and RER values did not change with training in either group. In addition, VT obtained pre-training occurred at $54.7\pm1.1\%$ of $\dot{V}O_2$peak for men and $55.3\pm0.8\%$ for women (p <0.05). The VT expressed relative to $\dot{V}O_2$peak did not change after training in either group. The moderate-intensity exercise bout (50% test) performed pre- and post-training was lower than the power output corresponding to VT for each subject.

The $\dot{V}O_2$ data measured during the 50% tests was well described by the single-term exponential equation (equation 1). There was no significant difference between the asymptotic amplitude ($A'_2$) when compared to the $\dot{V}O_{2\text{EE}}$ value measured before or after training for either men or women. In addition, there was no change from pre- to post-training in $A'_2$ or the $\dot{V}O_{2\text{EE}}$ value measured during the 50% test in either group. There were no gender differences in $\tau_2$ obtained for the 50% test, and $\tau_2$ did not change with training in either men or women. End-exercise HR (HR\text{EE}) obtained during the final 10 s of the 50% test was not different between men and women and did not change significantly with training.
Table 1. Physical characteristics of the subjects and peak exercise values obtained during incremental cycling before and after training in men and women.

<table>
<thead>
<tr>
<th></th>
<th>Men (n=6)</th>
<th>Pre-training</th>
<th>Post-training</th>
<th>Women (n=6)</th>
<th>Pre-training</th>
<th>Post-training</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>24±2</td>
<td>-</td>
<td>25±2</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Height (cm)</td>
<td>176.8±1.1*</td>
<td>167.8±2.7</td>
<td></td>
<td>78.8±3.3*</td>
<td>79.4±3.5*</td>
<td>62.3±1.3</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>30.6±1.4*</td>
<td>62.7±1.3</td>
<td>30.9±1.6*</td>
<td>62.3±1.3</td>
<td>21.3±0.9</td>
<td>21.1±0.5</td>
</tr>
<tr>
<td>Active muscle mass (kg)</td>
<td>3.61±0.21†</td>
<td>30.9±1.6*</td>
<td>2.41±0.08</td>
<td>3.84±0.21†</td>
<td>2.44±0.07</td>
<td></td>
</tr>
<tr>
<td>VO2peak (L·min⁻¹)</td>
<td>117.6±2.0†</td>
<td>113.7±2.0</td>
<td>191±3</td>
<td>115.7±3.1</td>
<td>124.4±2.3*</td>
<td>193±2</td>
</tr>
<tr>
<td>VO2peak (mL·kgAMM⁻¹·min⁻¹)</td>
<td>191±3</td>
<td>193±2</td>
<td>383±34†*</td>
<td>195±4</td>
<td>124.4±2.3*</td>
<td>1.23±0.09</td>
</tr>
<tr>
<td>Peak Power (W)</td>
<td>431±38*</td>
<td>248±14†</td>
<td>2.41±0.08</td>
<td>277±16</td>
<td>383±34†*</td>
<td>1.26±0.08</td>
</tr>
</tbody>
</table>

Values are means±standard error of the mean. *Male subjects significantly different to female subjects, p < 0.05. †Pre-training significantly different to post-training, p < 0.05. VO2peak = peak oxygen uptake; AMM = active muscle mass; Peak HR = peak heart rate; RER = respiratory exchange ratio.

Oxygen uptake kinetics during cycling at 50% and 110% of pre-training VO2peak. The parameters describing the VO2 response to cycling before and after training are presented in Table 2 for male and female subjects. As expected, male subjects reached significantly greater absolute VO2EE values than female subjects during the 50% and the 110% tests. Phase I of the VO2 response (TD2) ended between 17-19 s for both groups during the 50% and the 110% tests. The duration of phase I kinetics did not change with training in either group.
Table 2. Oxygen uptake kinetics during cycling at 50% and 110% of pre-training \( \dot{V}O_2 \) peak in men and women before and after HIT.

<table>
<thead>
<tr>
<th></th>
<th>Men (n=6)</th>
<th>Pre-training</th>
<th>Post-training</th>
<th>Women (n=6)</th>
<th>Pre-training</th>
<th>Post-training</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>50% of pre-training ( \dot{V}O_2 ) peak</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Work rate (W)</td>
<td>131±16*</td>
<td>-</td>
<td></td>
<td>82±2</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>( \dot{V}O_2 ) UL (L min(^{-1}))</td>
<td>0.75±0.09*</td>
<td>0.73±0.08*</td>
<td></td>
<td>0.63±0.13</td>
<td>0.62±0.14</td>
<td></td>
</tr>
<tr>
<td>TD(_2) (s)</td>
<td>18.2±0.9</td>
<td>18.1±1.1</td>
<td></td>
<td>18.9±1.4</td>
<td>18.3±1.2</td>
<td></td>
</tr>
<tr>
<td>( \tau )(_2) (s)</td>
<td>29.0±3.3</td>
<td>28.8±2.2</td>
<td></td>
<td>30.1±2.4</td>
<td>28.4±2.2</td>
<td></td>
</tr>
<tr>
<td>( A_2' ) (L min(^{-1}))</td>
<td>1.78±0.19*</td>
<td>1.83±0.07*</td>
<td>1.12±0.11</td>
<td>1.16±0.09</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \dot{V}O_2 ) EE (L min(^{-1}))</td>
<td>1.84±0.12*</td>
<td>1.84±0.15*</td>
<td>1.18±0.05</td>
<td>1.19±0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \dot{V}O_2 ) EE (% ( \dot{V}O_2 ) peak)</td>
<td>51.0±0.5†</td>
<td>47.6±1.5</td>
<td>49.0±1.3</td>
<td>48.8±0.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HREE (beats min(^{-1}))</td>
<td>125±4</td>
<td>124±2</td>
<td>123±7</td>
<td>120±10</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>110% of pre-training ( \dot{V}O_2 ) peak</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Work rate (W)</td>
<td>346±22</td>
<td>-</td>
<td>218±7</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \dot{V}O_2 ) UL (L min(^{-1}))</td>
<td>0.76±0.04*</td>
<td>0.78±0.05*</td>
<td>0.66±0.05</td>
<td>0.66±0.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TD(_2) (s)</td>
<td>17.4±1.2</td>
<td>17.3±0.7</td>
<td>17.7±0.5</td>
<td>17.9±0.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \tau )(_2) (s)</td>
<td>40.1±1.9†</td>
<td>36.4±1.6*</td>
<td>45.5±2.2</td>
<td>44.8±2.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( A_2' ) (L min(^{-1}))</td>
<td>3.67±0.12†</td>
<td>3.90±0.14*</td>
<td>2.33±0.22</td>
<td>2.39±0.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \dot{V}O_2 ) EE (L min(^{-1}))</td>
<td>3.50±0.14†</td>
<td>3.84±0.15*</td>
<td>2.28±0.08</td>
<td>2.28±0.09</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \dot{V}O_2 ) EE (% ( \dot{V}O_2 ) peak)</td>
<td>96.9±1.3†</td>
<td>99.9±1.1*</td>
<td>94.8±2.8</td>
<td>93.5±2.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HREE (beats min(^{-1}))</td>
<td>180±3†</td>
<td>172±2</td>
<td>180±6†</td>
<td>174±6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means±standard error of the mean. Parameter estimates are determined from a single-term exponential equation. *Men significantly different to women, p < 0.05. †Pre-training significantly different to post-training, p < 0.05. \( \dot{V}O_2 \) peak = peak oxygen uptake; \( \dot{V}O_2 \) UL = oxygen uptake (L min\(^{-1}\)) measured during the final 60 s of unloaded cycling; TD\(_2\) = phase II time delay; \( \tau \)\(_2\) = phase II time constant for oxygen uptake; \( A_2' \) = the sum of the phase II amplitude and the \( \dot{V}O_2 \) achieved at the end of the phase I response; \( \dot{V}O_2 \) EE = end-exercise oxygen uptake; HREE = end-exercise heart rate. End-exercise values were calculated as an average of the last 10 s of exercise.
Figure 1 illustrates the $\dot{V}O_2$ response during 3 min of cycling at 110% of pre-training $\dot{V}O_2$peak in one male and one female subject before and after training. Pre-training, the $\dot{V}O_2$ response to exercise at 110% of $\dot{V}O_2$ peak was well described by a single-term exponential model in both men and women. Inspection of the residual plots revealed no obvious trend and the “goodness-of-fit” was not significantly improved for the two-term, compared to the single-term model, in any of the subjects pre-training ($p > 0.05$). $\dot{V}O_2$EE was significantly lower than the asymptotic projection ($A_2$') predicted by the model in both groups, whereas $A_2$' was not different from $\dot{V}O_2$peak in either group pre-training ($p < 0.05$). The mean $\tau_2$ value of 40.1±1.8 s obtained for male subjects was significantly faster than the $\tau_2$ value of 45.5±2.2 s determined for female subjects pre-training.

![Figure 1](image1.png)

Figure 1. The oxygen uptake response to 3 min of cycling at 110% of pre-training $\dot{V}O_2$ peak in one male subject (left panel) and one female subject (right panel) pre- (open circles) and post-training (closed circles). Subjects cycled unloaded before the work rate was applied at time 0 (dashed line). Individual data points are 1-s values obtained from the average of two repetitions.
Following 8 wk of training, the \( \dot{V}O_2 \) response to the 110% test was well described by a single-term exponential in female subjects. No significant change in \( A_2' \) or \( \dot{V}O_2EE \) was apparent in female subjects. In addition, no significant change in \( \tau_2 \) was demonstrated in female subjects. However, \( \dot{V}O_2EE \) was significantly higher in male subjects after training compared to before training. In addition, \( \dot{V}O_2EE \) was not different to \( A_2' \) or to post-training \( \dot{V}O_2 \) peak in male subjects. Furthermore, \( \tau_2 \) described by the single-term exponential was significantly faster after training than before training in male subjects.

Table 3. Oxygen uptake kinetics obtained during the 110% test in men: parameter estimates determined from a single- and a two-term exponential equation.

<table>
<thead>
<tr>
<th>Sub-group 1 (single-term; n=4)</th>
<th>Sub-group 2 (two-term; n=2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-training</td>
</tr>
<tr>
<td>Work rate (W)</td>
<td></td>
</tr>
<tr>
<td>( \dot{V}O_2UL ) (L min(^{-1}))</td>
<td>352±30</td>
</tr>
<tr>
<td>TD(_2) (s)</td>
<td>0.76±0.06</td>
</tr>
<tr>
<td>( \tau_2 ) (s)</td>
<td>16.3±0.6</td>
</tr>
<tr>
<td>( A_2' ) (L min(^{-1}))</td>
<td>37.6±1.3(^{\dagger})</td>
</tr>
<tr>
<td>TD(_3) (s)</td>
<td></td>
</tr>
<tr>
<td>( \dot{V}O_2EE ) (L min(^{-1}))</td>
<td>3.71±0.13(^{\dagger})</td>
</tr>
<tr>
<td>Residuals (L min(^{-1}))</td>
<td>0.09±0.04</td>
</tr>
</tbody>
</table>

Values are means±standard error of the mean. \(^{\dagger}\)Pre-training significantly different to post-training for Sub-group 1, \( p < 0.05 \). \( \dot{V}O_2UL \) = oxygen uptake (L min\(^{-1}\)) measured during the final 60 s of unloaded cycling; TD\(_2\) = phase II time delay; \( \tau_2 \) = phase II time constant for oxygen uptake; \( A_2' \) = the sum of the phase II amplitude and the \( \dot{V}O_2 \) achieved at the end of the phase I response; TD\(_3\) = the phase III (\( \dot{V}O_2 \) slow component) time delay; \( \dot{V}O_2EE \) = end-exercise oxygen uptake. End-exercise values were calculated as an average of the last 10 s of exercise. Residuals = the square root of the sum of squared errors of the interpolated data from the model fit, divided by the number of data points over the 3 min period (i.e., 180).
Peak $\dot{V}O_2$ was significantly increased in male subjects with training. Thus, the exercise work rate applied during the 110% test after training corresponded to 103.2±0.8% of post-training $\dot{V}O_2$peak. Incidentally, two of the six male subjects (Sub-group 2) demonstrated a significantly improved “goodness-of-fit” when the two-term equation was used to model the $\dot{V}O_2$ kinetics compared to the single-term model ($F = 11.68$ and 19.74; $p < 0.05$). Table 3 shows the mean parameter estimates for Sub-group 2 that describe the $\dot{V}O_2$ kinetics during the 110% test post-training. New mean values were calculated for the remaining four subjects (Sub-group 1) pre- and post-training and have been presented in Table 3. Using a Wilcoxon Signed-Ranks z-test, $\tau_2$ described by the single-term equation was significantly faster after training than before training in Sub-group 1. This confirms the results obtained from the ANOVA performed on the original group of male subjects ($n=6$).

Finally, HR measured during the final 10 s of the 110% test was not different between men and women before or after training. In addition, HR was significantly lower after training in both male and female groups. The $\tau_2$ determined for the $\dot{V}O_2$ response during the 110% tests (single-term exponential) was not significantly correlated with $\dot{V}O_2$peak (mL·kgAMM$^{-1}$.min$^{-1}$) in either men (pre-training $r = -0.19$; post-training $r = -0.28$, $p > 0.05$) or women (pre-training $r = -0.12$; post-training $r = 0.24$, $p > 0.05$). Also, the increase in $\dot{V}O_2$peak from pre- to post-training was not significantly correlated to the change in the $\tau_2$ found in men during the 110% test after training ($r = 0.27$, $p > 0.05$).

4.3 DISCUSSION

This is the first study that has reported the parameters of $\dot{V}O_2$ kinetics in men and women before and after HIT. The findings of the present study show that $\tau_2$ was significantly faster in male compared to female subjects during exercise performed at 110% of $\dot{V}O_2$peak. In contrast, $\dot{V}O_2$ kinetics were not different between men and women during the 50% test. Consistent with the hypothesis, this investigation demonstrated a speeding of $\tau_2$ in men, but not in women after 8 wk of HIT. However,
faster kinetics were only demonstrated for the 110% test. No change in the $\dot{V}O_2$ response was observed for either men or women during cycling at 50% of pre-training $\dot{V}O_2$peak. This study supports the notion that oxidative adaptations to short-term HIT are gender specific (Weber and Schneider, 2002) and that changes in $\dot{V}O_2$ kinetics after training are dependent on the intensity of the exercise bout at which the $\dot{V}O_2$ response is observed (Carter et al., 2000, Brandenberg et al., 1999).

Using multiple regression analysis, Chilibeck et al. (1996a) reported that gender was significantly related to phase II $\dot{V}O_2$ kinetics during moderate-intensity cycling independent of the gender differences found in aerobic power. In contrast, the time constant for the primary component during the 50% test in the present study was not different between men and women. These results are in agreement with the findings of two previous studies including a subsequent investigation performed by Chilibeck and colleagues (Chilibeck et al., 1996b; Fawkner et al., 2002). Therefore, the majority of evidence now suggests that phase II $\dot{V}O_2$ kinetics are not different in men and women during exercise performed below the VT.

There is a considerable amount of research to suggest that a greater supply of oxygen to the active musculature has no effect on the time course of $\dot{V}O_2$ at the onset of exercise (Grassi, 2000; Burnley et al., 2000). Perhaps the most compelling evidence that supports this suggestion is the findings from isolated in situ dog gastrocnemius preparations. Grassi et al. (1998) and others (Piiper et al., 1968) examined the $QO_2$ kinetics under conditions in which the delay in the adjustment of oxygen supply was eliminated by having the muscle pump-perfused. The findings of their study indicate that the time course of $\dot{QO}_2$ during the rest-to-exercise transition is unchanged even when the supply of oxygen to the active muscle is elevated. Despite relatively higher maximal $\dot{Q}$ values (Wiebe et al., 1998, Reybrouck and Fagard, 1999) and hemoglobin concentrations (Cureton et al., 1986; Green et al., 1999) previously reported in men, male subjects in the present study did not demonstrate faster $\tau_2$ values compared to female subjects during the 50% test either before or after training. Thus, the findings of the present study extend previous investigations that suggest a greater capacity to supply oxygen is not a limiting factor for $\dot{V}O_2$ kinetics during work rates performed below the VT.
In contrast to the 50% test, the $\tau_2$ of 40.1±1.8 s obtained for men during the 110% test pre-training was significantly faster than the $\tau_2$ of 45.5±2.2 s obtained for women in the present study. No previous study has compared the phase II $\dot{V}O_2$ time constant determined during supramaximal exercise between untrained men and women. The findings of the present study indicate that under conditions of maximal oxygen demand, the adjustment of cardiovascular and metabolic mechanisms underlying the fast exponential increase in pulmonary $\dot{V}O_2$ are faster in male than in female subjects. It is appropriate however, to recognize that based on a small number of subjects, the conclusions of the present study should be considered with some caution despite their statistical significance.

Indeed, oxygen supply might become progressively more important when the step increase in work rate is above the VT. Gerbino et al. (1996) showed that $\dot{V}O_2$ kinetics were speeded during a second bout of heavy exercise. It was proposed that residual vasodilatation at the onset of the second bout improved the delivery and distribution of oxygen to the working muscles. Furthermore, MacDonald et al. (1997) demonstrated faster $\dot{V}O_2$ kinetics in hyperoxia than in normoxia at the onset of heavy exercise performed above the VT. Increased arterial oxygen content was suggested to improve the transport of oxygen to the working muscle. Therefore, a greater capacity to deliver oxygen under conditions of severe oxygen demand may diminish the limitation of systemic oxygen supply and contribute in part, to faster $\dot{V}O_2$ kinetics in male compared to female subjects during supramaximal cycling. In the absence of $Q$ and ($a-\bar{v}$)$O_2$ difference measurements, no precise conclusion can be made about the effect of oxygen supply mechanisms on the gender differences observed in $\dot{V}O_2$ kinetics in the present study.

Significant levels of oxygen saturation observed in the venous blood of maximally working human muscle indicates that oxygen extraction is incomplete and might reflect a diffusive limitation of oxygen supply (Wagner, 1988). However, few studies have examined the gender-specific differences that affect transport processes that occur at the microvascular level. Porter et al. (2002) demonstrated that women possess lower capillarization of type II muscle fibers compared to men. Furthermore, it has been suggested that type II fibers are recruited simultaneously, if not before
slow twitch fibers during sprinting (Abernethy et al., 1990). A greater capillary density observed in the type II skeletal muscle fibers of men would reduce the effect of diffusive limitations on $\dot{Q}_O_2$ (McGuire and Secomb, 2001) and increase the rate of $\dot{V}O_2$ adjustment at the onset of severe exercise. Moreover, Green et al. (1984) and other investigators (Borges and Essen-Gustavsson, 1989; Saltin et al., 1977; Nygaard, 1981) have reported that the maximal activity of succinate dehydrogenase (SDH) and citrate synthase (CS) is lower in active female than in male subjects. If oxygen utilization is rate-limiting to $\dot{V}O_2$ at the onset of exercise, increased enzyme activation and a greater volume density of mitochondria in men (Coggan et al., 1992a; 1992b; Hoppeler et al., 1973) might contribute to the faster $\dot{V}O_2$ kinetics observed during supramaximal cycling in male than in female subjects. Nevertheless, similar $\tau_2$ values obtained for men and women during the 50% test in the present study suggest that any rate-limiting affect of oxidative enzyme activity on $\dot{V}O_2$ kinetics is independent of gender during moderate-intensity exercise.

Regardless of the gender differences previously reported in oxygen supply and utilization, $\dot{V}O_2$ peak normalized for AMM was not significantly different between male and female subjects pre-training in the present study. This suggests that the relative capacity of the aerobic energy system was not different between male and female subjects. However, Hill and Stevens (2001) demonstrated that at the onset of supramaximal exercise, $\dot{V}O_2$ is initially driven toward the oxygen demand and is not limited by the achievable $\dot{V}O_2$ peak. Thus, the limitations of $\dot{V}O_2$ peak do not appear to influence $\dot{V}O_2$ kinetics until about 45-60 s after the onset of exercise. It is quite possible that the gender-related differences in central and peripheral reflexes could be more important to the supply of oxygen at the onset of exercise than the limitations set by maximal $\dot{Q}$ and the oxygen carrying capacity of the blood. Considering the arterial and cardiopulmonary baroreflexes are involved in the control of autonomic adjustments at the onset of exercise, gender-related differences in baroreceptor sensitivity could potentially affect sympathetic nerve activity during exercise. Preliminary studies offer insights into the potential gender-related differences in the autonomic regulation of muscle sympathetic nerve activity and in the cardiovagal baroreflex gain in humans (Evans et al., 2001; Beske et al., 2001).
Furthermore, sympathetic $\beta$-adrenergic receptors may play a role in increasing active muscle blood flow (Buckwalter, 1998; Robergs and Roberts, 1997; Vander et al., 1994). If increased sympathoadrenal outflow affects vasomotor tone during exercise, vasodilation in the working muscle may be augmented. Consequently, blood flow to the working muscles would be improved resulting in a faster $\dot{V}O_2$ response during exercise regardless of the change in $Q$. Slower $\dot{V}O_2$ kinetics observed with $\beta$-adrenergic blockage (Kowalchuk and Hughson, 1990) as well as an increased sympathoadrenal response to hypoxia (Mazzeo et al., 2001) support the suggestion that increased sympathetic outflow might affect blood flow and oxygen supply to the working muscles. In a previous study (Weber and Schneider, 2000), we found significantly higher plasma epinephrine concentrations following cycling at 120% of $\dot{V}O_2$ peak in untrained men compared to women. This finding suggests a greater sympathoadrenal response to this type of exercise in male than in female subjects. Whether neural and hormonal mediators are related to the gender differences observed in the time course of $\dot{V}O_2$ during supramaximal cycling in the present study remains to be investigated.

A higher $\dot{V}O_2$ peak and faster $\dot{V}O_2$ kinetics during the 110% test demonstrated in male subjects might indicate increased oxygen supply and/or oxygen extraction after 8 wk of HIT. In contrast, no significant improvement in $\dot{V}O_2$ peak or $\dot{V}O_2$ kinetics occurred in female subjects. Several researchers have shown that the $\dot{V}O_2$ response to submaximal exercise is speeded in men after a period of endurance training (Hickson et al., 1978; Phillips et al., 1995; Berry and Moritani, 1985). In contrast, Brandenberg et al. (1999) found no change in $\dot{V}O_2$ kinetics during cycling at a work rate above the lactate threshold in healthy sedentary female subjects after 3 mo of endurance training. However, these researchers demonstrated that $\dot{V}O_2$ kinetics were 30% faster during moderate-intensity cycling after training in the same group. The findings of these studies are in contrast to the results of the present investigation that show no improvement in $\dot{V}O_2$ kinetics during moderate-intensity exercise in either male or female subjects after HIT training. It is possible that the impact of endurance training on the mechanisms that drive $\dot{V}O_2$ kinetics during moderate-intensity exercise are different to the changes in oxygen supply and/or extraction caused by HIT.
McKenna et al. (1997) and Harmer et al. (2000) clearly demonstrated enhanced aerobic metabolism during supramaximal exercise after sprint training in male subjects. It was suggested by Harmer et al. (2000) that the greater proportion of ATP produced aerobically after training may be due to a greater flux of pyruvate and/or intramuscular triglyceride oxidation. They also propose that increased levels of β-hydroxyacyl-CoA-dehydrogenase, CS, and SDH activities previously reported after HIT in men (Pilegaard et al., 1999; Jacobs et al., 1987; MacDougall et al., 1998), indicate increased mitochondrial density and an enhanced capacity for oxygen utilization. McKenna et al. (1997) demonstrated thatVO₂ was increased in men during a 30-s sprint cycling bout after HIT. Despite being unable to confirm a greater arteriovenous oxygen content difference, McKenna et al. (1997) speculated that small increases in skeletal muscle blood flow might account for the elevated peak pulmonary VO₂. It was also highlighted that an increased maximal Q has been found in sprint-trained rats (Hilty et al., 1989), suggesting that a similar adaptation may occur in humans after HIT. Increased capillarization and mitochondrial volume density were also suggested as potential skeletal muscle adaptations to sprint-type training. Direct measurements of the cardiovascular and skeletal muscle adaptations to HIT in female subjects are scarce. Nevertheless, the results presented in experiment two of this thesis (Weber and Schneider, 2002) clearly indicate increased AO₂ uptake in men, but not in women during supramaximal cycling after 8 wk of HIT. Thus, failure to improve VO₂peak as well as phase II VO₂ kinetics during the 110% test in the present experiment supports the findings of experiment two and suggests that oxidative metabolism is unchanged in female subjects after short-term HIT.

Unchanged VO₂ kinetics during the 50% test with a concurrent speeding of the phase II time constant during the 110% test in men, suggests that oxygen supply, rather than oxygen utilization was improved with training. Research demonstrating that VO₂ kinetics are speeded with elevated oxygen supply during heavy, but not moderate-intensity exercise support this notion (MacDonald et al., 1997). Reduced peak HR values obtained for men during the 110% test in the present study indicate improved cardiovascular function during supramaximal exercise with HIT. No change in peak HR was observed in male subjects during the 50% test post-training.
However, similar changes in HR were observed in female compared to male subjects whereas no improvement in $\tau_2$ was found during the 110% test after training in women. Furthermore, the phase I $\dot{V}O_2$ response was not significantly different pre- and post-training in the present study, suggesting that the immediate $\dot{Q}$ adjustments to exercise were unchanged with HIT in both groups. These findings suggest that adaptations to cardiac function cannot explain the improvement in $\dot{V}O_2$ kinetics found during supramaximal cycling in male subjects in the present study.

Hughson et al. (2000) suggested that a noticeable third phase was evident after about 40 s of cycling at 125% of $\dot{V}O_2$ peak for approximately 1 min. In agreement with these findings, Carter et al. (2002) suggest that the $\dot{V}O_2$ response during 6 min of exercise performed at 100% of $\dot{V}O_2$ peak is best fit with a model that identifies an additional third phase. However, Özyener et al. (2001) demonstrated that the $\dot{V}O_2$ response (after phase I) to cycling at 110% of $\dot{V}O_2$ peak was well described by a single-term exponential model. The fundamental decision of whether to include a higher order exponential term in the model may lie with the knowledge of the presence of a $\dot{V}O_{25c}$ during supramaximal exercise. However, without this knowledge one can only depend on statistical processes to determine the most appropriate model (Hughson et al., 2000; Carter et al., 2002; Özyener et al., 2001).

To make a valid comparison of $\dot{V}O_2$ kinetics across genders, exercise intensity was normalized with reference to peak $\dot{V}O_2$ determined for each subject. This methodological approach has previously been used to compare $\dot{V}O_2$ kinetics between cycling and running (Carter et al., 2000) as well as $\dot{V}O_2$ kinetics between boys and men (Hebestreit et al., 1998). However, it has been demonstrated that the $\dot{V}O_2$ response to a step increase in work rate is faster in individuals with a higher $\dot{V}O_2$ peak (Hickson et al., 1978; Hagberg et al., 1980; Cooper et al., 1985; Powers et al., 1985; Chilibeck et al., 1996a). Thus, it could be argued that faster $\dot{V}O_2$ kinetics found in untrained male compared to untrained female subjects pre-training is due simply to the greater absolute aerobic power in men. However, in agreement with the findings of the present study, Carter et al. (2000) failed to demonstrate a significant relationship between the phase II time constant and $\dot{V}O_2$ peak for either moderate- or heavy-intensity exercise. To limit the effect of cardiorespiratory fitness
on \( \dot{VO}_2 \) kinetics, subjects in the present study were matched for training status. Moreover, the \( \dot{VO}_2 \) peak expressed relative to AMM for cycling was not different between the two groups. Therefore, the faster phase II \( \dot{VO}_2 \) kinetics demonstrated in male compared to female subjects in the present study was not due to a greater aerobic power in men.

In summary, the present study demonstrated that phase II \( \dot{VO}_2 \) kinetics during moderate-intensity exercise are not different between men and women. However, faster \( \dot{VO}_2 \) kinetics during supramaximal exercise observed in male subjects reflects gender-specific differences in the mechanisms that facilitate \( \dot{QO}_2 \) during conditions of severe oxygen demand. Furthermore, an increase in \( \dot{VO}_2 \) peak and the speeding of \( \dot{VO}_2 \) kinetics during the 110% test found after training in male subjects only, indicates that 8 wk of HIT increases aerobic energy production in men but not in women. However, it is apparent that any improvement in oxygen supply and/or oxygen utilization after HIT does not affect \( \dot{VO}_2 \) kinetics during moderate-intensity exercise. Furthermore, improved \( \dot{VO}_2 \) kinetics after HIT in men may be dependent on the intensity of the exercise bout at which the \( \dot{VO}_2 \) response is examined. Finally, faster \( \dot{VO}_2 \) kinetics during supramaximal cycling after HIT in men, are probably not due to an improvement in cardiac function.
CHAPTER 5: Statement of conclusions

5.1 REVIEW OF THE FINDINGS

Summary of the findings. The determination of the maximal accumulated oxygen deficit (MAOD) for cycling was highly repeatable at 110% and 120% of peak oxygen uptake ($\dot{V}O_2$peak) in both untrained male as well as untrained female subjects. In addition, MAOD was not different when measured during exhaustive cycling at 110% compared to 120% of $\dot{V}O_2$peak in untrained subjects. These findings indicate that MAOD is not influenced by exercise intensity or exercise duration when the time to exhaustion (TE) of the supramaximal exercise bout is longer than ~2 min. Furthermore, both untrained men and untrained women can achieve maximal accumulated oxygen (AO2) deficit values during supramaximal cycling of between 2 and 4 min. The TE during supramaximal cycling performed at 110% and 120% of $\dot{V}O_2$peak was also highly reproducible in untrained male and female subjects.
Absolute MAOD values were significantly greater in untrained male compared to female subjects. Men achieved a higher mean MAOD than women even when values were expressed relative to the estimated active muscle mass for cycling. However, the percent increase in MAOD was not different between men and women after 4 and 8 wk of high-intensity interval training (HIT). The percent increase in MAOD achieved by both men and women was greater in the first 4 wk compared to the last 4 wk of training. Moreover, 8 wk of HIT augmented oxidative metabolism in men, indicated by the improvement in VO2peak and greater AO2 uptake measured during constant-load supramaximal cycling after compared to before training. No significant improvement in VO2peak or AO2 uptake was found in female subjects with training. However, male and female subjects demonstrated similar reductions in blood lactate concentration ([La-]) and peak heart rate (HR) values during the post-training timed test when compared to the blood [La-] and HR values obtained during the MAOD test pre-training.

The phase II VO2 time constant (τ) was significantly faster in untrained male compared to untrained female subjects during exercise performed at 110% of VO2peak. However, the VO2 response at the onset of cycling at 50% of VO2 peak was not different between men and women. There was no change in the adjustment of VO2 at the onset of exercise after HIT for either men or women during moderate-intensity cycling. In contrast, the τ determined during cycling at 110% of pre-training VO2peak was speeded in male, but not in female subjects after 8 wk of HIT.

**Future considerations.** It is difficult to speculate about the biological mechanisms responsible for the gender-specific adaptations in VO2peak and VO2 kinetics after HIT. However, the role of estrogen in skeletal muscle adaptation to training warrants further discussion. Previous studies have reported the effect of menstrual cycle phase and/or the use of oral contraceptives (OC) on the metabolic response to exercise. Of particular note are the studies by Notelovitz et al. (1987) and Casazza et al. (2002) who found that VO2peak (L·min⁻¹) decreased by about 10% after 4 to 6 mo of OC administration. It was suggested that a decrease in stroke volume was unlikely to be responsible for the OC-induced negative effect on VO2peak because
estrogen replacement therapy has been shown to increase stroke volume. Furthermore, no difference in resting blood hemoglobin concentrations measured in subjects using OC compared to control subjects suggests that $\dot{V}O_2$ peak was not limited by blood oxygen carrying capacity (Larsson et al., 1992). Alternatively, Casazza and colleagues (2002) suggested that the reduced $\dot{V}O_2$ peak observed in subjects using OC might be a result of a blunting of sympathetic nervous system activity. During exercise, increased sympathetic nervous system activity and the resultant vasoconstriction in non-active tissue is essential for increasing blood flow to the working muscle. In agreement with Casazza et al. (2002), the findings of a number of other researchers suggest a suppressive effect of estrogen on the neural control of skeletal muscle circulation (Charkoudian, 2001; Minson et al., 2000; Weitz et al., 2001). These studies clearly show reduced specific sympathetic nervous activity with increased circulating estrogen during acute experimental observations. Whether the effect of estrogen on sympathetic nervous activity can be related to metabolic and/or cytological adaptations that occur with HIT has not been previously explored.

Considerable research has been performed examining estrogen as an antioxidant in muscle during exercise and its potential to modify post-exercise muscle damage (Yagi and Komura, 1986; Tiidus et al., 1993; Tiidus, 1995). In addition, estrogen has been associated with improved muscle force development and reduced fatigability in repeated strength trials (Phillips et al., 1993; Sarwar et al., 1996). Indeed, the capacity for estrogen to modify muscle force and fatigue might, in part be related to its ability to protect muscle from oxidative stress. Although this mechanism of reducing oxidative stress appears favorable for maintaining force production, other physiological consequences of increased oxidative stress engendered by exercise are unclear. There is evidence emerging which suggests that an increased production of free radicals may act as signals for the adaptive response in skeletal muscle, resulting in the increased production of antioxidant enzymes and heat shock proteins (McArdle et al., 2002). Furthermore, the nature in which free radical species influence gene expression has been the topic of recent research (Jackson, 1999). In this context, it is possible that oxidative stress is a trigger for a signaling of events that lead to metabolic and/or cytological adaptations after exercise. Therefore, the
role of estrogen in reducing oxidative stress and damage may prevent signals from turning on other physiological adaptations such as increased oxidative enzyme activity and/or capillarization.

In light of the findings in the present thesis, it is possible that the failure of untrained women to improve $\dot{V}O_2$peak with HIT was mediated in part, by attenuation of oxidative stress, or through a reduction in sympathetic outflow. However, the influence of estrogen on skeletal muscle adaptation with training remains purely speculative at this time.

5.2 CONCLUSION

Activities performed at supramaximal work rates dominate the physical demands of many athletic events. In fact, few sports events in the United States require a sustained all-out effort longer than 30 to 60 s (deVries, 1986). Surprisingly, supramaximal exercise has previously received little attention from exercise physiology researchers. Furthermore, the metabolic adaptations that occur in men subsequent to HIT have been generalized to the larger population without further question or investigation into the effect of gender. This thesis provides new evidence to suggest that some of the metabolic responses to supramaximal exercise and adaptations to HIT are different between men and women.

As with any objective measure, we need to be certain that the results can be repeated with a high level of reliability. The MAOD is a highly reliable measure that can be used to quantify anaerobic capacity (AC). It is suggested that the measurement of MAOD can be used to determine differences in anaerobic energy production between two groups and to monitor changes in AC with training in both male and female subjects. It should be noted that controlled testing conditions are important to prevent systematic bias in successive trials in the measurement of MAOD. Furthermore, although MAOD is highly repeatable in untrained male and
female subjects at both 110% and 120% of \( \dot{V}O_2 \)peak, it is recommended that the preparation and execution of the MAOD test be carefully standardized.

The percent increases in MAOD and blood [La\(^-\)], observed during exhaustive supramaximal cycling in response to HIT, was not different between male and female subjects. Thus, it is suggested that men and women possess a similar ability to increase anaerobic ATP production in response to 8 wk of HIT. It could be suggested to coaches and exercise specialists that male and female athletes will demonstrate similar improvements in AC when exposed to the same HIT program. However, it should also be recommended that further consideration be given to the contribution of the aerobic energy system to supramaximal exercise and the oxidative changes that occur in response to HIT. Faster \( \dot{V}O_2 \) kinetics observed during supramaximal cycling in untrained male compared to untrained female subjects in the present thesis reflects gender-specific differences in the mechanisms that facilitate skeletal muscle oxygen consumption during conditions of severe oxygen demand. The \( \tau_2 \) was not different between untrained male and female subjects during cycling at 50% of \( \dot{V}O_2 \)peak. Thus, the gender differences in \( \dot{V}O_2 \) kinetics are dependent on the intensity of the experimental exercise bout at which the \( \dot{V}O_2 \) response is observed.

The higher \( \dot{V}O_2 \)peak, greater AO\(_2\) uptake and faster \( \tau_2 \) determined after compared to before training in male subjects clearly indicates the augmentation of oxidative pathways in response to HIT in men. However, failure of female subjects to demonstrate increases in any of these parameters with training suggests that HIT does not facilitate changes in the aerobic energy system in women. Nevertheless, it is apparent that any improvement in the aerobic energy system with HIT in men, does not change \( \dot{V}O_2 \) kinetics during moderate-intensity exercise. Moreover, similar reductions in peak HR observed during the post-training timed test (experiment two) and after 3 min of cycling at 110% of pre-training \( \dot{V}O_2 \) peak (experiment three) between male and female subjects indicate that changes to oxidative metabolism with HIT are probably not due to an improvement in cardiac function in men.
Based on the findings of the present thesis, I confirm and support the notion that the metabolic response to supramaximal exercise and training is in part, gender-dependent. Finally, I maintain the recommendations made by Professor Clarence A. Weber in 1930 and suggest that men and women require specifically designed training programs where improvement in both anaerobic and aerobic metabolism is desired.
CHAPTER 6: Methodology

6.1 PREPARATION FOR EXERCISE TESTING

Subject selection. All of the subjects were selected from volunteers to participate in these experiments based on the following criteria:

- No documented history or clinical signs or symptoms of pulmonary, cardiovascular or metabolic disorder
- Absence of any medication that would interfere with exercise responses or blood lactate concentrations ([La\(^-\)])
- All subjects were aged between 18 and 35 yr
- Subjects were considered untrained if they were not training and had not regularly participated or competed in a sport for 2 yr. No subject had a history of highly-competitive sport
- Demonstrated a peak oxygen uptake ($\text{VO}_2\text{peak}$) of 25-45 mL·kg$^{-1}$·min$^{-1}$ for female subjects and 30-50 mL·kg$^{-1}$·min$^{-1}$ for male subjects
- All female subjects reported having a regular menstrual cycle

**General procedures.** Volunteers were asked to visit the laboratory and speak with the investigator who provided additional information about the research study. Individuals wishing to continue were asked to complete a Physical Activity Readiness Questionnaire (PAR-Q). This form of screening before participation in exercise has been widely accepted as a preliminary medical screen (American College of Sports Medicine, 2000). In addition, a detailed medical history questionnaire was completed before the investigator explained all testing procedures and the related risks and benefits associated with the experiment. Once subjects had completed the PAR-Q and the medical history questionnaire, eligible subjects were asked to sign an informed consent document specific to each experiment. The subjects were then familiarized with the testing equipment such as the cycle ergometers and mouthpiece. All experimental procedures were approved by the Griffith University Ethics Committee for Human Experimentation. Subjects were requested to continue their normal diet throughout the testing period. All exercise tests were conducted early in the morning following an overnight fast and participants were instructed not to perform intense physical exercise for 24 hr before each exercise test.

**Medical history and informed consent.** Subjects were provided with written details explaining the time commitments of each session and the purpose of each session. All potential risks as well as the benefits of the testing sessions were outlined and explained in detail by the investigator. A comprehensive statement of the experimental procedures was supplied to each subject. Subjects were required to complete a PAR-Q and an additional medical history questionnaire in order to highlight any illness or any other factor that may have prevented the subject from participating in the study. Subjects were requested to sign a document clearly indicating that they had volunteered to continue their participation in the study.
Subject familiarization. Subjects were familiarized with the two cycle ergometers. Seat height and handlebar adjustments were made while the subject was instructed to pedal at low revolutions to ensure that they were comfortable with all settings on the cycle ergometer. These settings were recorded for each subject and used in subsequent exercise tests and training. At the same time, the subject was familiarized with the mouthpiece and nose clip. Before inserting the mouthpiece, subjects were informed of hand signals that would be used throughout all testing sessions to ensure adequate communication between investigator and subject. Once the mouthpiece was fitted comfortably, a nose clip was placed on the subject. Subjects were allowed ample time to become comfortable and familiar with the testing equipment.

Subject information and safety. Before experimental testing, each subject was informed of the safety precautions. Subjects were requested and encouraged to stop exercising if they experienced any of the following symptoms during exercise:

- Moderate chest pain (angina)
- Dizziness or confusion
- Nausea

Subjects were monitored throughout all testing procedures and the test was immediately terminated if any of the following signs or symptoms were observed:

- A change in skin color such as pallor (whitened) or cyanosis (blueness around the mouth)
- Ventricular tachycardia
- Exercise-induced heart block
- Significant ST-segment depression or elevation

In the very unlikely case of a misadventure, the investigator was present during all tests to either prevent a problem or deal with any problem that may have occurred.
Subjects were encouraged to stop the test if they felt any discomfort such as chest pain, dizziness, or nausea. After each exercise test, the mouthpiece and nose clip were removed and the subject remained pedaling unloaded at low revolutions until their heart rate decreased to less than 110 beats-min\(^{-1}\). The subject then dismounted the cycle ergometer and sat quietly in an armchair. Subjects were encouraged to drink water as they wished.

**Laboratory conditions.** Temperature, relative humidity and barometric pressure were monitored (Perception II, Davis Instruments, Hayward, CA, USA) and recorded before all sessions. These values were used in the calculation of metabolic parameters measured by the metabolic measurement system. Laboratory temperature was maintained at 21±1°C and the relative humidity at 55±5%.

### 6.2 EQUIPMENT used for collecting and analyzing physiological data

**Metabolic measurement system.** Oxygen uptake (\(\dot{V}_O_2\); STPD), carbon dioxide output (\(\dot{V}_C_O_2\); STPD) and minute ventilation (\(V_E\); BTPS) were determined by open-circuit spirometry using a MedGraphics Cardiorespiratory Diagnostic System (Medical Graphics Corporation, St.Paul, MN, USA). Subjects inhaled room air through a MedGraphics mouthpiece with inspired and expired air passing through a bi-directional differential pressure pneumotachograph (preVent, Medical Graphics Corporation, St. Paul, MN, USA). The pneumotachograph determined flow by measuring the pressure differential between two screens inside the pneumotachograph. The pneumotachograph was calibrated at five varying inspiration and expiration flow rates between 30 and 360 L\cdot min^{-1} before each test using a 3-L syringe (Medical Graphics Corporation, St. Paul, MN, USA). Calibration was repeated if the error at any flow rate was greater than 2.00%. Expired air was continuously sampled from a line placed just in front of the pressure sensor in the pneumotachograph for percent oxygen and carbon dioxide. The percent oxygen was measured using a fast-response zirconia transducer oxygen analyzer. Percent carbon dioxide was measured using a fast-response infrared transducer carbon
dioxide analyzer. The oxygen and carbon dioxide analyzers were integrated using an IBM compatible computer using the BREEZE EX software package (Medical Graphics corporation, St. Paul, MN, USA). Oxygen uptake, $\dot{V}_{CO_2}$, $V_\text{E}$ and respiratory exchange ratio (RER) were measured breath-by-breath. The oxygen and carbon dioxide analyzers were calibrated before and after each test using precision reference (oxygen 21.0%; carbon dioxide 0.0%) and calibration gases (oxygen 12.0%; carbon dioxide 5.0%).

**Cycle ergometers.** The Lode electronically-braked cycle ergometer (Excalibur Sport V2.0, Groningen, Netherlands) was used for all exercise testing sessions. The Lode cycle ergometer demonstrates a high level of precision and accuracy at work rates over 100 W (<2%). These characteristics make this type of ergometer well suited for supramaximal exercise such as the MAOD (maximal accumulated oxygen deficit) test. The Lode cycle ergometer incorporates a feedback mechanism so that work rate is independent of variations in pedal rate between 30 and 120 rev-min$^{-1}$. Therefore, the work rate during exercise remains constant regardless of minor changes in pedal rate. In addition, during the MAOD test, the work rate was programmed to increase up to 1000 W in less than 1 s, allowing the predetermined work rate to be applied almost immediately. Calibration of the Lode cycle ergometer requires specialized equipment that is routinely performed by the manufacturer.

A basket-loading cycle ergometer (Monark 824 E, Varberg, Sweden) was used for all training sessions. The Monark cycle ergometer allowed the work rate to be applied immediately and to be easily readjusted for interchanging subjects with different training work rates. Another benefit of the basket-loading ergometer is the fact that no mechanical calibration is necessary as the resistance applied by the basket-loading ergometer is a direct function of the weight hanging in the basket.

**Height stadiometer and weight scale.** Subject height was measured using a wall mounted stadiometer and was recorded to the nearest 0.5 cm. Body mass was
measured to the nearest 0.1 kg before each testing session using a calibrated electronic scale (CH-150K, AND Scales, Sydney, NSW, Australia).

**Electrocardiogram and electrodes.** Heart rate (HR) and rhythm were monitored continuously during each cycling test using a CM5 electrode configuration and an electrocardiograph (M607, Lohmeier, Munchen, Germany). The skin was prepared before electrode application by lightly rubbing with an ethanol-soaked cotton gauze swab and where necessary chest hair was shaved. Transpore surgical tape was used to secure the electrodes to minimize artifact caused by subject movement. The ECG signal was transferred into the metabolic measurement system for HR storage. During training sessions in experiment two and three, HR was monitored during each 2-min cycling interval using a Polar Beat HR monitor (Polar Electro Oy, Kempele, Finland).

**Blood lactate analyzer.** Blood lactate was collected during experiment two of this thesis. After the final blood collection point, the blood samples in Eppendorf tubes were frozen at -70°C until the samples could be analyzed for blood lactate concentration ([La\textsuperscript{-}]). Blood [La\textsuperscript{-}] was measured using an automated blood lactate analyzer (2700 SELECT, Yellow Springs Instruments, Yellow Springs, Ohio, USA). Four calibration standards of known [La\textsuperscript{-}] of 0, 5, 15 and 30 mmol L\textsuperscript{-1} were used to manually calibrate the analyzer before blood lactate measurement. In addition, the YSI analyzer automatically self-calibrated with a calibration standard of 5.06 mmol L\textsuperscript{-1} prior to use, and again after every five samples or 15 min. A 2.0% agreement between the repeated calibration currents was required for successful auto-calibration.

After calibration, the investigator presented each blood sample to the aspirator needle on the YSI analyzer. Twenty-five µl of the blood sample was aspirated from the Eppendorf tube. The remaining sample was kept on ice until all samples had been analyzed. The blood entered the YSI chamber and was stirred and diluted with a buffer solution. The substrate (lactate) diffused through a thin polycarbonate
membrane, combining with oxygen to produce hydrogen peroxide and pyruvate. The hydrogen peroxide produced diffused toward the electrochemical platinum anode where it was oxidized. The subsequent flow of electrons was linearly proportional to the hydrogen peroxide concentration. The measured current at the platinum anode was linearly proportional to the concentration of blood lactate. Each blood sample took about 3 min to analyze. The YSI microprocessor and diagnostic software package performed the calculations and displayed the blood \([La^-]\) in \(\text{mmol}\cdot\text{L}^{-1}\). The value displayed was then multiplied by the dilution factor (x3) for the initial dilution (50 \(\mu\text{L}\) buffer solution, 25 \(\mu\text{L}\) whole blood) of the blood sample.

**Dual-energy X-ray absorptiometry.** A clear understanding of the concepts is important to the appropriate use and interpretation of dual-energy X-ray absorptiometry (DXA). Below is a brief outline of the DXA method that will aid in the appropriate interpretation of measurement results and may stimulate important research questions. In the present thesis, the Norland XR-36 (Fort Atkinson, WI, USA) was used to determine whole-body and leg measurements of fat and lean mass. There are substantial technical differences among the different DXA manufactures (Hologic, Lunar, Norland) as they use different hardware, X-ray sources, X-ray spectra, detectors, calibration standards and software algorithms. Nevertheless, the basic principles of DXA are the same (Lehmann et al., 1981; Mazess et al., 1990; Wahner et al., 1988).

The X-ray source of the Norland XR-36 directs a pencil beam of two distinct X-ray energies through the body of the subject. The Norland system produces energy peaks at maxima of 40 and 80 keV. The X-ray detector system (2 NaI scintillation detectors in pulse-counting mode) measures the intensity of the X-ray beam that has passed through the body of the subject. It is possible to differentiate dissimilar components of body composition using the two X-ray energies (Pietrobelli et al., 1998). Specifically, the body is grouped into three components with respect to their X-ray properties: bone mineral, fat, and lean tissue (non-fat soft tissue). Bone mineral contains a large percent of calcium and phosphorus, whereas soft tissue is composed nearly completely of hydrogen, carbon and oxygen. In addition, lean
mass components contain traces of potassium, chlorine, sulfur and calcium, whereas the fat compartment contains none. The non-bone containing pixels are analyzed for fat and lean mass, whereas the bone-containing pixels are analyzed for bone and soft tissue. The specific mix of fat and lean tissue that is treated as 'soft tissue' in the bone pixels is estimated, since it cannot be measured.

**General procedures.** The DXA machine was calibrated before the whole-body scan with an automated 77-step calibration standard. On arrival to the laboratory, the subject’s body mass and height were recorded and they were required to remove any metal objects such as belts and jewelry. The subject was then instructed to lie supine on the DXA table ensuring that he/she was positioned entirely within the scan limit border. The technician then directed commands to the DXA machine through an IBM compatible PC computer (500 MHz Intel microprocessor) with a Windows 3.11 operating system. The subject’s body mass and height were entered into the computer and the whole-body scan was selected from the scan type selection screen display. The subject was instructed to remain motionless throughout the scan. Whole-body scan time was 5 min and the radiation exposure was approximately 0.4 mRems. Following analysis, a report screen was displayed on the computer monitor. Each body region was calculated separately as a total mass (g) and independently for bone mineral content (BMC) (g), fat (g and % of total body mass) and lean body mass (LBM).

The white lines defining the separate body segments were adjusted so that the LBM of the gluteal muscle group was included in the calculation of active muscle mass (AMM). The horizontal line marking the top of the pelvis was moved to just above, but not touching, the iliac crest. The auto-grid application was used to ensure that the line position could be repeated accurately. The lines passing through the femoral necks and just below the ischium were adjusted so that the triangular area representing the pelvis mass was included in the right and left leg analysis.

**Sources of error in the assessment of body composition using DXA.** The extent to which the hydration status and the estimation of the composition of soft tissue in bone mineral-containing pixels affect the assessment of body composition cannot be
succinctly summarized. In addition, the strategies used to account for known sources of error would vary among DXA manufactures. Nevertheless, the DXA method has technical limitations that may influence measurements of body composition. The most important of these is the fact that the composition of the soft tissue concealed by bone has to be extrapolated from the composition of the adjacent soft tissues (Tothill and Nord, 1995). For example, in some areas such as the lumbar spine, the soft tissue is 'hidden' by the bone and is assumed to be the same composition as the surrounding soft tissue.

Another recognized weakness in the assessment of body composition by DXA is the assumption that non-bone lean tissue has fixed water and protein fractions. Thus, one source of error in the estimation of body composition by DXA is the hydration status of the subject. The ingestion of ~2.0 kg of fluid are reflected by DXA as changes in non-bone lean mass, whereas bone mineral and fat mass are not affected. However, in most individuals the magnitude of error in fat or fat-free mass would not exceed 0.5 kg, even under conditions of extreme physiological variance in hydration status (Kohrt, 1997). The error in vivo for soft tissue changes are approximately 0.2 kg for total soft tissue mass and approximately 1.0 kg for changes in fat mass and fat-free mass (Gotfredsen et al., 1997). The coefficient of variation for the Norland XR-36 4500 DXA is <1.0% and reliability trials have reported intra-class correlation coefficient of 0.998 (Norton and Olds, 1996).

6.3 CALCULATIONS AND FORMULA

Calculation of the ventilatory threshold using the V-slope method. Oxygen uptake and $\dot{V}CO_2$ data collected during incremental cycling to exhaustion was used to determine the ventilatory threshold (VT). The computerized and simplified V-slope methods of detecting the VT have been well described previously by Schneider et al. (1993). In the present study, we used the simplified V-slope method developed by Sue et al. (1988). Carbon dioxide output was plotted against $\dot{V}O_2$ on equal axes. A line parallel to the line of identity was visually, and then physically drawn through the
data points in view of minimizing the residual sum of squares. The VT was visually identified as the point at which $\dot{V}CO_2$ began to increase more rapidly (i.e., slope $>1.00$) than $\dot{VO}_2$. Thus, VT was determined as the point at which $\dot{V}CO_2$ departed from the line. This process was performed by two independent investigators and the $\dot{VO}_2$ value corresponding to this point was used as the VT. When the point at which VT occurred differed by more than 20 s between investigators, a third independent researcher was consulted.

**Examination of the maximal accumulated oxygen deficit.** There is some uncertainty regarding the validity of the accumulated oxygen (AO$_2$) deficit. Here, I have endeavored to present findings that support the use of these methods during whole body exercise to quantify anaerobic ATP production.

*The $\dot{VO}_2$-power relationship.* Medbø and colleagues have reported that in well-controlled studies, steady-state $\dot{VO}_2$ is found after 8-10 min of constant-intensity exercise performed at both low and high (above VT) submaximal work rates. Medbø and colleagues (1988) suggest that this relationship can be linearly extrapolated to predict the oxygen demand of supramaximal work rates. However, there is substantial evidence indicating that for independent constant-load exercise tests, a non-linear $\dot{VO}_2$-power relationship is observed when exercise is performed both below and above VT (Barstow and Mólé, 1991; Bangsbo et al., 1993). Bangsbo et al., (1993) reported higher $\dot{VO}_2$ values during high submaximal running speeds than that estimated from extrapolating the linear relationship between $\dot{VO}_2$ and power at lower running speeds. The finding of “excessive” $\dot{VO}_2$ at high-intensity submaximal exercise suggests that the energy demand during supramaximal intensities may be underestimated when extrapolated from the $\dot{VO}_2$-power relationship. Therefore, the calculation of the oxygen demand during supramaximal exercise from submaximal exercise intensities including several above the VT, remains in doubt.

In a recent study by Weber and Schneider (2000), steady-state $\dot{VO}_2$ was averaged over minutes 9 and 10 during six constant-load, submaximal cycling bouts between 25% and 75% of $\dot{VO}_2$ peak. The Pearson’s correlation coefficient for the $\dot{VO}_2$-power
relationship of the six submaximal cycling bouts was $0.996 \pm 0.001$ for male subjects and $0.995 \pm 0.001$ for female subjects. In agreement with the findings of previous work, Pearson’s correlation coefficients of between 0.995 and 0.999 were obtained for both male and female subjects in experiment one and two of the present thesis. These findings suggest that a strong linear relationship exists between the $\dot{V}O_2$ and power output of six submaximal exercise bouts measured between 25% and 75% of $\dot{V}O_2$ peak for cycling. However, it is acknowledged that without a statistical comparison of other regression models, no conclusions can be made regarding the $\dot{V}O_2$-power relationship based on the Pearson’s correlation coefficient.

It is accepted that some error might be present when predicting the oxygen demand of supramaximal work rates from the $\dot{V}O_2$-power relationship due to the non-linear characteristics previously demonstrated in work rates above the VT. However, findings from experiment one of this thesis suggest that the mean values for the slope (trial 1 $0.010 \pm 0.000$; trial 2 $0.010 \pm 0.000$ L·min$^{-1}$·W$^{-1}$) and y-intercept (trial 1 $0.46 \pm 0.02$; trial 2 $0.45 \pm 0.03$ L·min$^{-1}$) did not differ significantly between the first and the second $\dot{V}O_2$-power relationships determined. Also, experiment one clearly demonstrates the repeatability of MAOD measured in both male and female subjects. Therefore, the methods used to calculate MAOD described by Medbø et al. (1988) and Weber and Schneider (2000) are useful for comparing MAOD in male and female groups. In support of these comments, Bangsbo (1996) has suggested that MAOD can be used for comparative purposes and that changes in anaerobic performance may be demonstrated by the response in MAOD before and after training. It was suggested that for accurate within-subject comparisons, the same $\dot{V}O_2$-power relationship should be used before and after training, suggesting that the MAOD test must be performed at the same exercise intensity before and after training (Bangsbo, 1996).

**Validity of the maximal accumulated oxygen deficit.** Both theoretical and biochemical measures of the anaerobic energy yield have been favorably compared with MAOD measurements (Bangsbo et al., 1990; Medbø et al., 1988; Withers et al., 1991). Several studies have supported the concept of a limited anaerobic energy source by demonstrating that the MAOD is unchanged with exhaustive supramaximal exercise.
bouts ranging from 2-16 min (Karlsson and Saltin, 1970, Medbø et al., 1988). Furthermore, Linnarsson et al. (1974) and more recently Medbø et al. (1988) showed no change in MAOD under hypoxic conditions despite a reduction in both exercise duration and the $\text{AO}_2$ uptake during the MAOD test. These findings suggest that MAOD is unaffected by changes in inspired oxygen concentration, indicating that anaerobic capacity (AC) is independent of aerobic power. Moreover, significantly larger MAOD values are measured in sprint-trained athletes when compared to both endurance-trained and untrained subjects (Gastin and Lawson, 1994; Medbø et al., 1988; Saltin, 1989; Scott et al., 1991). Differences of 15-36% have been reported, suggesting that sprint-trained athletes are capable of achieving a higher MAOD due to their greater capacity to produce adenosine triphosphate (ATP) anaerobically.

Graham and McLellan (1989) demonstrated that oxygen deficit was significantly correlated ($r = 0.89$) to time to exhaustion during supramaximal exercise. Based on these findings, they concluded that the calculated oxygen deficit is a valid estimate of the energy demands that are met by anaerobic energy sources. Scott et al. (1991) demonstrated significant correlations between the 30-s Wingate Anaerobic Test and oxygen deficit among sprinters, middle-distance runners, distance-runners and control subjects. In addition to these investigations, the comparison of MAOD with biochemical measures is viewed as the best method of validation (Gastin, 1994). Precise quantitative measures of the decrease in ATP and creatine phosphate (CrP) concentrations as well as the accumulation of pyruvate and lactate in the muscle provide a model to study the anaerobic energy yield. Bangsbo et al. (1990) utilized this method to make comparisons with MAOD values calculated during intense one-legged, dynamic knee extensor exercise. Anaerobic energy production was 91.2 mmol·kg$^{-1}$ wet wt compared to 91.6 mmol·kg$^{-1}$ wet wt when measured using the $\text{AO}_2$ deficit expressed in the same units. Medbø and Tabata (1993) also reported that the $\text{AO}_2$ deficit was significantly correlated ($r = 0.94$) with the rate of anaerobic energy release during maximal cycling over 2 min in untrained males. Due to the similarity of the values measured for the muscle metabolite and oxygen deficit method, there is support for the use of MAOD as a quantitative measure of AC during exhaustive supramaximal exercise (Bangsbo et al., 1990; Medbø and Tabata, 1993).
Modeling oxygen uptake kinetics during moderate-intensity and supramaximal exercise. This section examines the procedures used to analyze breath-by-breath $\dot{V}O_2$ data collected during both moderate-intensity and supramaximal exercise bouts.

Detection of the end of the phase I $\dot{V}O_2$ response. The increase in pulmonary blood flow during the first $\sim 15$ s of exercise (phase I) is the main mechanism accounting for the increase in $\dot{V}O_2$. Phase I results in an increase in $\dot{V}CO_2$ that approximates the change in $\dot{V}O_2$. Thus, at the onset of exercise, RER remains similar to that observed immediately before the work rate transition (Whipp et al., 1982). Phase I lasts until the gas contents of mixed venous blood perfusing the pulmonary capillaries begins to change as a result of active muscle metabolism and increased muscle gas exchange. However, $\dot{V}CO_2$ kinetics appear to be slowed relative to $\dot{V}O_2$. Slowed $\dot{V}O_2$ kinetics have been attributed to the larger capacity to store carbon dioxide compared to oxygen in the periphery (Farhi and Rahn, 1955). Therefore, when blood in the muscle at the onset of exercise reaches the lungs, a sudden downturn in RER may be observed due to delayed increase in $\dot{V}CO_2$ and the concomitant increase in $\dot{V}O_2$. This downturn in RER approximates the end of the phase I $\dot{V}O_2$ response.

Middle-five-of-seven breaths. This technique was used to smooth breath-by-breath $\dot{V}O_2$ data before the linear interpolation was performed. The middle five-of-seven averaging process is an option that can be selected from the MedGraphics data acquisition program (BREEZE EX software package; Medical Graphics corporation, St. Paul, MN, USA). The raw $\dot{V}O_2$ data is collected and recorded breath-by-breath. The middle five-of-seven technique results in the same number of $\dot{V}O_2$ values as when collected breath-by-breath. For every seven breaths, the highest and the lowest $\dot{V}O_2$ values are omitted and the remaining five breaths are averaged. For example:

\[
X_1 = 0.99 \text{ L-min}^{-1} \\
X_2 = 0.97 \text{ L-min}^{-1} \\
X_3 = 1.01 \text{ L-min}^{-1} \\
X_4 = 1.05 \text{ L-min}^{-1} \\
X_5 = 1.03 \text{ L-min}^{-1} \\
X_6 = 1.04 \text{ L-min}^{-1} \\
X_7 = 1.17 \text{ L-min}^{-1} \\
X_8 = 1.06 \text{ L-min}^{-1}
\]
The first seven breaths are selected \((X_1 - X_7)\) where \(X_2\) (0.97 L·min\(^{-1}\)) is the lowest value and \(X_7\) (1.17 L·min\(^{-1}\)) is the highest value. Therefore, \(X_2\) and \(X_7\) are omitted and the remaining five \(\dot{V}O_2\) values \((X_1, X_3, X_4, X_5, X_6)\) are averaged and presented as the first data point (1.02 L·min\(^{-1}\)). The next seven values \((X_2 - X_8)\) are then selected and the highest and lowest values omitted whereas the remaining five values are averaged and presented as the second data point.

**Linear interpolation of \(\dot{V}O_2\) data.** Similar to breath-by-breath, data that has been smoothed using the middle five-of-seven averaging technique results in \(\dot{V}O_2\) values that are irregularly spaced. In other words, the data set does not demonstrate \(\dot{V}O_2\) values at 1-s intervals. Before combining two or more data sets, \(\dot{V}O_2\) data can be linearly interpolated second-by-second to give a \(\dot{V}O_2\) value for each second of the exercise bout (i.e., a 3 min exercise bout would result in 180 data points). Linear interpolation replaces missing values in a series of irregularly spaced data. Where one missing value is recorded between two measured \(\dot{V}O_2\) values, the missing value is simply the average of the two measured \(\dot{V}O_2\) values. Particularly during moderate-intensity exercise, breath-by-breath \(\dot{V}O_2\) data may have several periods of two or more consecutive seconds that do not have a corresponding measured \(\dot{V}O_2\) value. Where there are two or more consecutive missing values, the missing values are weighted according to their position. For example, the following data series is a 5-s period that includes only two measured \(\dot{V}O_2\) values \((X_6\) and \(X_{10}\)):

\[
\begin{align*}
X_6 &= 0.74 \text{ L·min}^{-1} \\
X_7 &= \, \, ? \\
X_8 &= \, \, ? \\
X_9 &= \, \, ? \\
X_{10} &= 0.83 \text{ L·min}^{-1}
\end{align*}
\]

where, \(X_6\) is the last measured \(\dot{V}O_2\) value before and \(X_{10}\) is the first measured \(\dot{V}O_2\) value measured after the missing values; \(X_7, X_8,\) and \(X_9\). The missing values are calculated as shown below.

\[
\begin{align*}
X_6 &= 0.74 \text{ L·min}^{-1} \\
X_7 &= X_6 + 0.25*(X_{10} - X_6) \\
X_8 &= X_6 + 0.50*(X_{10} - X_6) \\
X_9 &= X_6 + 0.75*(X_{10} - X_6) \\
X_{10} &= 0.83 \text{ L·min}^{-1}
\end{align*}
\]
Thus, the interpolated data set would contain the following \( \dot{V}O_2 \) values:

\[
\begin{align*}
X_6 &= 0.74 \text{ L-min}^{-1} \\
X_7 &= 0.76 \text{ L-min}^{-1} \\
X_8 &= 0.79 \text{ L-min}^{-1} \\
X_9 &= 0.81 \text{ L-min}^{-1} \\
X_{10} &= 0.83 \text{ L-min}^{-1} \\
\end{align*}
\]

Comparing the fits of two models. Curve fitting of physiological responses can incorporate physiological correlates, or it can simply attempt to minimize the error of the distribution of the data points about the line of best fit. Nevertheless, unnecessary terms included in the model make the model more flexible than it should be, allowing it to fit some of the random variation in the data as if it were deterministic structure. Including unnecessary terms in the model causes uncertainties associated with incorrect physiological conclusions that might be drawn from the analysis of the data. During supramaximal cycling, it is not possible to confirm or reject the presence of a \( \dot{V}O_{2\text{SC}} \). Therefore, I justify my model selection by the absence of a statistically significant improvement in fit between a single-term exponential model and a model that includes an extra parameter to account for a \( \dot{V}O_{2\text{SC}} \).

The F test employed in this thesis (1), was used to calculate the ratio of the residual sums of squares for each model, corrected for the appropriate degrees of freedom. In other words, the F test quantified the relationship between the relative increase in the residual sum of squares with the relative increase in degrees of freedom (going from the single- to the two-term model).

\[
F = \frac{(ss1 - ss2)/ss2}{(df1 - df2)/df2}
\]

The P value associated with the F statistic confirmed or rejected the appropriateness of using the single-term model.

Slow component during supramaximal cycling. As previously introduced, submaximal exercise performed above the VT is associated with a slow increase in
that may begin between approximately 90-150 s after the onset of exercise (Barstow and Molé, 1991; Paterson and Whipp, 1991). However, it is not clear if a slow component ($\dot{V}O_2_{SC}$) exists during severe-intensity exercise that is performed at a work rate above peak $\dot{V}O_2$. This uncertainty raises questions about the validity of using either a single- or two-term exponential model to describe the phase II and phase III $\dot{V}O_2$ response to supramaximal exercise. During supramaximal exercise, $\dot{V}O_2$ kinetics have been well described by both a single-term (Özyener et al., 2001) and a two-term (Hughson et al., 1998) exponential equation. Some researchers have attempted to avoid this difficulty by modeling only the first 75 s of $\dot{V}O_2$ data, therefore minimizing the effect of the $\dot{V}O_2_{SC}$ (Hebestreit et al., 1998; Hill and Stevens, 2001).

In a study by Hill and Stevens (2001), untrained subjects cycled to fatigue at a work rate predicted to elicit exhaustion between 3 to 4 min. The phase II $\dot{V}O_2$ response was evaluated using a single-term exponential (excluding phase I). For each subject, the $\dot{V}O_2$ response was described using all ~3.5 min of data after the phase I response. In addition, the data were reanalyzed (excluding phase I) up to 90 s and also up to 75 s. Interestingly, there was no difference in the $\dot{V}O_2$ amplitude predicted from 75 s or 90 s of the $\dot{V}O_2$ response when compared to the amplitude predicted using 3.5 min of data. In addition, the time constant based on the first 75 s as well as 90 s of data was not significantly different to the time constant based on all of the data. If a $\dot{V}O_2_{SC}$ was manifest in the later stages (i.e., 90-150 s) of the exercise bout, the amplitude of the 3.5 min data set would have been higher and the time constant slower than that which was predicted by the 75 s or the 90 s data sets. The study by Hill and Stevens (2001) supports the findings of Özyener et al. (2001) and the results presented in experiment three of this thesis for untrained men and women; no $\dot{V}O_2_{SC}$ can be detected during 3 min of cycling at 110% of $\dot{V}O_2$ peak.

Poole (1994) has shown that increased skeletal muscle oxygen consumption might account for 86% of the $\dot{V}O_2_{SC}$. This finding suggests that the increased recruitment of metabolically inefficient type II skeletal muscle fibers is the primary cause of the $\dot{V}O_2_{SC}$. Sprint exercise is associated with a preferential depletion of glycogen from type IIb then type IIa and type I fibers (Abernethy et al., 1990). This suggests that
type II fibers are recruited simultaneously, if not before type I fibers during sprinting. Thus, the magnitude of the $\dot{VO}_{2SC}$ may well be higher with increasing exercise intensities (Carter et al., 2002). However, it has not been established if the onset of the $\dot{VO}_{2SC}$ occurs progressively earlier with increasing work rates. It is possible that the $\dot{VO}_{2SC}$ is present during supramaximal exercise, but begins simultaneously with the primary component. Earlier mathematical descriptions of $\dot{VO}_2$ during heavy-intensity exercise (Linnarsson, 1974; Miyamoto et al., 1982) incorporated two exponential terms that began together after a common time delay. In this case, the two time constants would converge to a mono-exponential curve, albeit at a higher $\dot{VO}_2$ L·min⁻¹·W⁻¹. Whereas, recent studies have shown that for heavy exercise, the onset of the second term may be delayed relative to the start of exercise and to the start of the first exponential term, it is possible that a $\dot{VO}_{2SC}$ begins without delay during supramaximal exercise intensities. However, this hypothesis remains to be investigated.


References


REFERENCES


REFERENCES

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REFERENCES


SYMBOLS AND ABBREVIATIONS

Dot [ · ] above any symbol indicates a time derivative
Dash [ - ] above any symbol indicates a mean value

SYMBOLS

\[ \alpha \] alpha
\[ \beta \] beta
\[ \Delta \] delta; change or difference
\[ \mu \] micro
\[ \tau \] tau; time constant
\[ r \] pearson’s correlation coefficient
\[ r^2 \] ‘goodness of fit’
\[ % \] percent
**SYMBOLS AND ABBREVIATIONS**

### UNITS OF MEASUREMENT

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
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<td>beats-min⁻¹</td>
<td>beats per minute</td>
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<td>days</td>
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<tr>
<td>Cr</td>
<td>creatine</td>
</tr>
<tr>
<td>CrP</td>
<td>creatine phosphate</td>
</tr>
<tr>
<td>FADH$_2$</td>
<td>flavin adenine dinucleotide</td>
</tr>
<tr>
<td>GDP</td>
<td>guanosine diphosphate</td>
</tr>
<tr>
<td>GTP</td>
<td>guanosine triphosphate</td>
</tr>
<tr>
<td>H$^+$</td>
<td>hydrogen ion</td>
</tr>
<tr>
<td>H$_2$O</td>
<td>water</td>
</tr>
<tr>
<td>HAD</td>
<td>3-hydroxyacyl-CoA dehydrogenase</td>
</tr>
<tr>
<td>IMP</td>
<td>inosine monophosphate</td>
</tr>
<tr>
<td>K$^+$</td>
<td>potassium</td>
</tr>
<tr>
<td>LDH</td>
<td>lactate dehydrogenase</td>
</tr>
<tr>
<td>Na$^+$</td>
<td>sodium</td>
</tr>
<tr>
<td>NAD$^+$</td>
<td>nicotinamide adenine dinucleotide, oxidized form</td>
</tr>
<tr>
<td>NADH</td>
<td>nicotinamide adenine dinucleotide, reduced form</td>
</tr>
<tr>
<td>NaHCO$_3$</td>
<td>sodium bicarbonate</td>
</tr>
<tr>
<td>NH$_4^+$</td>
<td>ammonium</td>
</tr>
<tr>
<td>PCO$_2$</td>
<td>partial pressure of carbon dioxide</td>
</tr>
<tr>
<td>PO$_2$</td>
<td>partial pressure of oxygen</td>
</tr>
<tr>
<td>PDH</td>
<td>pyruvate dehydrogenase</td>
</tr>
<tr>
<td>PFK</td>
<td>phosphofructokinase</td>
</tr>
<tr>
<td>PHOS</td>
<td>phosphorylase</td>
</tr>
</tbody>
</table>
SYMBOLS AND ABBREVIATIONS

\[ P_i \quad \text{inorganic phosphate} \]
\[ \text{SDH} \quad \text{succinate dehydrogenase} \]

VARIABLES AND ABBREVIATED TERMS

\[ \dot{V}_{CO_2} \quad \text{carbon dioxide output} \]
\[ \dot{V}O_2 \quad \text{oxygen uptake} \]
\[ \dot{V}O_2\text{peak} \quad \text{peak oxygen uptake} \]
\[ \dot{V}O_2\text{max} \quad \text{maximal oxygen uptake} \]
\[ \dot{V}O_{2\text{SC}} \quad \text{slow component of oxygen uptake} \]
\[ \dot{V}O_2\text{EE} \quad \text{oxygen uptake measured end-exercise} \]
\[ \dot{V}O_2\text{UL} \quad \text{oxygen uptake measured during unloaded cycling} \]
\[ \dot{V}_E \quad \text{minute ventilation} \]
\[ (a−\overline{v})O_2 \text{ difference} \quad \text{arteriovenous oxygen content difference} \]
\[ Q \quad \text{cardiac output} \]
\[ \dot{Q}O_2 \quad \text{skeletal muscle oxygen consumption} \]
\[ \text{La}^- \quad \text{lactate} \]
\[ [\text{La}] \quad \text{blood lactate concentration} \]
\[ [\text{La}]_{3\text{ min}} \quad \text{blood lactate concentration measured 3 min post exercise} \]
\[ A \quad \text{amplitude} \]
\[ \text{AC} \quad \text{anaerobic capacity} \]
\[ \text{AMM} \quad \text{active muscle mass} \]
\[ \text{ANOVA} \quad \text{analysis of variance} \]
\[ \text{AO}_2 \quad \text{accumulated oxygen} \]
\[ \text{BC} \quad \text{buffering capacity} \]
\[ \text{BF} \quad \text{body fat} \]
\[ \text{BM} \quad \text{body mass} \]
\[ \text{BMC} \quad \text{bone mineral content} \]
\[ \text{BTPS} \quad \text{body temperature and pressure saturated with water vapor} \]
\[ \text{DXA} \quad \text{dual-energy X-ray absorptiometry} \]
\[ \text{ECG} \quad \text{echocardiograph} \]
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ETC</td>
<td>electron transport chain</td>
</tr>
<tr>
<td>$F_2O_2$</td>
<td>fractional concentration oxygen in inspired gas</td>
</tr>
<tr>
<td>GMG</td>
<td>gluteal muscle group</td>
</tr>
<tr>
<td>HIT</td>
<td>high-intensity interval training</td>
</tr>
<tr>
<td>HR</td>
<td>heart rate</td>
</tr>
<tr>
<td>HREE</td>
<td>heart rate measured end-exercise</td>
</tr>
<tr>
<td>LBM</td>
<td>lean body mass</td>
</tr>
<tr>
<td>Leg-LBM</td>
<td>leg lean body mass</td>
</tr>
<tr>
<td>MAOD</td>
<td>maximal accumulated oxygen deficit</td>
</tr>
<tr>
<td>OC</td>
<td>oral contraceptive</td>
</tr>
<tr>
<td>PAR-Q</td>
<td>physical Activity Readiness Questionnaire</td>
</tr>
<tr>
<td>PCO$_2$</td>
<td>partial pressure of carbon dioxide</td>
</tr>
<tr>
<td>PO$_2$</td>
<td>partial pressure of oxygen</td>
</tr>
<tr>
<td>RER</td>
<td>respiratory exchange ratio</td>
</tr>
<tr>
<td>SA node</td>
<td>sinoatrial node</td>
</tr>
<tr>
<td>SNS</td>
<td>sympathetic nervous system</td>
</tr>
<tr>
<td>STPD</td>
<td>standard temperature (0°C) and pressure (760 mm Hg) dry</td>
</tr>
<tr>
<td>SV</td>
<td>stroke volume</td>
</tr>
<tr>
<td>TCA cycle</td>
<td>tricarboxylic acid cycle</td>
</tr>
<tr>
<td>TD</td>
<td>time delay</td>
</tr>
<tr>
<td>TE</td>
<td>time to exhaustion</td>
</tr>
<tr>
<td>UWW</td>
<td>underwater weighing</td>
</tr>
<tr>
<td>VT</td>
<td>ventilatory threshold</td>
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</table>