THE EFFECTS OF TYPE II MUSCLE FIBRE GLYCOGEN DEPLETION ON THE SLOW COMPONENT OF OXYGEN UPTAKE

By

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Abstract

The present study was undertaken to investigate the role of the recruitment of type II muscle fibres in contributing to the $\dot{V}O_2$ slow component. Glycogen depletion of the type II muscle fibres was utilized as the mechanism through which the contribution of the type II motor units to the slow component could be manipulated. Eight healthy male subjects (19-25 yrs) performed constant-load exercise tests, at moderate and heavy intensity workrates under both control (CON) and glycogen depleted (GD) conditions. The glycogen depletion protocol consisted of ten 1-minute bouts of cycle ergometer exercise at 130% $\dot{V}O_{2max}$, each separated by 5 minutes rest. After 1-hour recovery, 8 minutes of moderate (80% T_{VE}) and 10 minutes heavy (Δ 40%) intensity constant-load exercise were performed, each from a baseline of loadless cycling. $\dot{V}O_2$ was measured breath-by-breath, and fit using a 2 component exponential model for moderate and a 3 component exponential model for heavy intensity exercise. Muscle biopsy samples were obtained at rest and following the GD protocol, for determination of glycogen concentration. Arterialized venous blood was sampled from a dorsal hand vein and analyzed for plasma [La^-]. All muscle fibre types showed uniform glycogen content at rest (dark PAS staining intensity). Following the GD protocol, mean glycogen concentration was decreased by 65%, and glycogen depletion was greatest in type IIb muscle fibres, with intermediate depletion in type IIa muscle fibres and little or no glycogen depletion in type I fibres, as indicated by PAS staining intensity. Plasma [La^-] was not different between conditions in either moderate or heavy intensity exercise. There were no differences in the $\dot{V}O_2$ on-kinetics between CON and GD in moderate intensity exercise. In heavy intensity exercise, τ_2 was larger in the GD condition than in

CON (24 ± 5 s vs 28 ± 7 s). The $\dot{V}O_2$ slow component ($\Delta \dot{V}O_{2(10\text{-}3min)}$) was not different between CON and GD (228 ± 84 vs 182 ± 116 ml/min for CON and GD respectively), nor was the amplitude of the third phase (A_3) different between conditions (459 ± 209 vs 394 ± 253 ml/min for CON and GD respectively). However, when an apparent outlier was excluded from the analysis, the magnitude of the $\dot{V}O_2$ slow component (A_3) was significantly smaller ($P<0.03$) after glycogen depletion. Type II muscle fibre glycogen depletion does not effect the $\dot{V}O_2$ slow component, suggesting that the contribution of type II motor units to the slow component is still unclear.

Keywords: gas-exchange kinetics; type II muscle fibres; glycogen depletion; ventilatory threshold; muscle biopsy; lactate

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Chapter I

Introduction

Following the onset of constant-load exercise below ventilatory threshold (T_{VE}; moderate exercise), $\dot{V}O_2$ increases exponentially with first order kinetics to achieve a new steady state value (Barstow, 1994; Barstow & Molé, 1991; Paterson & Whipp, 1991), the gain of which is a linear function of workrate (Gaesser & Poole, 1996; Poole & Richardson, 1991).

However, following the onset of constant load exercise above T_{VE} (>T_{VE}; heavy exercise), during which there is a sustained rise in blood lactate concentration, the exercise $\dot{V}O_2$ response becomes more complex. Unlike moderate exercise, the $\dot{V}O_2$ response to heavy exercise is characterized by three kinetic components (Paterson & Whipp, 1991). Evident in the rise in $\dot{V}O_2$ is a secondary slower component, which causes $\dot{V}O_2$ to project above the apparent steady-state value of the underlying primary monoexponential response (Casaburi et al., 1987; Barstow & Molé, 1991; Gaesser et al., 1994; Paterson & Whipp, 1991; Poole et al., 1991; Whipp and Wasserman, 1972). This slow component leads to the end-exercise $\dot{V}O_2$ being greater than that predicted from the below T_{VE} $\dot{V}O_2$ / Workrate relationship (Whipp, 1987). The onset of the slow component is delayed and does not begin until several minutes after the onset exercise (Barstow, 1994; Paterson & Whipp, 1991). The $\dot{V}O_2$ slow component is typically quantified as the difference between the end- exercise $\dot{V}O_2$ and that at minute 3 of the exercise bout (Gaesser & Poole, 1996), and more recently by parameter estimation (amplitude; τ) using

computer modeling techniques (Scheuermann et al., 1998). Thus, the $\dot{V}O_2$ slow component represents additional O_2 required during heavy exercise.

Understanding the cause of the $\dot{V}O_2$ slow component is important for two general reasons. First, the work tolerance in individuals with a cardiac or ventilatory limitation is restricted due to the high $\dot{V}O_2$ demands associated with the slow component (Poole et al., 1994; Gaesser and Poole, 1996). Second, early onset of fatigue is associated with exercise at power outputs engendering a slow component, and as such, understanding its mechanism is fundamental to understanding the limitations to exercise performance (Poole et al., 1994a; Poole et al., 1994b; Womack et al., 1995).

The aetiology of the slow component during heavy exercise is unclear. Several factors have been proposed to contribute to the slow component. These include: a) High lactate concentrations during heavy exercise may stimulate gluco/glyconeogenesis, thus accelerating the metabolic rate and subsequently increasing the O_2 cost of exercise (Casaburi et al., 1987; Gaesser, 1994; Gaesser et al., 1992; Gaesser et al., 1994; Poole et al., 1994b; Poole et al., 1990; Womack et al., 1995). Alternatively, Wasserman (1994), contends that it is not lactate per se, but rather the accompanying metabolic acidosis which causes the $\dot{V}O_2$ slow component. A rightward shift in the oxyhemoglobin dissociation curve, via the Bohr effect, leads to an increase in capillary PO_2, and thus driving pressure for O_2 into the mitochondria, allowing $\dot{V}O_2$ to increase (Stringer et al., 1994; Wasserman et al., 1994). b) Elevated muscle temperature could stimulate metabolism (and $\dot{V}O_2$), via the Q_{10} effect (Gaesser and Poole, 1996; Hagberg et al., 1978; Koga et al., 1997; Poole et al., 1991). c) Increased plasma epinephrine levels that occur during heavy exercise are known to stimulate the metabolic rate, which in turn could

increase $\dot{V}O_2$ (Gaesser et al., 1994; Poole et al., 1991). d) The increased O_2 cost of respiratory muscle work for pulmonary ventilation during heavy exercise may contribute to the $\dot{V}O_2$ slow component (Billat et al., 1998; Casaburi et al., 1987; Gaesser, 1994; Paterson and Whipp, 1991; Whipp, 1994; Womack et al., 1995). e) Recruitment of accessory muscle groups as occurs with increased stabilization of the body, or with an increase in body sway during heavy exercise (Whipp, 1994), may increase the O_2 cost of heavy exercise. f) Recruitment of less efficient type II muscle fibres could lead to a greater O_2 cost of exercise for a given rate of ATP utilization (Barstow, 1994; Barstow et al., 1996; Coyle et al., 1992; Poole et al., 1994; Whipp, 1994; Willis and Jackman, 1994). In addition, type II muscle fibres have inherently slower kinetics for $\dot{V}O_2$ (Barstow et al., 1996). During heavy exercise, type II muscle fibres are progressively recruited in addition to type I muscle fibres (Vollestad & Blom, 1985), in order to maintain the high workload and thus maintain a constant power output. Type II muscle fibres are less energetically efficient relative to type I muscle fibres, and as a result have a higher O_2 cost for force production (Kushmerick et al., 1992). Thus, type II muscle fibres require a greater amount of O_2 to produce the same amount of ATP and consequently could contribute to the $\dot{V}O_2$ slow component.

While several of these factors may be involved in the mechanism of the slow component, none have been adequately substantiated. Many investigators have proposed that the recruitment of type II muscle fibres may provide a viable mechanism for the $\dot{V}O_2$ slow component (Barstow, 1994; Barstow et al., 1996; Coyle et al., 1992; Poole et al., 1994; Whipp, 1994; Willis & Jackman, 1994). However, the role that type II fibre recruitment plays in contributing to the $\dot{V}O_2$ slow component has not been extensively

investigated. Thus, the present study was designed to investigate the role type II muscle fibres play in contributing to the $\dot{V}O_2$ slow component. In an attempt to manipulate recruitment of type II muscle fibres, selective glycogen depletion of type II muscle fibres was undertaken. It has been documented that high intensity interval exercise causes glycogen depletion of type II muscle fibres (Gollnick et al., 1973; Gollnick et al., 1974a; Gollnick et al., 1974b; Thomson et al., 1979), which may alter the force producing capabilities of these fibres. Following this type II muscle fibre glycogen depletion protocol, a decreased total muscle glycogen content and specifically a reduction in the glycogen content of type II muscle fibres was expected. Because glycogen is the primary substrate for type II muscle fibres, glycogen depletion of type II muscle fibres may alter the fatigue profile of these fibres. Thus, while the type II muscle fibres may still be recruited following glycogen depletion, they may not be able to produce the required force. If the force production is depressed, then other muscle fibres may be recruited in order to maintain the total power output. Grisdale and colleagues (1990), demonstrated that following glycogen depletion and/or previous exercise, force production was depressed and during a 50% maximal voluntary contraction (MVC), and the EMG signal was increased, suggesting that there was increased muscle fibre recruitment in order to maintain the required power output. Therefore, glycogen depletion in the type II muscle fibres may result in altered fibre type recruitment, specifically an increase in the recruitment of type I muscle fibres.

The primary purpose of the current study was to examine the effect of the glycogen depletion protocol on the slow component of oxygen uptake. It was hypothesized that in the glycogen depletion condition, there would be a decreased

oxygen uptake kinetics (τ_2) for heavy exercise. Thus, in the present study, if glycogen depletion results in the recruitment of primarily type I muscle fibres, then a speeding of kinetics (τ_2) may occur as a result of glycogen depletion. Segal and Brooks (1979) have reported that glycogen depletion was associated with a greater initial $\dot{V}O_2$ response at the onset of moderate and heavy intensity exercise. This greater initial $\dot{V}O_2$ response suggests a speeding of $\dot{V}O_2$ on-kinetics following glycogen depletion. Thus, the results of Segal and Brooks (1979) provide further support to suggest that muscle glycogen levels may influence the $\dot{V}O_2$ on-kinetics.

Chapter II

Review of Literature

2.1 Oxygen Uptake Kinetics During the On-Transient of Moderate Intensity (Below Ventilatory Threshold) Exercise

During the on-transient of moderate intensity exercise that would elicit a $\dot{V}O_2$ less than the $\dot{V}O_2$ at the ventilatory threshold (T_{VE}), oxygen uptake ($\dot{V}O_2$) increases towards a steady state with first order kinetics, as a linear function of workrate (WR; $\Delta \dot{V}O_2 / \Delta$ WR ~ 10 L·min/W). (Gaesser and Poole, 1996, Gerbino et al., 1996; Paterson and Whipp, 1991; Poole et al., 1991; Poole et al., 1994b; Poole and Richardson, 1997; Womack et al., 1995). During moderate-intensity exercise, (all exercise below the ventilatory threshold which does not induce a sustained lactic acidosis), $\dot{V}O_2$ increases monoexponentially with a time constant (tau; τ) of approximately 30 seconds, reaching steady state within 2-3 minutes. This time constant does not normally vary at different moderate intensity work rates (Barstow et al., 1996; Barstow and Mole, 1991; Belardinelli, 1995; Gaesser and Poole, 1996; Gaesser et al., 1994; Whipp, 1994). This increase in $\dot{V}O_2$ can be described mathematically by the equation: $\dot{V}O_2(t) = \dot{V}O_2(ss) + A \cdot (1 - e^{-[t-TD]/\tau})$, where $\dot{V}O_2(t) = \dot{V}O_2$ at any time t, $\dot{V}O_2(ss) = \dot{V}O_2$ at steady state, prior to the step increase in workrate; A = the initial amplitude in $\dot{V}O_2$, TD= the time delay (Gaesser and Poole, 1996).

During a transition to moderate intensity exercise, the $\dot{V}O_2$ response has been described by three phases. Each phase of the increase in $\dot{V}O_2$ represents underlying physiological mechanisms. Phase I, known as the cardiodynamic phase,

begins at exercise onset and is described as an early, rapid increase in $\dot{V}O_2$ (Whipp, 1994; Whipp et al., 1982; Yoshida et al., 1995). Phase I precedes the exponential rise (Phase II) and represents the first 15-25 seconds of exercise (Barstow, 1994). Throughout phase I, there is a rapid increase in $\dot{V}O_2$, $\dot{V}CO_2$ and V_E (Casaburi & Wasserman, 1986; Linnarsson, 1974), while end tidal oxygen and carbon dioxide partial pressures ($P_{ET}O_2$ and $P_{ET}CO_2$) remain constant (Weissman, 1982). The constant $P_{ET}O_2$ and $P_{ET}CO_2$ levels indicate that the increase in oxygen utilisation at the level of the muscle has not been recognised at the level of the mouth. The rise in $\dot{V}O_2$ during Phase I is due to a) the augmented cardiac output and pulmonary blood flow (Casaburi et al., 1989; Gaesser and Poole, 1996; Whipp et al., 1982), b) increased ventilation and c) changes in mixed venous O_2 content and lung gas stores (Barstow, 1994; Barstow and Mole, 1991; Whipp et al., 1982). Phase I is called the cardiodynamic phase because the increase in O_2 uptake principally reflects the increase in cardiac output as the venous blood from the exercising muscles has not yet reached the lung. Thus, phase I represents the transit time delay (approximately 20 s) of the venous return from the active muscles to the lung (Grassi et al., 1996; Whipp & Ward, 1990).

Following phase I, which is manifest by alterations in $P_{ET}O_2$ and $P_{ET}CO_2$ the de-oxygenated blood from the exercising limbs arrives at the lungs.. This indicates the onset of phase II. Phase II corresponds to a monoexponential increase in $\dot{V}O_2$ with a time constant of 30-45 seconds (Barstow, 1994; Linarsson, 1974; Whipp et al., 1982). In Phase II the increase in $\dot{V}O_2$ is due to a) the continued increase in cardiac output (Poole and Richardson, 1997), and b) the arrival at the

lung of venous blood from the exercising limbs. Thus, the increased $\dot{V}O_2$ represents increased O_2 delivery, increased O_2 extraction and tissue oxidation (and resultant decreased venous O_2 content) (Barstow and Mole, 1991; Gaesser and Poole, 1996). Pulmonary $\dot{V}O_2$ in Phase II is believed to reflect $\dot{V}O_2$ of the working tissue (Barstow et al., 1994; Grassi et al., 1996). This is supported strongly by modelling studies and also the apparent temporal correspondence between Phase II pulmonary $\dot{V}O_2$ changes and those of phosphocreatine, (PCr) within exercising muscle (Barstow, 1994; Gaesser and Poole, 1996; McCreary et al., 1996;).

During moderate intensity exercise, phase III of the response occurs once a new steady state level of $\dot{V}O_2$ has been achieved, usually within 2-3 minutes, or approximately four time constants (Barstow, 1994; Paterson & Whipp, 1991; Whipp et al., 1982).

2.2 Oxygen Uptake Kinetics During the On-Transient of Heavy Intensity (Above Ventilatory Threshold) Exercise.

For constant-load exercise above the ventilatory threshold ($T_{\dot{V}E}$), (heavy or severe exercise intensities that result in a sustained lactic acidosis), the $\dot{V}O_2$ response becomes more complex and ceases to be a simple function of work rate. During heavy exercise the $\Delta \dot{V}O_2 / \Delta$ WR relationship is increased ($\Delta \dot{V}O_2 / \Delta$ WR > 10 L·min/W) and becomes non-linear (Paterson & Whipp, 1991; Whipp, 1986; Zoladz et al., 1995). Above $T_{\dot{V}E}$, three kinetic components characterize the $\dot{V}O_2$ response (Gaesser et al., 1994; Paterson and Whipp, 1991). An additional, or slowly developing component of $\dot{V}O_2$ is evident that is of delayed onset. (Casaburi et al., 1987; Gaesser and Poole, 1996; Gaesser et al.,

1994; Gerbino et al., 1996; Hagberg et al., 1978; Poole et al., 1994b; Paterson & Whipp, 1991; Poole et al., 1991; Whipp and Wasserman, 1972). This slowly developing component results in the steady-state $\dot{V}O_2$, if achieved, or end-exercise $\dot{V}O_2$ being greater than that predicted from the below T_{VE} $\dot{V}O_2$/WR relationship. (Gaesser, 1994; Paterson and Whipp, 1991; Poole et al., 1994a; Poole et al., 1991; Womack et al., 1995). The slow component of $\dot{V}O_2$ represents an additional O_2 requirement of above-threshold exercise. The slow component of $\dot{V}O_2$ kinetics is of delayed onset and becomes manifest approximately 80 to 110 s following exercise onset (Barstow, 1994; Barstow & Mole, 1991; Barstow et al., 1996; Gaesser, 1994; Poole, 1994; Poole et al., 1994a; Whipp, 1994). The $\dot{V}O_2$ slow component may achieve a delayed steady state, or may continue to rise as a function of time for several minutes until either exercise is terminated, or exhaustion ensues, and can drive $\dot{V}O_2$ to its maximum, equivalent to the individual's maximal aerobic power (Barstow, 1994; Barstow et al., 1996).

The slow component has been quantified as the difference between the end-exercise $\dot{V}O_2$ and that at minute 3 of the exercise bout (Gaesser, 1994), or by parameter estimation by modelling techniques (Scheuermann et al., 1998). The $\dot{V}O_2$ slow component represents the extra oxygen needed during constant-load exercise above the ventilatory threshold in order to maintain a constant power output (Heck, et al., 1998). The magnitude of the slow component usually increases as a function of work-rate above T_{VE}. The slow component can be as high as 1.0L·min^{-1} above that predicted on the basis of the below threshold $\dot{V}O_2$-workrate relation (Gaesser and Poole, 1996). The slow component of $\dot{V}O_2$ is

associated with parallel increases in heart rate, body temperature, and ventilation (V_E). As such, the metabolic cost of these activities will be incorporated into the $\dot{V}O_2$ slow component (Poole et al., 1994a)

The slow component implies that ATP demand is increasing, i.e. the efficiency of ATP utilization is decreasing, or that mitochondrial ADP/O is falling, or both. During aerobic metabolism, the rate of ATP breakdown must be matched by an equal rate of mitochondrial ATP production, which, in turn requires a proportional O_2 consumption rate as determined by the ADP/O ratio (Willis and Jackman, 1994).

It has been shown that exercise training reduces the $\dot{V}O_2$ slow component (Casaburi et al., 1987; Womack et al., 1995). Training can also elevate the workrate achieved at T_{VE}, which increases the range of workrates at which exercise can be performed without eliciting a slow component (Gaesser et al., 1992). This could be beneficial for certain patient populations with cardiac or ventilatory functional limitations (Gaesser and Poole, 1996). For example, for individuals with a cardiac limitation, T_{VE} occurs at a very low workrate and, as a result the $\dot{V}O_2$ slow component is present in relatively light exercise which forces a $\dot{V}O_{2max}$ at a low workrate. Reducing the slow component with exercise training increases T_{VE}, and thus there is a resultant increase in workrate to be achieved by these individuals before reaching $\dot{V}O_{2max}$ (Poole et al., 1994a).

2.3 Physiological significance of the $\dot{V}O_2$ slow component

Many exercise physiology textbooks fail to acknowledge the existence of the $\dot{V}O_2$ slow component. This may be due to the fact that the slow component

weakens fundamental concepts in exercise physiology, such as steady state and the O_2 deficit. If the concept of steady state no longer exists during heavy exercise, then the idea of caloric equivalents is threatened. Further, heavy intensity exercise, engendering a $\dot{V}O_2$ slow component can lead to exercise fatigue. Thus, understanding the slow component is essential to understanding exercise energetics and the limitations of performance (Poole et al., 1994a; Poole et al., 1994b; Womack et al., 1995).

2.4 Potential Mechanisms Responsible for the $\dot{V}O_2$ Slow Component

The aetiology of the slow component of $\dot{V}O_2$ has yet to be elucidated. Many researchers have investigated the slow component in an attempt to determine what causes the increase in $\dot{V}O_2$ during heavy exercise. Many theories have been put forward to explain the underlying physiology of the slow component, but one conclusive mechanism remains to be found. Several of the proposed mechanisms for the $\dot{V}O_2$ slow component will be reviewed.

The $\dot{V}O_2$ slow component was thought to be due to increases in blood lactate concentration, as the $\dot{V}O_2$ slow component is only evident for exercise intensities above the T_{VE} when a sustained lactic acidosis is present. Many studies have investigated the role lactate may play in contributing to the $\dot{V}O_2$ slow component (Casaburi et al., 1987; Gaesser et al., 1992; Gaesser et al., 1994; Poole et al., 1994b; Poole et al., 1991; Poole et al., 1990; Womack et al., 1995). Ryan et al., (1979), infused lactate in humans during rest and exercise and demonstrated that both blood lactate and $\dot{V}O_2$ were elevated over control values. The magnitude

and temporal characteristics of the $\dot{V}O_2$ slow component appear to be related to the magnitude and time course of the increase in blood lactate concentration during heavy exercise (Gaesser et al., 1992; Gaesser and Poole, 1996; Roston et al., 1987; Whipp, 1994). The high lactate concentrations during heavy exercise may stimulate gluco/glyconeogenesis (ATP requiring processes), thus accelerating the metabolic rate, and subsequently increasing the O_2 cost of exercise (Gaesser, 1994; Poole et al., 1994b). Furthermore, exercise training reduces the blood lactate concentration during heavy exercise, which is highly correlated with the decrease in the magnitude of the $\dot{V}O_2$ slow component after training (Casaburi et al., 1987; Poole et al., 1994b; Whipp, 1994; Womack et al., 1995). Casaburi and colleagues (1987) investigated the effect of endurance training on the $\dot{V}O_2$ slow component. The training program utilised by these researchers consisted of cycle ergometry exercise for 5 days/week, 45 minutes per session, over an 8-week period. Casaburi et al. (1987) reported that the exercise training resulted in an increase in $\dot{V}O_{2max}$ of 15%, a reduction in the $\dot{V}O_2$ slow component, marked reduction in end-exercise plasma lactate, slightly reduced rectal temperature, a decrease in both norepinephrine and epinephrine, and a reduced increase in ventilation (Casaburi et al., 1987). These authors concluded that lactate and the $\dot{V}O_2$ slow component are related; endurance training produces a lower $\dot{V}O_2$ with a lower level of blood lactate. The investigators proposed that the decreases in body temperature and catecholamine levels do not seem to be changed with training in proportion to the changes in the $\dot{V}O_2$ slow component. Thus, from this study it seemed that the $\dot{V}O_2$

slow component was significantly correlated to blood lactate levels and pulmonary ventilation (Casaburi et al., 1987).

However, there is recent and compelling evidence that lactate is not the primary cause of the $\dot{V}O_2$ slow component. It has been proposed by several investigators that the close correlation between lactate and the slow component is correlational and not causal (Gaesser, 1994; Poole et al, 1994b; Scheuermann et al., 1998; Whipp, 1994). Blood lactate concentration can be elevated significantly during exercise via lactate infusion or epinephrine infusion, without any effect on exercise $\dot{V}O_2$ (Gaesser, 1994; Gaesser and Poole, 1996; Whipp, 1994). For example, Poole and colleagues (1994b) looked at the effects of infused lactate into working dog gastrocnemius muscle in order to directly investigate the role of lactate in the slow component. These researchers demonstrated that in electrically stimulated isolated dog gastrocnemius muscle, with O_2 delivery, pH, and temperature held constant, increased blood and muscle lactate concentrations by La^- infusion were not associated with an increase in pulmonary $\dot{V}O_2$ (Poole et al., 1994b). Poole and co-workers (1994b), concluded that lactate per se was not the stimulating factor for the increase in $\dot{V}O_2$ in heavy exercise. However, the close association between blood lactate and the slow component may indicate a temporal relationship between some metabolic event and the route of lactate metabolism involved in the $\dot{V}O_2$ slow component (Poole et al., 1994b).

Recently, in our laboratory, (Scheuermann et al., 1998) the role of lactate in the slow component was investigated through the inhibition of carbonic anhydrase (CA) with an acute administration of acetazolamide (ACZ). CA inhibitors such as

ACZ function to inhibit the enzyme CA from catalysing the interconversion of CO_2 and HCO_3^-. These authors demonstrated that with ACZ (CA inhibition), the plasma [La^-] was reduced during both moderate and heavy intensity exercise without any effect on $\dot{V}O_2$ kinetics or the amplitude of $\dot{V}O_2$ during either moderate or heavy intensity exercise (Scheuermann et al., 1998). Thus, it appears that neither elevated nor decreased lactate concentrations have any affect on the slow component.

Gaesser (1994) proposed that lactate may serve as a marker for increased recruitment of lower-efficiency, type II motor units at high exercise intensities, which could increase $\dot{V}O_2$ and elevate lactate concentration (Gaesser, 1994). Thus, it could be that lactate itself does not cause the slow component directly, but may serve as a proxy-variable for some La^- related mechanism whose result is the $\dot{V}O_2$ slow component.

In a related study, Heck and associates (1998) examined the idea that the slow component is due to a decrease in intramuscular pH, which may inhibit muscle force production. A decrease in muscle pH has been shown previously to inhibit force production of active muscle fibres (Donaldson, 1983). This could result in higher threshold, less efficient motor units being recruited to maintain a constant power output. Heck et al. (1998) attempted to determine if sodium bicarbonate ingestion would reduce the magnitude of the $\dot{V}O_2$ slow component during constant-load exercise, through the reduction in H^+ ion concentration in active muscle fibres. The results of the study indicate that despite differences in plasma acid-base status between treatments (blood pH was higher in the

bicarbonate condition compared to the control condition) there were no differences in $\dot{V}O_2$ or the magnitude of the $\dot{V}O_2$ slow component between conditions. Thus, it was concluded that induced blood alkalosis did not attenuate the $\dot{V}O_2$ slow component during constant-load exercise above the ventilatory threshold (Δ 50%) (Heck et al., 1998).

Wasserman and colleagues (Stringer et al., 1994; Wasserman, 1994; Wasserman et al., 1995) suggested that the $\dot{V}O_2$ slow component could be a result of a) a developing inefficiency in aerobic regeneration of ATP, b) an adaptation to an anaerobic state where the O_2 supply to the muscles improves, thus facilitating aerobic regeneration of ATP or c) a combination of these processes (Wasserman et al., 1995, p. 187.) The authors present an interesting hypothesis, that the $\dot{V}O_2$ slow component may actually be an adaptive mechanism in response to an improvement in O_2 supply to the active muscles. Wasserman and co-workers contention is that it is not lactate per se, but rather the accompanying metabolic acidosis that causes the slow component of $\dot{V}O_2$. The decrease in pH accompanying the lactic acidosis shifts the oxyhemoglobin dissociation curve to the right, via the Bohr effect, which leads to an increase in capillary PO_2 and thus driving pressure for O_2 into the mitochondria, causing $\dot{V}O_2$ to increase. Thus, when the capillary PO_2 falls below some critical level. (15-20 Torr; Wittenberg & Wittenberg, 1989) aerobic ATP production is impaired causing anaerobic ATP production and lactic acid production increases. The ensuing lactic acidosis promotes O_2 unloading from the haemoglobin, and raises capillary PO_2 to a level that is adequate to maintain aerobic ATP production. Wasserman and colleagues hypothesis is that lactic

acidosis is fundamental during heavy exercise as it provides the mechanism through which critical capillary PO_2 can be reached and thus maximal O_2 extraction in the muscle cells can occur (Stringer et al., 1994; Wasserman, 1994; Wasserman et al., 1995).

The oxygen cost of respiratory muscle work required to increase ventilation has been proposed to contribute to the $\dot{V}O_2$ slow component. The energetic cost of breathing dictates the percent of the $\dot{V}O_2$ slow component that can be ascribed to the additional O_2 requirement for ventilation. Ventilation probably accounts for between 14-30% of the total $\dot{V}O_2$ slow component, (thus a relatively small proportion) (Gaesser, 1994; Gaesser and Poole, 1996). Hagberg et al., (1978), determined that there was an increase in ventilation during heavy exercise, which could increase the $\dot{V}O_2$ of the respiratory muscles. The role of ventilation in causing the slow component could be appreciated by the decrease in the $\dot{V}O_2$ slow component after training, which is highly correlated to the reduction in ventilation that occurs with training (Casaburi et al., 1987; Gaesser, 1994; Hagberg et al., 1978).

Womack and colleagues (1995) studied the effects of endurance training on the $\dot{V}O_2$ slow component and concluded that there is a minimal contribution from ventilation in the $\dot{V}O_2$ slow component, however, as an epinephrine infusion increased ventilation by 10 L/min, without any concomitant increase in $\dot{V}O_2$ (Womack et al., 1995). Furthermore, Paterson and Whipp (1991), found that the $\dot{V}O_2$ response in heavy exercise is asymmetric. That is, the off-transient

kinetics were found to be faster than those of the on-transient. During the off-transient, $\dot{V}O_2$ was either monoexponential, or had a reduced slow component compared to that of the on-transient. These results suggest that the limitations to O_2 utilisation during exercise are less prominent at the off-transient. As the O_2 cost of respiratory and cardiac work should be seen in both the on- and off- transients, a significant impact of these factors on the slow component seems unlikely (Paterson & Whipp, 1991; Whipp, 1994). Thus, while the O_2 cost of ventilatory work plays a role in contributing to the slow component it does not seem to be the primary factor.

The excess $\dot{V}O_2$ could also be a result of increased work by accessory muscles during heavy exercise such as pulling more forcefully on handlebars or increased body swaying (Whipp, 1994). The $\dot{V}O_2$ slow component is lower in running than in cycling (Billat et al., 1997; Billat et al., 1998). In running exercise, more accessory muscles (i.e. postural muscles) would be active from the onset of exercise thus, one would expect a higher $\dot{V}O_2$ cost of running than cycling exercise. If the $\dot{V}O_2$ slow component is due in part to recruitment of these accessory muscles, a smaller slow component would be expected with running compared to cycling, as accessory muscles are not progressively recruited with time of exercise.

Epinephrine (Epi) has been considered to have a potential role in the slow component. Plasma Epi levels increase during exercise above $T_{\dot{V}E}$ and plasma epinephrine concentration rises gradually with power output (Gaesser et al., 1994; Poole et al., 1991). Further, Epi is known to increase the metabolic rate, which

would stimulate increased $\dot{V}O_2$ (Gaesser and Poole, 1996; Gaesser et al., 1994). To elucidate the role of Epi, Gaesser and colleagues (1994) studied the effects of infused Epi on the $\dot{V}O_2$ slow component. If Epi is responsible for the $\dot{V}O_2$ slow component, then its infusion should augment the slow component. Subjects (n = 6) completed two 20-minute constant-load tests at Δ20%, under a control condition and with continuous intravenous infusion of Epi, at a rate of 100 ng/kg/minute, beginning at the end of the 10^{th} min of exercise. Despite raising plasma epinephrine significantly (2190 ± 410 pg/ml vs. 600 ± 280 pg/ml) higher than Epi levels in the control condition, epinephrine infusion had no effect on exercise $\dot{V}O_2$, although blood lactate and pyruvate concentrations were increased, and blood pH was reduced. Also, $\dot{V}CO_2$ and RER were both increased with Epi infusion, however, the infused epinephrine failed to influence $\dot{V}O_2$ (Gaesser, 1994). Gaesser and associates (1994) concluded that Epi does not contribute significantly to the $\dot{V}O_2$ slow component.

Furthermore, Womack and colleagues (1995) also looked at the effects of infused epinephrine during their training study. After completing a training protocol (6 weeks of training, 4 days a week, 2 days or 40 min cycling @ 70% peak power and 2 days of interval training) subjects completed another 20-minute constant load test, in which Epi was infused intravenously (at 100ng/kg/min). The Epi infusion successfully raised plasma epinephrine levels (12 fold), but had no effect on either the end-exercise $\dot{V}O_2$ or the slow component of $\dot{V}O_2$ (Womack et al., 1995). Womack and colleagues concluded that the attenuation in the slow

component following training was not the result of reduced epinephrine concentration after training. Consequently, there seems to be convincing evidence that epinephrine does not play a major role in contributing to the $\dot{V}O_2$ slow component (Gaesser et al., 1994; Womack et al., 1995).

During exercise, both core and muscle temperature increase (Hagberg et al., 1978; Gaesser and Poole, 1996; Poole et al., 1991). The increase in temperature may contribute to the rise in $\dot{V}O_2$ in heavy intensity exercise, via the Q_{10} effect (Gaesser and Poole, 1996; Koga et al., 1997). The Q_{10} value is an expression of the relationship between the rate of a reaction and the temperature. It is defined as "the relative increase in enzyme activity with a 10 degree increase in temperature" (Robergs and Roberts, 1997, pg. 41). For most enzyme-catalysed reactions, the range of the Q_{10} effect is from 1.5 to 2.5. The increased muscle temperature elevates O_2 consumption of mitochondria by a Q_{10} effect and by decreasing the phosphorylation potential (ADP/O ratio) (Koga et al., 1997; Willis and Jackman, 1994). Theoretically, the Q_{10} effect could be a large contributor to the slow component. The "Q_{10} effect could increase $\dot{V}O_2$ in proportion to the attendant metabolic rate and the working muscles are generating 80-90% of that metabolic rate, then the Q_{10} effect has the potential to increase the $\dot{V}O_2$ 5-10 times that occurring at rest" (Gaesser and Poole, 1996).

Support for the involvement of the Q_{10} effect in the slow component of O_2 uptake came from Hagberg et al. (1978). In this study, subjects exercised at 65% and 80% of $\dot{V}O_{2max}$ for 20 minutes each. The authors found that the increase in rectal temperature with heavy exercise could account for 31% of the rise in $\dot{V}O_2$ at

both workloads. This finding led Hagberg et al. (1978) to conclude that the slow component of $\dot{V}O_2$ is due primarily to an increase in muscle temperature during heavy exercise. Further, Poole et al, (1991) hypothesized from their study looking at the contribution of the exercising legs during the slow component, that the Q_{10} effect could play a significant role in the $\dot{V}O_2$ slow component. Poole and colleagues estimated (with a Q_{10} effect of 2.5) that during heavy exercise, a 1°C rise in muscle temperature could account for approximately 39% of the slow component arising from the exercising limbs (Poole et al., 1991).

Willis and Jackman (1994) also support the idea that a rise in muscle temperature could explain the $\dot{V}O_2$ slow component. Willis and Jackman found that an increase in temperature from 37° to 40°C reduced the ADP/O ratio by approximately 10%, with a further 10% decrease when the temperature increased from 40° to 43°C. This decrease in ADP/O ratio resulted in a concomitant increase in $\dot{V}O_2$ to effect a given ATP production rate (Willis & Jackman, 1994). From this study it appears that as muscle temperature increases a given level of ATP resynthesis requires an increase in $\dot{V}O_2$. It should be noted, however, that this study was not performed on human subjects but in rat and rabbit muscle tissue.

However, several investigators contend that the rise in muscle temperature does not cause the $\dot{V}O_2$ slow component (Casaburi et al., 1987; Koga et al., 1997). Casaburi et al. (1987) determined that rectal temperature does increase with work rate, and that endurance training decreased the size of the increase in temperature (as well as the $\dot{V}O_2$ slow component). However, the change in rectal temperature

did not correlate to the size of the $\dot{V}O_2$ slow component. Further, the time course for the $\dot{V}O_2$ slow component and the increase in rectal temperature were dissimilar (Casaburi et al., 1987). Recently, Koga and colleagues (1997) also investigated the effect of increased muscle temperature on $\dot{V}O_2$. Despite effectively raising the muscle temperature prior to exercise onset, the researchers found that the increment in $\dot{V}O_2$ between the 3rd and 6th minute of heavy exercise was significantly *smaller* for the increased muscle temperature condition than in the normal temperature condition. Thus, increased muscle temperature was associated with a reduction in the $\dot{V}O_2$ slow component (Koga et al., 1997). These results make it seem unlikely that the Q_{10} effect plays any significant role in the mechanism for the $\dot{V}O_2$ slow component.

Perhaps the most compelling evidence for the mechanism of the $\dot{V}O_2$ slow component is related to the recruitment of lower-efficiency, type II motor units. Because the exercise intensity required to elicit the slow component is relatively high (> T_{VE}), it is likely that type II muscle fibres will be recruited at these intensities in order to maintain a constant power output. There are well-documented metabolic differences between slow twitch (type I) and fast twitch (type II) muscle fibres. Slow twitch muscle fibres have higher activities of mitochondrial oxidative enzymes than fast twitch muscle fibres and a lower capacity for anaerobic metabolism, whereas fast twitch muscle fibres have greater activities of glycolytic enzymes (i.e. phosphofructokinase; PFK) and much lower oxidative potential than slow twitch muscle fibres (Essen et al., 1975; Saltin & Gollnick, 1983). More importantly for this review on the $\dot{V}O_2$ slow component,

type II muscle fibres are energetically less efficient than type I muscle fibres in that the high energy phosphate produced per oxygen molecule consumed (P/O ratio) is less than in the type I muscle fibres (Barstow et al., 1996; Kushmerick et al., 1992; Gaesser and Poole, 1996; Poole et al., 1994a). Thus type II muscle fibres require a greater amount of oxygen to produce the same amount of ATP. Further, type II muscle fibres have slower $\dot{V}O_2$ kinetics than type I muscle fibres (Barstow et al., 1996; Crow & Kushmerick, 1982).

During metabolism, nicotinamide adenine dinucleodtide (NADH) is required in order for glycolysis to proceed. However the inner mitochondrial membrane is impermeable to NADH. In order for NADH to be transferred into the mitochondria for oxidative phosphorylation, the transfer of reducing equivalents into mitochondria via the malate-aspartate (MA) and alpha-glycerophosphate (α-GP) shuttles are required (Schantz & Henriksson, 1987). The enzymes of these two shuttle systems differ between muscle fibre types. Fast twitch muscle fibres have higher concentrations of α-GP enzymes and thus preferentially utilise the α-GP shuttle, whereas slow twitch muscle fibres have higher MA shuttle enzyme concentrations and favour the MA shuttle (Schantz & Henriksson, 1987). The α-GP shuttle bypasses one phosphorylation site, and thus to produce the same amount of ATP fast twitch fibres require more O_2 than slow twitch fibres. During heavy intensity exercise, a larger proportion of fast twitch muscle fibres are recruited than during moderate intensity exercise. Thus it seems plausible that the recruitment of type II muscle fibres could contribute to the $\dot{V}O_2$ slow component due to the extra O_2 requirement of these fibres.

There are several mechanisms through which the recruitment of type II muscle fibres could account for the slow component. a) The predominantly type IIa and IIb muscle fibres of the mouse extensor digitorum muscle have a longer time constant for the rise in $\dot{V}O_2$ than that of the soleus muscle (mostly type I and IIa) (Crow & Kushmerick, 1982). b) Type II muscle fibres in vitro, produce more heat (50-600%) and consume more O_2 for the same tension development when compared to type I muscle fibres (Crow & Kushmerick, 1982; Gibbs et al., 1972; Wendt & Gibbs, 1973). c) In type II muscle fibres, the calcium ATP dependent pump activity is 5-10 fold higher than in type I muscle fibres (Gibbs et al., 1972; Wendt & Gibbs, 1973). d) Lastly, isolated mitochondria from type II muscle fibres exhibit an 18% lower P/O ratio, which predicts a greater $\dot{V}O_2$ for any given ATP resynthesis rate (Willis & Jackman, 1994). This difference in P/O ratio may be due to a greater reliance on the α-GP shuttle over the MA shuttle in type II muscle fibres (Schantz & Henriksson, 1987).

While it seems physiologically possible that the type II muscle fibres could contribute to the $\dot{V}O_2$ slow component, there is further support for this mechanism in that the time course for type II muscle fibre recruitment seems to parallel the delayed onset of the $\dot{V}O_2$ slow component. First, the slow component increases progressively in an exercise bout (min 3-10), and the gradual increase of the integrated EMG in concert with the continued increase in $\dot{V}O_2$ supports an increase in fibre recruitment (Poole et al., 1994; Shinohara & Moritani, 1992). Second, it is likely that both slow and fast twitch muscle fibre populations are recruited simultaneously at the onset of heavy exercise. However because of slower kinetics,

the $\dot{V}O_2$ requirement of the fast twitch fibres may not become manifest for some minutes after exercise onset (Barstow & Mole, 1991; Crow & Kushmerick, 1982; Kushmerick et al., 1992; Poole et al., 1994). Third, heterogeneity of blood flow is present in exercising muscle at exercise onset. Fast twitch muscle fibres that are metabolically active at exercise onset may not participate in the exercise $\dot{V}O_2$ response until flow increases in their immediate adjacent capillaries (Piiper, 1992; Poole et al., 1994).

There is a great deal of support among researchers that type II fibre recruitment seems a viable mechanism for the slow component (Barstow, 1994; Barstow et al., 1996; Coyle et al., 1992; Gaesser and Poole, 1996; Poole, 1994; Poole et al., 1994a; Poole and Richardson, 1997; Poole et al., 1991; Whipp, 1994; Willis and Jackman, 1994). In 1991, Poole and associates determined through measuring both leg and pulmonary $\dot{V}O_2$ during heavy exercise that ~ 86% of the increase in pulmonary $\dot{V}O_2$ could be accounted for by the increase in leg $\dot{V}O_2$. These results demonstrate that the majority of the slow component results from the exercising limbs, which lends support to the theory of increased type II fibre recruitment, as the increased O_2 requirement would come directly from the exercising legs. Shinohara and Moritani (1992, as cited in Gaesser and Poole, 1996), found that during high intensity exercise integrated electromyogram (iEMG) of the exercising muscles, which indicates changes in motor unit recruitment, was positively correlated with the increase in pulmonary $\dot{V}O_2$. The authors then concluded that the $\dot{V}O_2$ slow component may well be due to the recruitment of more motor units, in particular, fast twitch muscle fibres. In the same regard,

Coyle et al. (1992), looked at the relationship between cycling efficiency and percent type I muscle fibres. They found that in trained cyclists, during heavy intensity constant-power exercise, those athletes with a higher percentage of type II muscle fibres had a greater $\dot{V}O_2$ requirement for exercise and lower cycling efficiency. Therefore motor unit recruitment during high intensity exercise may reflect the recruitment of type II motor units, which increases the $\dot{V}O_2$ cost of the exercise and could explain a large part of the slow component. Similarly, Barstow and associates (1996) investigated the influence of muscle fibre type and pedal frequency of O_2 uptake kinetics. The results of Barstow et al. (1996), indicated that the greater percentage type I muscle fibres, the smaller the size of the $\dot{V}O_2$ slow component. These findings suggest that fibre type distribution influences the $\dot{V}O_2$ slow component.

Endurance training causes attenuation of the $\dot{V}O_2$ slow component (Casaburi et al., 1987; Womack et al., 1995). Womack and colleagues (1995) determined that decreased blood lactate and plasma epinephrine levels following training appear to play no causal role in the reduced $\dot{V}O_2$ requirement for heavy exercise after training. It was suggested that perhaps the attenuated slow component could be accounted for by a change in motor unit recruitment after training (Womack et al., 1995).

Recently, in our laboratory, Bell and colleagues (1998) utilised older adults as a model to investigate the role of type II muscle fibre recruitment in the $\dot{V}O_2$ slow component. It has been demonstrated that with ageing there is a reduction in the proportion of fast twitch muscle fibres relative to slow twitch muscle fibres.

Thus, if older adults have a smaller proportion of fast twitch muscle fibres, they should have a smaller

$\dot{V}O_2$ slow component during heavy intensity constant-load exercise. The results of this study demonstrated an observable $\dot{V}O_2$ slow component in the elderly, and the magnitude of the slow component was smaller than in the young subjects. However, the smaller $\dot{V}O_2$ slow component in the older subjects appeared to be due to the lower absolute workrates of the older adults. If the exercise intensity was expressed as the same relative workrate between young and older subjects, the $\Delta \dot{V}O_2 / \Delta WR$ relationship was not different between groups. It is possible that the older adults in this study did not have a preferential loss of type II muscle fibres with ageing. However, this study does show that the efficiency of oxidative metabolism of older adults is similar to that of young adults. The results of this study seem to indicate that if older adults do have a loss of type II muscle fibres, then recruitment of these fibres may not be the mechanism for the $\dot{V}O_2$ slow component. However this possibility cannot be ruled out from this study.

Several investigators have endeavoured to manipulate the contribution of the different fibre types to power production during heavy exercise through altering pedalling cadence in order to study the theory of type II muscle fibre recruitment in causing the $\dot{V}O_2$ slow component. Gaesser and colleagues (1992) had subjects perform 18 min of constant-load heavy exercise at pedal rates of 50 rpm and at 100 rpm (same absolute power output) to determine if cadence had an effect on the $\dot{V}O_2$ slow component. It was found that the increase in $\dot{V}O_2$ ($\dot{V}O_2$ at min18 – min 3) was significantly higher at 100

rpm than at 50 rpm (0.67 ± 0.11 L/min vs 0.34 ± 0.07 L/min) (Gaesser et al., 1992). This seems to support the idea that there is an optimal velocity of fibre shortening and that it is necessary for recruiting fast twitch fibres at higher cadences (Poole et al., 1994). Barstow and co-workers (1996) also tested the theory that fibre recruitment may vary at different cadences. They hypothesized that type II motor until may be preferentially recruited at low pedal frequencies (40-50 rpm) when muscle tension is high and at high pedal rates (90-100 rpm) when contraction velocity is high. However, these researchers found no difference in the characteristics of the $\dot{V}O_2$ slow component with a change in pedal frequency (ranging from 45 rpm to 90 rpm). A further study by Zoladz and colleagues (1995) also examined the relationship between cycling cadence and the $\dot{V}O_2$ slow component. The protocol consisted of cycling at pedalling rates of 40, 60, 80, 100 and 120 rpm over which range it was expected to vary the proportion contribution of different fibre types to the power output. It was anticipated that at pedalling rates above 60 rpm there would be earlier recruitment and thus a progressive increase in the proportional contribution of the fast twitch fibres to the total power delivered (Sargeant, 1994). The results however, were in agreement with those of Barstow et al. (1996); there was no change in the magnitude or onset of the $\dot{V}O_2$ slow component in relation to pedalling rate despite higher lactate concentrations at higher cadences. Thus, findings of studies examining different pedalling rates on the hierarchical recruitment of different muscle fibres have proved equivocal. These results suggest that perhaps the recruitment of type II muscle fibres is not a significant factor in the $\dot{V}O_2$ slow component. However, Zoladz and colleagues (1995), suggested that because the mechanical efficiency-velocity relationship of muscle fibres is not well understood, the progressive recruitment of type II

muscle fibres cannot be ruled out. That is, while at higher pedalling rates, type II fibres may have a higher energy turnover, but they may simultaneously also have an increase in power output as they could be functioning at a velocity closer to the optimum for maximal efficiency. Clearly the role type II muscle fibre recruitment may play in the $\dot{V}O_2$ slow component is complex.

In a recent study James and Doust (1999) examined the $\dot{V}O_2$ response to high intensity running exercise following a bout of interval running. Subjects performed interval running exercise which consisted of 4 intervals of 800m at a velocity 1 km/hr less than velocity at $\dot{V}O_{2max}$, each bout separated by 3 min rest. Immediately prior to and 1 hour following the interval running, subjects performed constant-velocity high-intensity running at Δ 40. Results of this study demonstrated a significant increase in $\Delta \dot{V}O_{2\,(6\text{-}3min)}$ after interval running in an experimental group when compared to a control group that did not perform interval running. This greater magnitude of the $\dot{V}O_2$ slow component occurred in the absence of any change in body mass, core temperature or blood lactate between the two constant-velocity tests. The investigators concluded that this increase in the $\dot{V}O_2$ slow component could be due to progressively greater recruitment of type II muscle fibres following high intensity running (James & Doust, 1999).

In summary, there are many potential factors that could contribute to the $\dot{V}O_2$ slow component. While many investigations have sought to elucidate the exact mechanism, no one factor can be attributed to causing the slow component. The possibility of lactate and more recently the recruitment of type II muscle fibres have received most of the attention in research but, neither of these factors can be determined conclusively as the underlying physiological mechanism. The possibility that the slow

component could be due to the recruitment of type II muscle fibres seems to be the most convincing evidence to date. With continued research, hopefully the underlying physiological mechanism for this phenomenon can be resolved.

Chapter III

Methods

3.1 Subjects

Eight healthy male volunteers were recruited from the University community for participation in this study. Any volunteers taking medication known to influence cardiorespiratory function were excluded from participation. The study requirements were fully explained, in both written and verbal form, and informed consent was obtained from each participant. The study was approved by the University's Review Board for Health Sciences Research Involving Human Subjects (Appendix I).

3.2 Testing

3.2.1 Initial Test: $\dot{V}O_{2max}$

Preliminary testing of all subjects involved an incremental exercise test to volitional fatigue, on an electrically braked cycle ergometer (Lode, model H-300-R). After 4 minutes of loadless cycling (power output ~ 15 W), the workrate was increased as a ramp function at approximately 25-30 W/minute, depending on the fitness level of the participant. Fatigue was determined to be the point when the subject could no longer turn the pedals.

Gas exchange data were used to determine $\dot{V}O_{2max}$ and ventilatory threshold (T_{VE}). $\dot{V}O_{2max}$ was established by a leveling in $\dot{V}O_2$ with an increase in workload. $\dot{V}O_{2max}$ was taken as the highest $\dot{V}O_2$ achieved averaged over a 15-second interval of breath-by-breath measurements. Ventilatory threshold, T_{VE}, was determined from the relationship between $\dot{V}O_2$ and the ventilatory equivalent for $\dot{V}O_2$ ($V_E/\dot{V}O_2$), ventilatory equivalent for CO_2 output ($V_E/\dot{V}CO_2$), end-tidal PO_2 ($P_{ET}O_2$) and end-tidal PCO_2 (P_{ET}

CO_2) as well as the V-slope method ($\dot{V}CO_2$ vs $\dot{V}O_2$). T_{VE} was defined as the $\dot{V}O_2$ at which $V_E/\dot{V}O_2$ and $P_{ET}O_2$ increased systematically without a concomitant increase in $V_E/\dot{V}CO_2$ or decrease in $P_{ET}CO_2$ (Davies et al., 1982). The V-slope method (Beaver et al., 1986) was used in combination with these other methods to establish the T_{VE}. The V-slope method is a technique for determining ventilatory threshold from a ramped exercise test to exhaustion based on gas exchange data. The V-slope method is based on the fact that CO_2 is released as lactic acid is buffered by bicarbonate in the cells. This CO_2 is rapidly transported to the lungs, where it is detected as an increase in CO_2 output greater than the CO_2 produced from aerobic metabolism. The ventilatory threshold is then determined through plotting the breath-by-breath $\dot{V}CO_2$ data as a function of $\dot{V}O_2$ (the direct index of metabolism) during a ramped exercise test, and identifying the point at which $\dot{V}CO_2$ increases out of proportion to aerobic metabolism (Beaver et al., 1986).

3.3 Experimental Design

Participants were asked to abstain from ingesting any food or beverages containing caffeine and from exercise for a 12-hour period prior to testing. All participants were involved in regular exercise programs. Participation in this study required visiting the laboratory on five separate occasions, at approximately the same time each day.

Each participant was examined twice in each of the control and glycogen depletion conditions. The control and glycogen depletion trials were randomly assigned, and the tests were conducted at least 1 week apart. Seat height and handle bar positions on the cycle ergometer were adjusted at the first trial and replicated for each subsequent visit.

3.3.1 Glycogen Depletion Protocol

The glycogen depletion protocol used in this study was modified from the protocol described by Thompson and colleagues (1979), and was designed to deplete primarily the type II fibres. The protocol involved 10, one-min bouts of cycle ergometer exercise at 130% $\dot{V}O_{2max}$, with each bout of exercise separated by 5 min loadless cycling or stationary sitting, at the subject's discretion. Following the depletion protocol, the subjects rested for one hour. No food was allowed during this time, but the subjects were allowed to consume water.

Following the 1-hour rest period, subjects performed constant-load exercise protocols at power outputs both below and above their T_{VE} for determination of $\dot{V}O_2$ kinetics. The exercise protocol consisted of: 8 minutes of loadless cycling; 8 minutes moderate intensity exercise at a power output corresponding to 80% T_{VE}; 8 minute loadless cycling; 10 minutes heavy intensity exercise at a power output chosen to elicit a $\dot{V}O_2$ equal to 40 % of the difference between $\dot{V}O_{2max}$ and T_{VE} ($\Delta 40\%$; $\Delta 40 = [T_{VE} + (\dot{V}O_{2max} - T_{VE})\ 0.4]$); and 8 minutes recovery. Each power output was initiated as a step function without warning to the subject. Upon completion of the exercise protocol, subjects rested for 1 hour. Following 1-hour rest, the subjects repeated the exercise protocol. Refer to Figure 1 for a schematic diagram of the exercise protocol. This resulted in a total of 4 repeats for moderate and heavy exercise for the glycogen depletion condition (2 repeats per visit X 2 visits per condition). Ventilatory and gas exchange data were collected using the same apparatus as for the $\dot{V}O_{2max}$ test.

3.3.2 Control Condition

The exercise protocol for the control condition was identical to that of the glycogen depletion condition except that it was not preceded by any supramaximal exercise bouts to deplete muscle glycogen. Following the first set of constant-load tests, the subject rested for 1 hour before repeating the workrate transitions. This resulted in 4 repeats for moderate and heavy exercise for the control condition.

3.4 Muscle Biopsies

During one of the glycogen depletion trials, muscle biopsy samples were obtained from the vastus lateralis muscle of each subject at two times during the exercise protocol using the needle biopsy technique (Bergström, 1962). Initial biopsy samples were taken at rest before the glycogen depletion protocol in the supine position. A second biopsy was taken immediately following the glycogen depletion protocol (after the 10^{th} bout), within 5 minutes.

After the muscle sample had been excised, one sample was immediately frozen in liquid nitrogen and stored at –80°C. The second sample was mounted on a cork using an embedding medium and then frozen rapidly in isopentane, which had been chilled to approximately –160 °C with liquid nitrogen. The samples were stored at –80° C for later analysis.

Muscle was analyzed biochemically for glycogen concentration and histochemically for fibre type and fibre-specific glycogen content. Serial cross-sections of the muscle were cut in a cryostat at –20°C and mounted on cover glass for histochemical analysis. Muscle samples were stained using a periodic-acid Schiff (PAS) staining procedure according to the procedure of Brooke and Kaiser (1970) and muscle

fibres were classified according to 3 fibre types: type I, IIa and IIb. Myosin ATPase was determined through the procedure of Padykula & Herman (1955), and NADH tetrazolium reductase (diaphorase) was also performed on the muscle samples according to the procedure of Novikoff et al (1961). All histochemical procedures including PAS were performed during the same session under identical or nearly identical conditions. Biochemical analysis for total muscle glycogen was determined through the procedure of Lo et al. (1970). Although muscle fibre type was assessed using both myosin ATPase and NADH activity, the nomenclature chosen in the present study was type I, type IIa and IIb rather than slow twitch oxidative (SO), fast twitch oxidative-glycolytic (FOG) and fast twitch glycolytic (FG). While it is recognize that there may be slight differences, there is a general relationship between type I and SO, type IIa and FOG and type IIb and FG muscle fibres.

3.5 Blood Sampling

Blood samples were obtained during one of the control and glycogen depletion trials. Subjects rested while a percutaneous Teflon catheter (Angiocath, 21 gauge) was placed in a dorsal vein of the hand. The blood was arterialized by wrapping the hand and forearm in a heating pad. Blood was sampled at specific times throughout each exercise bout. Blood was sampled at minutes 0, 1, 2, 3, 4, 6, and 8 for the moderate intensity exercise, and at minutes 0, 1, 2, 3, 4, 6, 8 and 10 for the heavy intensity exercise.

The arterialized venous blood was drawn into 3 cc syringes containing lithium heparin and placed into an ice bath and analyzed after a short delay. Whole blood samples were analyzed (at 37 °C) for plasma lactate ($[La^-]_{pl}$) using selective electrodes (Stat Profile 9 Plus Blood gas-electrolyte analyzer, Nova biomedical, Canada). The electrodes were calibrated before each test and at regular intervals throughout testing.

3.6 Calibration, Equipment and Data Acquisition

Calibration of the equipment was completed prior to each testing session. Gas exchange data were collected on a breath-by-breath basis. Inspired and expired airflow and volumes were collected through a mouthpiece containing a low resistance, low dead space (90ml) bi-directional turbine (Alpha Technologies, VMM-110). The volume signal was calibrated before each test with a syringe of known volume (990 ml). Respired gases were sampled and collected continuously at the mouth by mass spectrometry (Perkin-Elmer, MGA-1100) for determination of fractional concentrations of O_2, CO_2, and N_2. The mass spectrometer was calibrated with precision-analyzed gas mixtures. The changes in gas concentration were aligned with the inspired and expired gas volumes by measuring the time delay for a square wave bolus of gas passing the turbine to the resulting changes in fractional gas concentrations as measured by the mass spectrometer. Analogue signals from the mass spectrometer and turbine transducer were sampled every 20 ms and stored on disk for later analysis. Alveolar $\dot{V}O_2$ data were calculated using the algorithms of Beaver et al., (1981). The signals were then analyzed breath-by-breath and processed on-line using First Breath Inc. software (St. Agatha, ON, 1993). Heart rate was monitored using a 3-lead electrocardiogram with electrodes in the modified V5 configuration.

3.7 Data Analyses

Breath-by-breath data obtained during step transitions in workrate were linearly interpolated at 1-second intervals, time aligned and ensemble averaged to provide a single response for each subject.

The gas-exchange data were mathematically modeled in order to describe the kinetic response to exercise. The control and glycogen depletion data were modeled separately for each subject. The model used to describe the kinetic response provides an estimate of the baseline (A_0), amplitudes (A_1, A_2, and A_3), time delays (TD_1, TD_2, and TD_3), and time constants (τ_1, τ_2, τ_3). A 2-component exponential model was used to model the kinetic parameters for moderate intensity exercise ($< T_{VE}$) according to:

$$VO_2 = A_0 + A_1 \cdot [1\text{-}e^{-(t\text{-}TD1/\tau1)}] \cdot u_1 + A_2 \cdot [1\text{-}e^{-(t\text{-}TD2/\tau2)}] \cdot u_2$$

Where $u_1 = 0$ for $t < TD_1$, $u_1 = 1$ for $t > TD_1$, $u_2 = 0$ for $t < TD_2$, and $u_2 = 1$ for $t > TD_2$.

The breath-by-breath data collected during the heavy intensity exercise ($> T_{VE}$) were fit using a 3- component exponential model with the equation:

$$VO_2 = A_0 + A_1 \cdot [1\text{-}e^{-(t\text{-}TD1/\tau1)}] \cdot u_1 + A_2 \cdot [1\text{-}e^{-(t\text{-}TD2/\tau2)}] \cdot u_2 + A_3 \cdot [1\text{-}e^{-(t\text{-}TD3/\tau3)}] \cdot u_3$$

Where $u_1 = 0$ for $t < TD_1$, $u_1 = 1$ for $t > TD_1$, $u_2 = 0$ for $t < TD_2$, $u_2 = 1$ $t > TD_2$, $u_3 = 0$ for $t < TD_3$, and $u_3 = 1$ for $t > TD_3$.

The curve fitting procedure involved the calculation of a modeled exponential output for test values of the various parameters determined by least squares non-linear regression, in which best fit was defined by the lowest residual sum of squares (RSS). The overall time course was determined from the mean response time (MRT) calculated from a weighted sum of TD and Tau for each component. MRT is equal to the time required to achieve approximately 63% of the difference between A_0 and the new steady state value for the entire $\dot{V}O_2$ response.

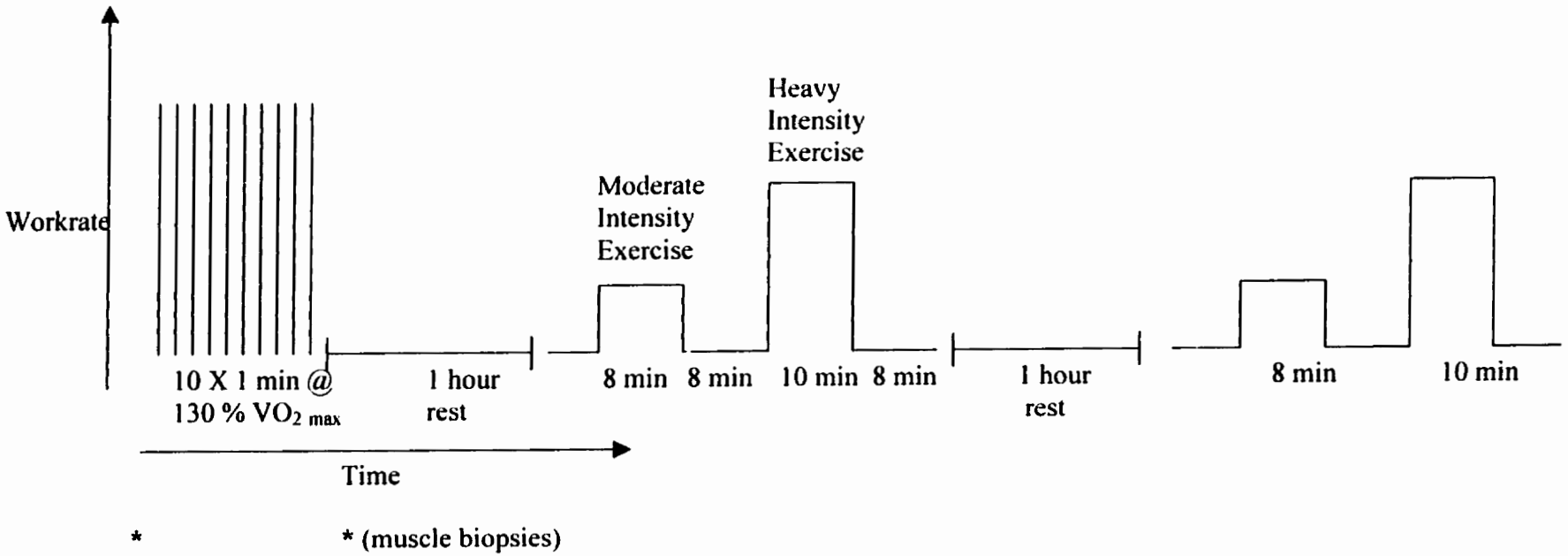
Magnitude and slope of the $\dot{V}O_2$ slow component were determined as the difference between the $\dot{V}O_2$ at the end of exercise and the $\dot{V}O_2$ at min 3 of exercise [$\Delta \dot{V}O_{2\,(10\text{-}3min)}$]. $\dot{V}O_2$ at min 3 was determined as the mean $\dot{V}O_2$ from 10 s before and 10 s

after 3 min, and end exercise $\dot{V}O_2$ established by the mean $\dot{V}O_2$ during the last 20 s of exercise. Further, the magnitude of the $\dot{V}O_2$ slow component was also determined by the amplitude term, A_3 of the three component model. Kinetics of the slow component were described by parameter estimates derived from the 3-component exponential model. Similarly, the change in blood lactate was described as the difference between plasma lactate [La^-] at the end of exercise and [La^-] at min 3 of exercise [$\Delta\ La^-_{(10\text{-}3\ min)}$] for heavy exercise.

3.8 Statistical Analyses

Gas exchange kinetic parameter estimates were analyzed using a one-way repeated-measures ANOVA for control vs. glycogen depletion conditions as the main effects. Blood lactate data were analyzed for condition and time effects using a two-way repeated measures ANOVA. $\Delta\ \dot{V}O_{2\ (10\text{-}3min)}$ and $\Delta\ [La^-]_{(10\text{-}3min)}$ were analyzed using Student's paired *t*-test. Correlation between percentage type I and type II muscle fibres and the magnitude of the slow component were analyzed using Pearson Product correlation. Statistical significance was set at $P < 0.05$.

Figure 1. Schematic diagram of the exercise protocol


Workrate
10 X 1 min @
130 % $VO_{2\ max}$
1 hour
rest
Moderate
Intensity
Exercise
8 min
8 min
Heavy
Intensity
Exercise
10 min
8 min
1 hour
rest
8 min
10 min
Time
*
* (muscle biopsies)

Chapter IV

Results

4.1 Subject Characteristics

Eight male subjects, of age 22.4 (± 2) years (mean ± SD; range = 19- 25 yrs) participated in this study. Subject physical characteristics, $\dot{V}O_2$ at T_{VE}, and $\dot{V}O_{2max}$ from the ramp exercise test are presented in Table 1.

Mean $\dot{V}O_{2max}$ for the participants was 52 (± 7) $ml \cdot kg^{-1} \cdot min^{-1}$. The individual work rates and $\dot{V}O_2$ values for constant load exercise tests are presented in Table 2 (moderate intensity exercise) and Table 3 (heavy intensity exercise). During each condition, subjects completed 4 repetitions at the work rates below T_{VE} and above T_{VE}, with the exception of two subjects who completed only two repetitions at each intensity during the glycogen depletion condition. The mean work rate utilized for the constant-load tests was 112 (± 36 W; 46.2 ± 6.8 % $\dot{V}O_{2max}$), for moderate intensity exercise and 234 (± 53 W; 80.1 ± 8.4 % $\dot{V}O_{2max}$) for heavy intensity exercise.

4.2 Muscle Fibre Type and Muscle Glycogen Content

All eight subjects completed the glycogen depletion protocol. However, several subjects maintained the required intensity (i.e. 130% $\dot{V}O_{2\,max}$) for only 40-50 s. Resting muscle biopsy samples were obtained from all eight subjects. However, biopsy samples were obtained from only seven of the eight subjects following the glycogen depletion protocol. After tissue preparation, it was determined that muscle samples from only 5 subjects were suitable for analysis.

The mean percentage of type I (slow twitch) muscle fibres of the group (n=5) averaged 44 (± 5)% (range 36-48%). Percentage of type IIa (fast twitch oxidative) and

type IIb (fast twitch fatigable) muscle fibres were 50 (± 5.2) and 6.1 (± 1.6) % respectively (range; type IIa 47-59%; type IIb 4-8%).

Total muscle glycogen concentration for each subject prior to the glycogen depletion protocol and following the depletion protocol are presented in Table 4. Total muscle glycogen concentration was significantly lower following the glycogen depletion protocol (1.21 ± 0.49 vs 0.42 ± 0.29 grams of glycogen/ 100 g tissue). This corresponded to a 65% overall depletion of muscle glycogen levels.

The pattern of glycogen depletion in individual muscle fibre types was determined by histochemical procedures (PAS staining). Plate 1 (A through F) shows the resting and post depletion biopsy samples of a representative subject for each of the histochemical tests performed. At rest, prior to the glycogen depletion protocol, the glycogen PAS stain intensity was uniformly dark in all muscle fibre types. Following the glycogen depletion protocol, it was evident that the PAS staining intensity of the type IIb muscle fibres was lightest (PAS negative) suggesting significant glycogen depletion in these fibres. The PAS staining intensity in the type I muscle fibres appeared unchanged compared to the resting sample, suggesting that these fibres did not experience significant glycogen depletion. The type IIa muscle fibres showed intermediate PAS staining intensity, suggesting some glycogen depletion following the glycogen depletion protocol.

4.3 Loadless Cycling

The group mean $\dot{V}O_2$, $\dot{V}CO_2$, RER and heart rate data for loadless cycling, prior to the onset of exercise for both the control and glycogen depletion conditions are presented in Table 5. There were no differences for $\dot{V}O_2$, $\dot{V}CO_2$, or heart rate during loadless

cycling between control and glycogen depletion conditions. However, the RER was significantly lower ($P<0.001$) following the glycogen depletion condition.

4.4 Plasma Lactate

The mean group responses for the change in plasma lactate concentration during moderate and heavy exercise intensities are presented in Figure 2. Blood samples were obtained from 5 of the 8 subjects.

During moderate intensity exercise, there were no differences in $[La^-]_{pl}$ between control and glycogen depletion conditions. There was no significant effect for either time or condition for $[La^-]_{pl}$ in moderate intensity exercise.

For heavy intensity exercise above T_{VE}, there was a progressive increase in $[La^-]_{pl}$ after exercise onset in both the control and glycogen depletion conditions ($P< 0.019$), with no differences in $[La^-]_{pl}$ between control and glycogen depletion conditions.

4.5 Respiratory Exchange Ratio

Respiratory exchange ratio (RER) values for individual subjects are presented in Table 6. RER during loadless cycling prior to the onset of moderate intensity exercise was higher in the control than the glycogen depletion condition; (0.88 (± 0.04) and 0.78 (± 0.03) for control and glycogen depletion conditions respectively, $P< 0.001$). However, the RER at the end of moderate intensity exercise was similar between conditions (0.90 ± (0.03) and 0.86 (± 0.04) for control and glycogen depletion respectively).

The RER for loadless cycling prior to the onset of heavy intensity exercise was significantly higher in the control than the glycogen depletion condition (0.90 (± 0.05) vs 0.83 (± 0.06) for control and glycogen depletion conditions respectively, $P < 0.001$). At

the end of heavy intensity exercise, RER was similar between conditions (0.97 (± 0.03) vs 0.95 (± 0.05) for control and glycogen depletion conditions, respectively).

4.6 $\dot{V}O_2$ Kinetics

A summary of the parameters of the model fit for the $\dot{V}O_2$ on-transient to a step increase to moderate ($<T_{VE}$) intensity exercise are presented in Table 7. Figure 3A depicts the group mean $\dot{V}O_2$ response for the on-transient to moderate intensity exercise. Figure 4 depicts the $\dot{V}O_2$ response for a single representative subject for moderate intensity exercise in the control condition, and Figure 5 shows the $\dot{V}O_2$ response for the same subject during moderate intensity exercise in the glycogen depletion condition. Baseline $\dot{V}O_2$ (A_0; 790 ± 81 and 836 ± 115 ml/min for control and glycogen depletion, respectively) and end-exercise $\dot{V}O_2$ (1831 ± 524 and 1872 ± 538 ml/min for control and glycogen depletion, respectively) for moderate intensity exercise were similar between control and glycogen depletion conditions. As a result the total amplitude in $\dot{V}O_2$ (TA = $A_1 + A_2$) from baseline to steady-state exercise was similar between control and glycogen depletion conditions (1069 ± 425 and 1103 ± 404 ml/min for control and glycogen depletion, respectively). There were no differences in parameter estimates for phase I (A_1, TD_1 and τ_1) or phase II (A_2, TD_2 and τ_2) kinetics for the $\dot{V}O_2$ on-transient between control and glycogen depletion conditions.

A summary of the model parameters for the on-transient to heavy intensity exercise ($>T_{VE}$) are presented in Table 7. Figure 3B shows the group mean $\dot{V}O_2$ response for the step transition to heavy intensity exercise. Figure 6 depicts the $\dot{V}O_2$ response of the same representative subject during heavy intensity exercise for the control condition,

while Figure 7 shows the $\dot{V}O_2$ response for this subject during heavy intensity exercise for the glycogen depletion condition. During loadless cycling, baseline $\dot{V}O_2$ (A_0) was similar between control and glycogen depletion conditions. After the onset of exercise, $\dot{V}O_2$ increased similarly in both conditions. There were no differences in the amplitudes (A_1, A_2 and A_3) between conditions. Likewise, end-exercise $\dot{V}O_2$ was also similar between conditions (3442 (± 656) and 3485 (± 614) for control and glycogen depletion, respectively).

At the onset of the step increase in work rate to heavy intensity exercise, τ_2 was significantly faster in the control condition than in the glycogen depletion condition (24.5 (± 5.3) vs 27.7 (± 7.4) s for control and glycogen depletion conditions respectively; $P<$ 0.015). There were no further differences observed between the two conditions in any other model parameters (TD or τ).

The group mean end-exercise ventilation (V_E), heart rate (HR), $\dot{V}CO_2$ and $\dot{V}O_2$ values are presented in Table 8. There were no differences in end-exercise V_E, HR, $\dot{V}CO_2$ or $\dot{V}O_2$ between control and glycogen depletion conditions during either moderate or heavy intensity exercise.

Cycling efficiency, determined as $\Delta \dot{V}O_2 / \Delta$ WR, for individual subjects is presented in Table 9. Cycling efficiency was similar between conditions during both moderate and heavy intensity exercise. Cycling efficiency (determined as $\Delta \dot{V}O_2$ (end-exercise-baseline)/ΔWR) was lower in moderate compared to heavy intensity exercise (11.2 ± 0.8 vs 12.2 ± 0.5 for moderate and heavy intensity exercise respectively for the control condition). However, when efficiency for heavy intensity exercise was expressed

as $\Delta \dot{V}O_2$ (projected steady state $\dot{V}O_2$ from the phase II increase in $\dot{V}O_2$ - baseline $\dot{V}O_2$), there was no difference in cycling efficiency between moderate and heavy intensity exercise (11.2 ± 0.8 vs 11.3 ± 0.5 for moderate and heavy intensity exercise respectively in the control condition).

4.7 $\dot{V}O_2$ Slow Component

The $\dot{V}O_2$ slow component for each subject is presented in Table 10. There was no difference in the slow component between conditions when expressed as $\Delta\dot{V}O_{2(10\text{-}3min)}$ (228 (± 84) vs 182 (± 116) ml/min for control and glycogen depletion conditions, respectively), or when quantified by the parameter estimate A_3 (Table 7; 459 (± 209) and 394 (± 253) ml/min for control and glycogen depletion conditions), or by the time course of the $\dot{V}O_2$ slow component (i.e. TD_3 ; CON 112 (± 32), GD 118 (± 32); and τ_3; CON 153 (± 44), GD 130 (± 40)) (Table 7) for both conditions. The parallel changes in plasma lactate concentration (Δ [La^-] $_{(10\text{-}3\ min)}$) during heavy intensity exercise are presented in Table 11 for the 5 subjects from whom blood samples were obtained. There was no difference in the Δ [La^-] $_{(10\text{-}3min)}$ between control and glycogen depletion conditions. Figure 8 depicts $\Delta \dot{V}O_{2\ (10\text{-}3min)}$ for the control condition versus the glycogen depletion condition. Six of the eight subjects have a $\Delta \dot{V}O_2$ control versus glycogen depletion that fell below the line of identity. Figure 9 depicts A_3 for the control versus the glycogen depletion condition. Similar to $\dot{V}O_{2(10\text{-}3min)}$, six of the eight subjects have an A_3 value that falls below the line of identity.

The amplitude of the $\dot{V}O_2$ slow component, expressed as the amplitude of the third component (A_3) was not correlated to the percentage of type I muscle fibres in either

the control ($r = 0.7$, $P>0.05$) or glycogen depletion condition ($r = 0.5$, $P > 0.05$), nor was A_3 correlated to the percentage of type II muscle fibres in either control ($r = 0.6$, $P>0.05$) or glycogen depletion ($r = 0.7$, $P>0.05$) conditions. Further, when the slow component is expressed as a relative contribution to the overall increase in $\dot{V}O_2$ ($A_3/A_1+A_2+A_3$) it was not correlated to the percentage type I or type II muscle fibres either (type I; CON $r = 0.7$, GD $r = 0.5$; type II, CON $r = 0.2$, GD $r = 0.5$, $P>0.05$).

Table 1. Subject physical characteristics and $\dot{V}O_{2max}$ for maximal ramp exercise test

Subject	Age (yr)	Height (cm)	Body mass (kg)	$\dot{V}O_2$ at T_{VE} ($L \cdot min^{-1}$)	$\dot{V}O_{2max}$ ($ml \cdot kg^{-1} \cdot min^{-1}$)	$\dot{V}O_{2max}$ ($L \cdot min$)
3697	23	181	101.5	2.40	43.4	4.41
3756	24	180	81.2	1.95	51.9	4.21
3832	20	175	69.0	2.50	60.9	4.20
3835	19	185	68.2	2.10	55.7	3.80
3868	22	191	79.5	3.40	55.0	4.37
3881	25	188	81.2	3.00	57.4	4.66
3884	23	180	81.2	2.20	48.6	3.95
3899	23	188	77.3	1.85	40.0	3.09
Mean (± SD)	22.4 (2)	184 (5)	80.0 (10)	2.43 (0.54)	52.0 (7.0)	4.09 (0.48)

T_{VE}; Ventilatory threshold; $\dot{V}O_{2max}$, maximal O_2 uptake

Table 2. Individual WR and $\dot{V}O_2$ responses for constant-load exercise below T_{VE}

Subject	Moderate Intensity Exercise		
	WR (W)	$\dot{V}O_2 < T_{VE}$ ($ml \cdot min^{-1}$)	% $\dot{V}O_{2max}$ (%)
3697	124	2048	46.5
3756	70	1518	36.0
3832	121	2031	48.0
3835	85	1565	42.0
3868	165	2467	56.0
3881	160	2494	54.0
3884	97	1581	40.0
3899	78	1446	47.0
Mean	112.5	1894	46.2
(± SD)	(36.3)	(428)	(6.8)

WR, work rate, $\dot{V}O_2 < T_{VE}$ determined as a 30 s average 3 min after the onset of exercise.

Table 3. Individual WR and $\dot{V}O_2$ responses for constant-load exercise above T_{VE}.

Subject	Heavy Intensity Exercise		
	WR (W)	$\dot{V}O_2 > T_{VE}$ (ml/min)	%$\dot{V}O_{2max}$ (%)
3697	266	3708	84.2
3756	220	3261	77.4
3832	280	3739	89.0
3835	185	2692	71.0
3868	300	3928	89.8
3881	280	3962	85.0
3884	180	2623	66.5
3899	164	2407	77.8
Mean	234.4	3290	80.1
(± SD)	(53.5)	(634.4)	(8.4)

WR, work rate; $\dot{V}O_2 > T_{VE}$ determined as a 30 s mean 3 min after the onset of exercise to avoid a significant slow component.

Table 4. Total muscle glycogen concentration at rest and after the glycogen depletion protocol

Subject	Rest (g glycogen/ 100 g tissue)	Following GD protocol (g glycogen/ 100 g tissue)
3697	1.02	0.20
3832	0.90	0.19
3835	0.86	0.32
3868	1.84	0.08
3881	1.98	0.65
3884	0.80	0.74
3899	1.15	0.72
Mean ± SD	1.21* (0.49)	0.42* (0.29)

GD, glycogen depletion
* Denotes significant difference between rest and after the glycogen depletion protocol.

Table 5. Loadless cycling HR, RER, $\dot{V}CO_2$ and $\dot{V}O_2$ for both control and glycogen depletion conditions.

	Loadless Cycling	
Variable	**CON**	**GD**
HR (bpm)	91 ± 12	97 ± 13
RER	0.88 ± 0.04	0.78 ± 0.04 *
$\dot{V}CO_2$ (ml/min)	697 ± 75	643 ± 74
$\dot{V}O_2$ (ml/min)	791 ± 83	873 ± 108

Values are mean ± SD. HR, heart rate; V_E, ventilation; $\dot{V}CO_2$, CO_2 production; $\dot{V}O_2$, O_2 uptake.
Loadless cycling is equivalent to 15 W.
* Denotes significant difference between control and glycogen depletion conditions ($P < 0.001$)

Table 6. Respiratory Exchange Ratio (RER) during moderate and heavy intensity exercise for both control and glycogen depletion conditions

Subject	Moderate Intensity Exercise			
	Loadless Cycling		End-Exercise	
	CON	GD	CON	GD
3697	0.89	0.79	0.91	0.94
3756	0.84	0.76	0.83	0.79
3832	0.87	0.77	0.89	0.87
3835	0.89	0.76	0.90	0.82
3868	0.93	0.84	0.91	0.88
3881	0.87	0.77	0.92	0.86
3884	0.93	0.79	0.95	0.86
3899	0.82	0.76	0.88	0.85
Mean (± SD)	0.88 (± 0.04) *	0.78 (± 0.03) *	0.90 (± 0.03)	0.86 (± 0.04)

Subject	Heavy Intensity Exercise			
	Loadless Cycling		End-Exercise	
	CON	GD	CON	GD
3697	0.92	0.84	0.98	0.97
3756	0.84	0.79	0.91	0.91
3832	0.89	0.81	0.96	0.96
3835	0.92	0.81	0.95	0.88
3868	0.93	0.97	0.99	0.97
3881	0.93	0.78	1.01	0.98
3884	0.95	0.84	0.99	0.97
3899	0.82	0.77	0.97	0.92
Mean (± SD)	0.90 (± 0.05) *	0.83 (± 0.06) *	0.97 (± 0.03)	0.95 (± 0.04)

CON, control condition; GD, glycogen depletion condition;
* Denotes significant difference between conditions ($P < 0.001$)

Table 7. Summary of parameter estimates of the model fit for VO_2 response during moderate intensity exercise below T_{VE} and during heavy intensity exercise, above T_{VE} for control and glycogen depletion conditions.

Moderate Intensity Exercise		
Parameter	**CON**	**GD**
A_0, ml/min	790.4 ± 81.1	835.9 ± 114.9
A_1, ml/min	423.4 ± 184.9	326.5 ± 208.7
A_2, ml/min	682.4 ± 301.5	771.5 ± 234.4
TD_1, s	4.1 ± 4.8	3.8 ± 2.1
TD_2, s	25.6 ± 5.6	19.9 ± 4.8
τ_1, s	10.6 ± 7.2	6.7 ± 7.7
τ_2, s	26.9 ± 19.4	27.3 ± 12.2
TLT	35.6 ± 6.8	35.8 ± 4.7
TA	1068.7 ± 425.1	1102.5 ± 403.6

Heavy Intensity Exercise		
Parameter	**CON**	**GD**
A_0, ml/min	828 ± 109.3	873.8 ± 127.4
A_1, ml/min	778.5 ± 367.3	777.2 ± 346.7
A_2, ml/min	1458.5 ± 417.9	1474.9 ± 580.8
A_3, ml/min	458.5 ± 208.9	394.1 ± 252.6
TD_1, s	3.3 ± 2.8	-1.2 ± 4.8
TD_2, s	18.7 ± 4.6	15.9 ± 4.7
TD_3, s	112.3 ± 32.1	118.2 ± 31.9
τ_1, s	8.7 ± 5.4	16.9 ± 13.5
τ_2, s	24.4 ± 5.3 *	27.8 ± 7.4 *
τ_3, s	152.5 ± 43.8	130.2 ± 39.4
TLT	72.2 ± 15.9	66.5 ± 18.4
TA	2695.5 ± 607.7	2646.2 ± 605.1

Values are mean ± SD. A, amplitude; TD, time delay; τ, time constant; TLT, total lag time; TA, total amplitude.

* Significant difference between control and glycogen depletion conditions ($P < 0.015$)

Table 8. End-exercise V_E, HR and $\dot{V}O_2$ for moderate and heavy intensity exercise for both control and glycogen depletion conditions.

Moderate Intensity Exercise		
Variable	**CON**	**GD**
V_E (L/min)	41 ± 9.7	40 ± 9.5
HR (bpm)	122 ± 13	127 ± 15
$\dot{V}O_2$ (ml/min)	1831 ± 524	1872 ± 538
$\dot{V}CO_2$ (ml/min)	1722 ± 412	1629 ± 410

Heavy Intensity Exercise		
Variable	**CON**	**GD**
V_E (L/min)	98 ± 25	102 ± 27
HR (bpm)	170 ± 13	173 ± 15
$\dot{V}O_2$ (ml/min)	3442 ± 656	3485 ± 614
$\dot{V}CO_2$ (ml/min)	3397 ± 697	3325 ± 680

Values are mean ± SD. V_E, ventilation; HR, heart rate; $\dot{V}O_2$, O_2 uptake; $\dot{V}CO_2$, CO_2 production.

Table 9. Cycling Efficiency; $\Delta \dot{V}O_2 / \Delta WR$

Subject	Moderate Intensity Exercise; $\Delta \dot{V}O_{2(EE)}/\Delta WR$	
	CON ($ml \cdot min^{-1} \cdot W^{-1}$)	GD ($ml \cdot min^{-1} \cdot W^{-1}$)
3697	11.9	11.7
3756	10.0	11.4
3832	11.6	11.5
3835	12.0	11.5
3868	10.3	10.9
3881	11.4	11.7
3884	12.0	10.7
3899	10.7	11.7
Mean (± SD)	11.2 (± 0.8) *	11.4 (± 0.4)

Subject	Heavy Intensity Exercise			
	$\Delta \dot{V}O_{2(EE)}/\Delta WR$ ($ml \cdot min^{-1} \cdot W^{-1}$)		$\Delta \dot{V}O_{2(Phase\ II)}/\Delta WR$ ($ml \cdot min^{-1} \cdot W^{-1}$)	
	CON	GD	CON	GD
3697	12.5	11.8	11.8	11.2
3756	12.4	12.3	11.7	11.2
3832	12.6	11.9	11.2	11.1
3835	12.3	12.1	11.6	11.4
3868	11.1	10.6	10.5	10.5
3881	12.2	12.9	11.4	11.7
3884	12.4	12.4	11.6	12.3
3899	12.4	12.9	10.9	11.3
Mean (±SD)	12.2 (± 0.5) *	12.1 (± 0.7)	11.3 (± 0.5)	11.3 (± 0.5)

CON, control condition; GD, glycogen depletion condition

$\Delta \dot{V}O_{2(EE)} / \Delta$ WR; (End exercise $\dot{V}O_2$ – baseline $\dot{V}O_2$) / (WR of constant load exercise - 15 W)

$\Delta \dot{V}O_{2(Phase\ II)}/\Delta WR$; ($\dot{V}O_2$ @ end of phase II –baseline $\dot{V}O_2$) / (WR of constant load-exercise-15W)

* Denotes significant difference between moderate and heavy intensity exercise

Table 10. $\dot{V}O_2$ Slow Component (end exercise $\dot{V}O_2$ – min 3 of exercise) for heavy intensity exercise

	CON (ml/min)		GD (ml/min)	
Subject	**$\Delta \dot{V}O_2$**	**A_3**	**$\Delta \dot{V}O_2$**	**A_3**
3697	217	309	148	253
3756	236	451	182	271
3832	399	700	275	533
3835	142	278	148	259
3868	193	373	49	221
3881	237	833	379	971
3884	129	249	31	307
3899	266	476	244	336
Mean	228	459	182	394
(± SD)	(± 84)	(± 209)	(± 116)	(± 253)
	*	*	ϕ	ϕ

CON, control condition; GD, glycogen depletion condition
A_3, amplitude of the third component of the model fit.
* Denotes $\dot{V}O_2$ significant difference for A_3 in control and ϕ denotes $\dot{V}O_2$ significant difference for A_3 in the glycogen depletion condition

Table 11. Δ [La $^{-}$] (end exercise [La $^{-}$] – min 3 of exercise) for heavy intensity exercise

Subject	CON (mmol/l)	GD (mmol/l)
3697	3.2	2.1
3835	2.9	3.0
3881	--	9.5
3884	1.3	1.2
3899	5.4	4.6
Mean (± SD)	3.2 (± 1.7)	4.1 (± 3.3)

CON, control condition; GD, glycogen depletion condition.

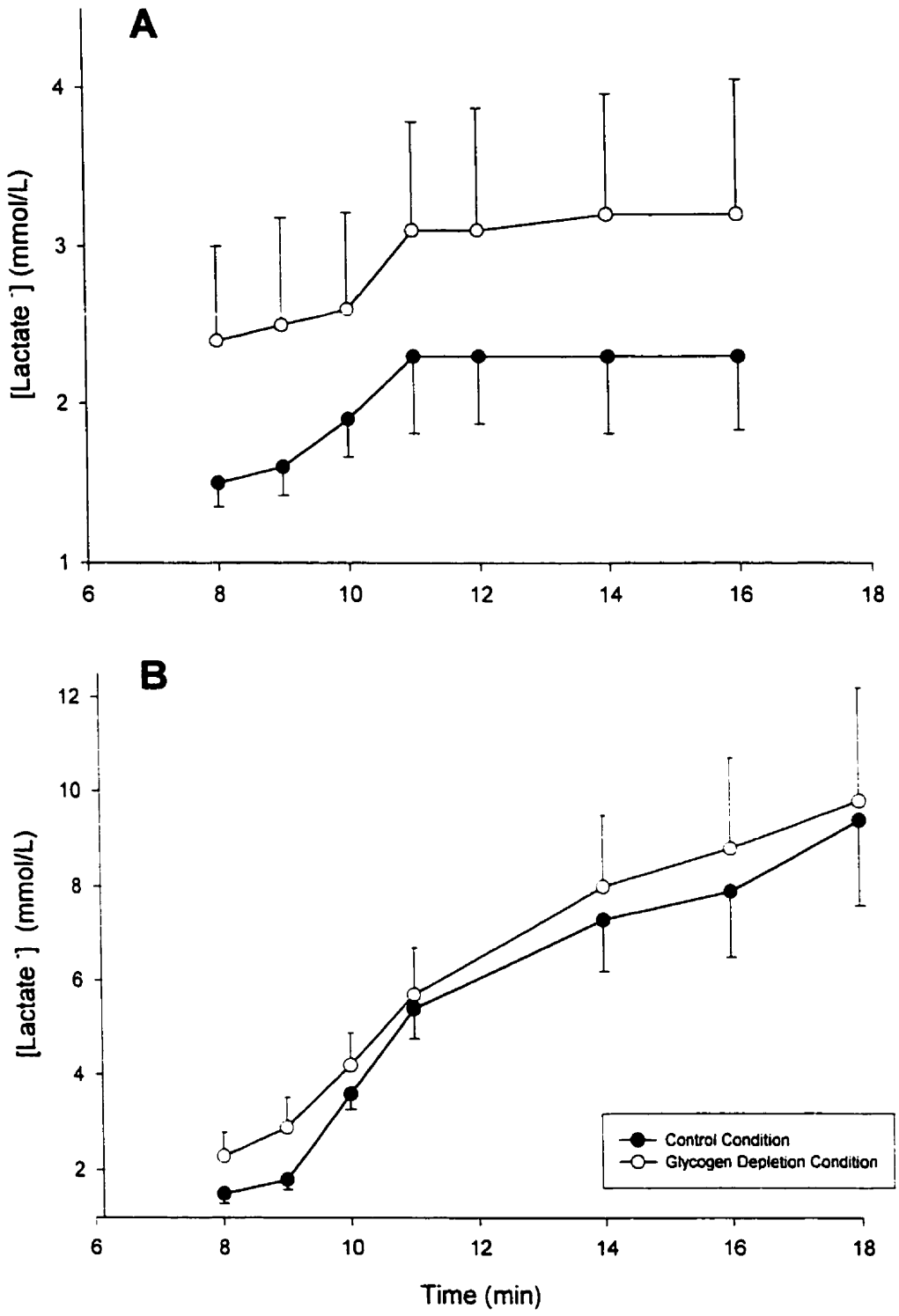


Figure 2. Group response, mean (SE) for plasma lactate concentration ([lactate$^-$]) during moderate (A) and heavy (B) intensity exercise for both control and glycogen depletion conditions. Exercise onset is at minute 8.

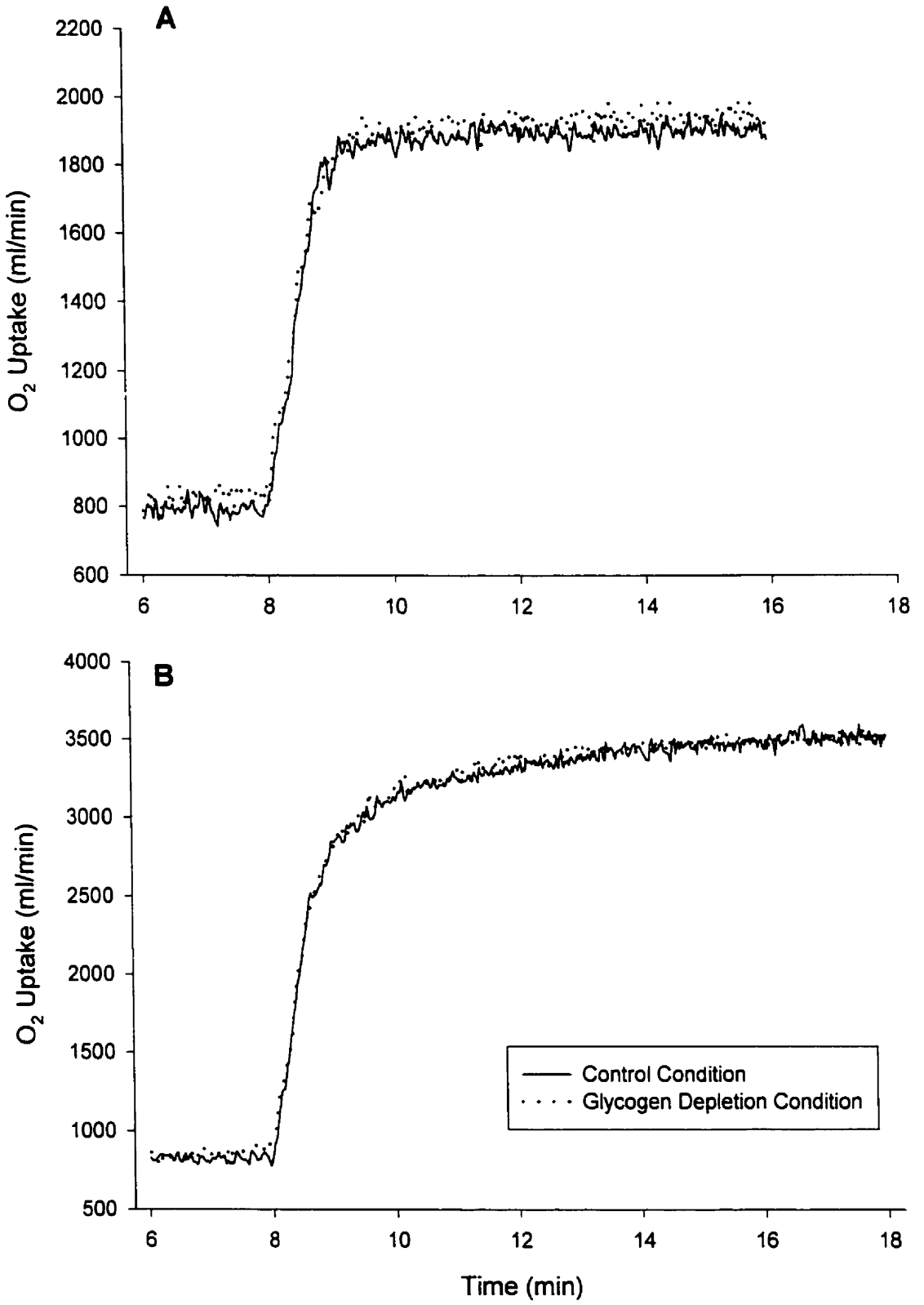


Figure 3. Group mean O_2 uptake on-transient response during moderate (A) and heavy (B) intensity exercise for control and glycogen depletion conditions.

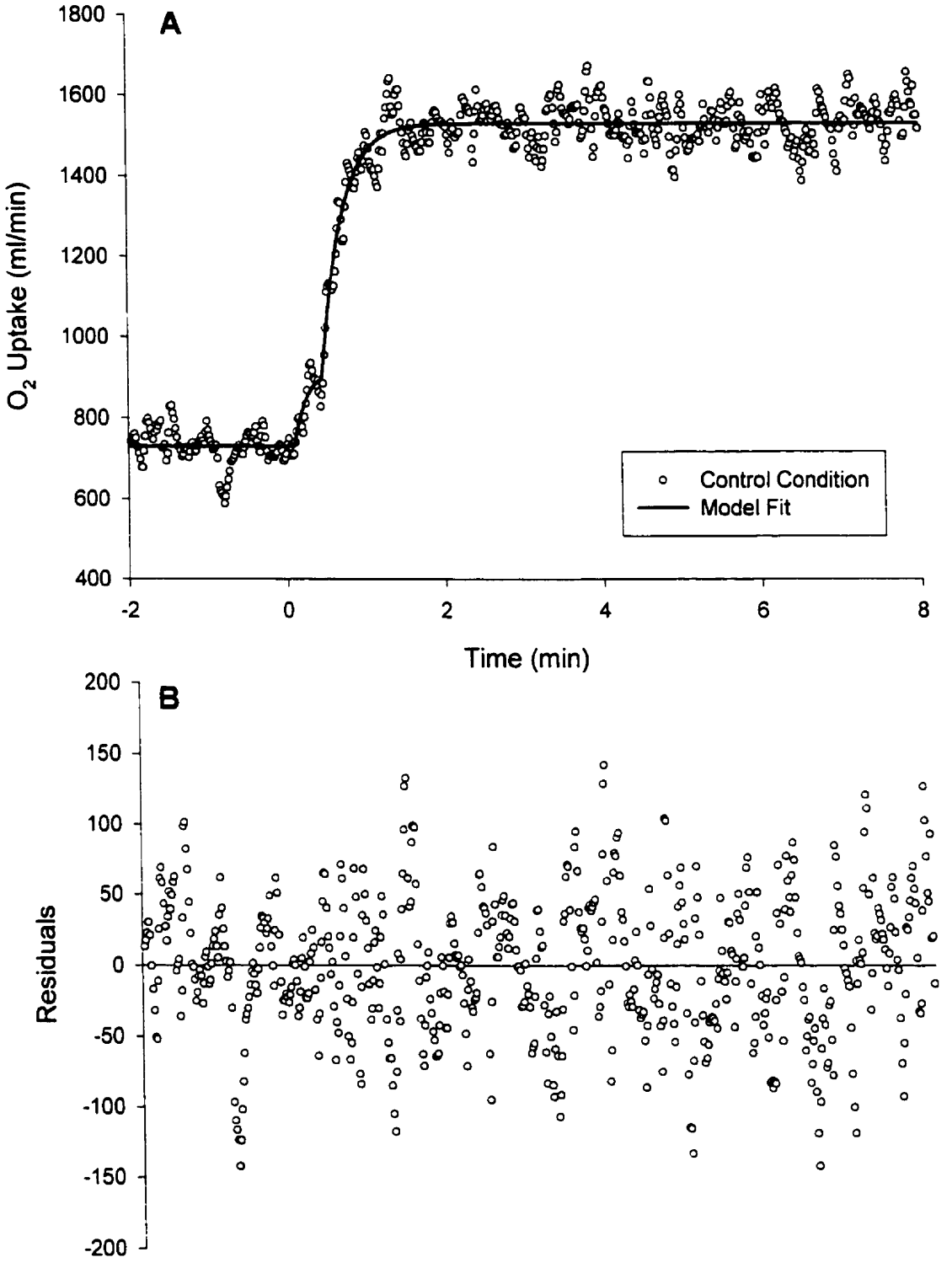


Figure 4. VO_2 on-transient for a single subject during moderate intensity exercise for the control condition (A). Dotted line is the breath-by-breath data; solid line is the line of best fit.
Panel B is the plot of the residuals.

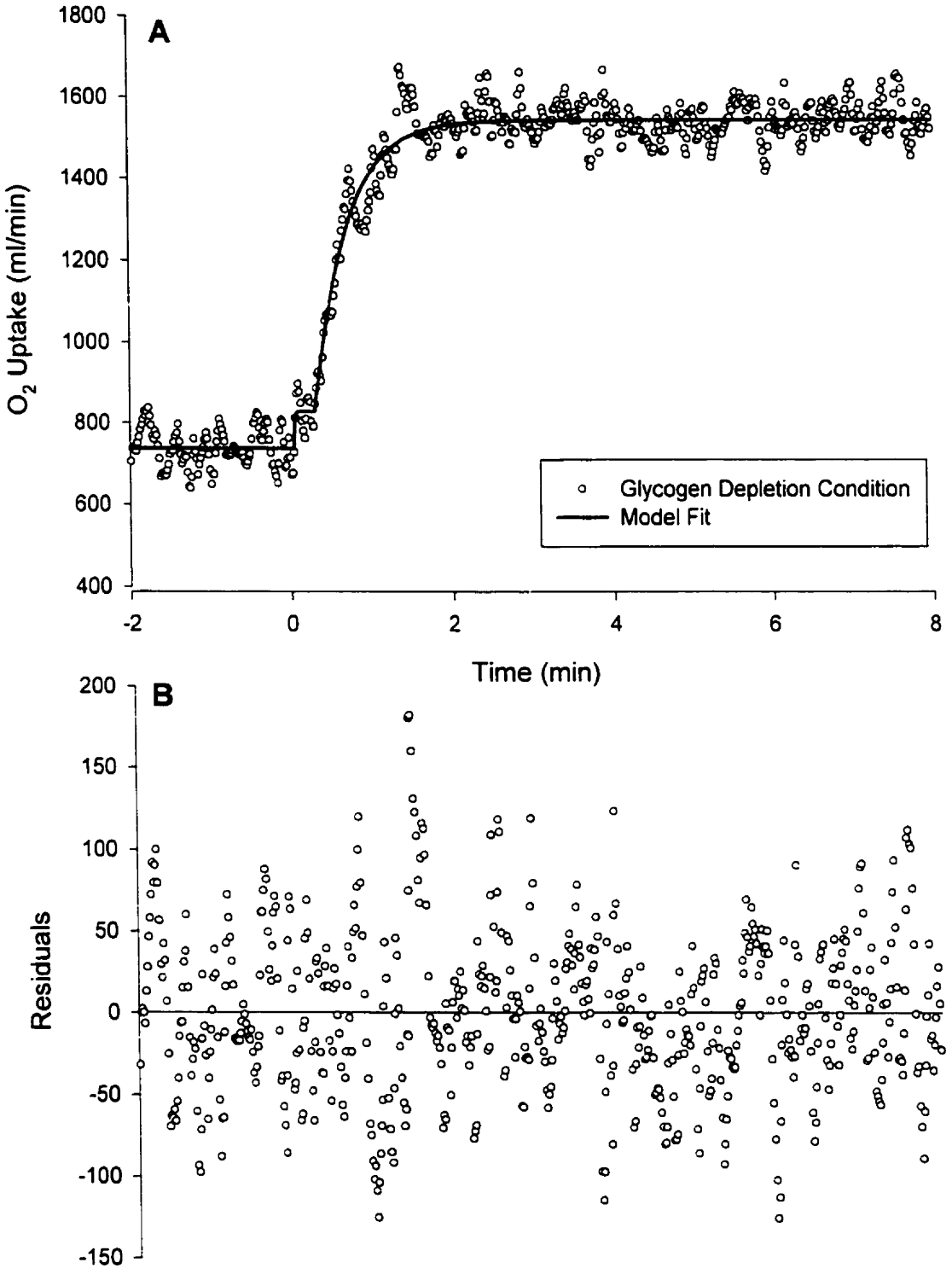


Figure 5. VO_2 on-transient for a single subject during moderate intensity exercise for the glycogen depletion condition (A). Dotted line is the breath-by-breath data; solid line is the line of best fit. Panel B is the plot of the residuals.

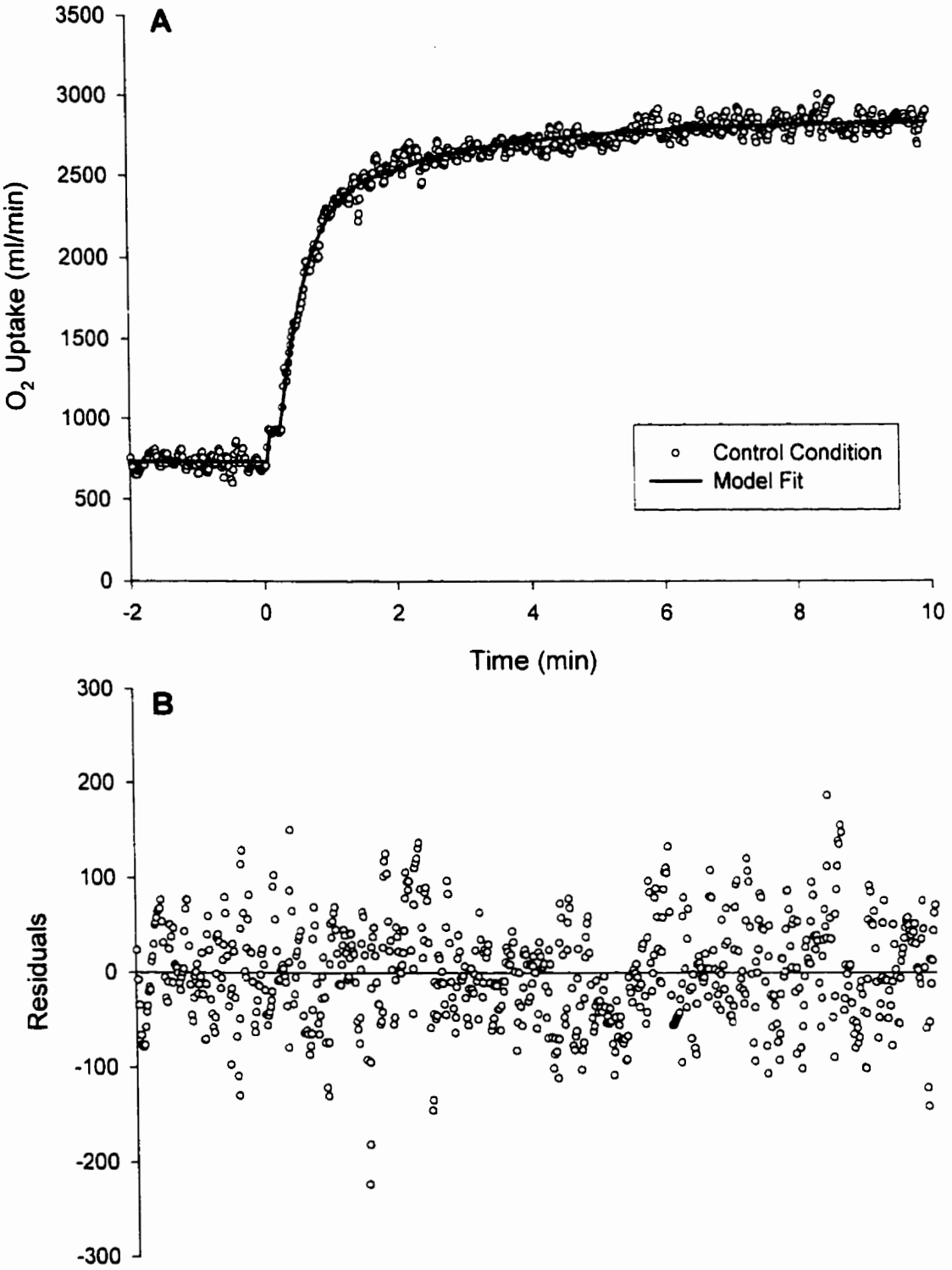


Figure 6 . VO_2 on-transient for a single subject during heavy intensity exercise for the control condition (panel A). Dotted line is the breath-by-breath data; solid line is the line of best fit.
Panel B is a plot of the residuals.

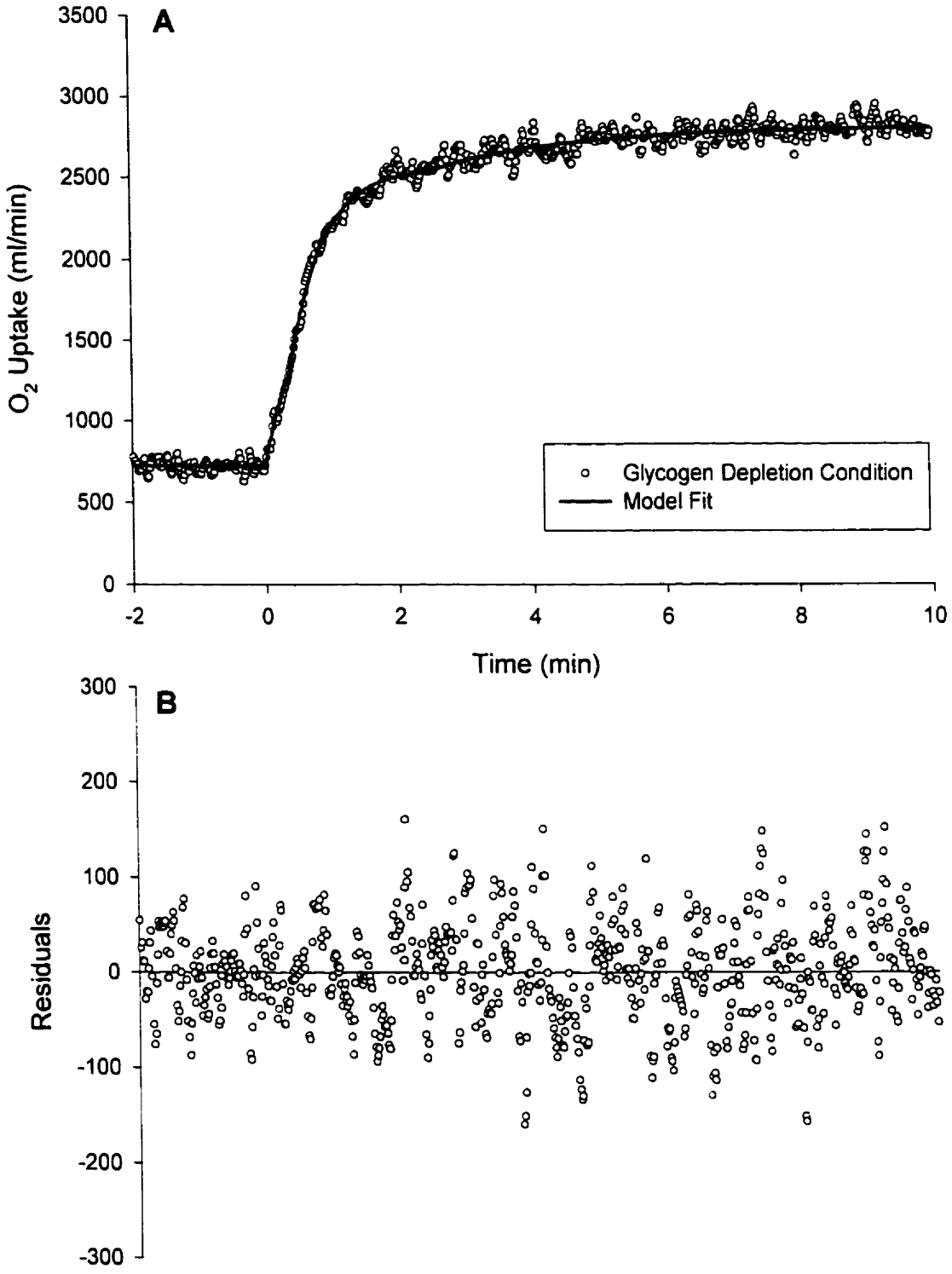


Figure 7. VO_2 on-transient for a single subject during heavy intensity exercise for the glycogen depletion condition (A). Dotted line is the breath-by-breath data; solid line is the line of best fit. Panel B is a plot of the residuals.

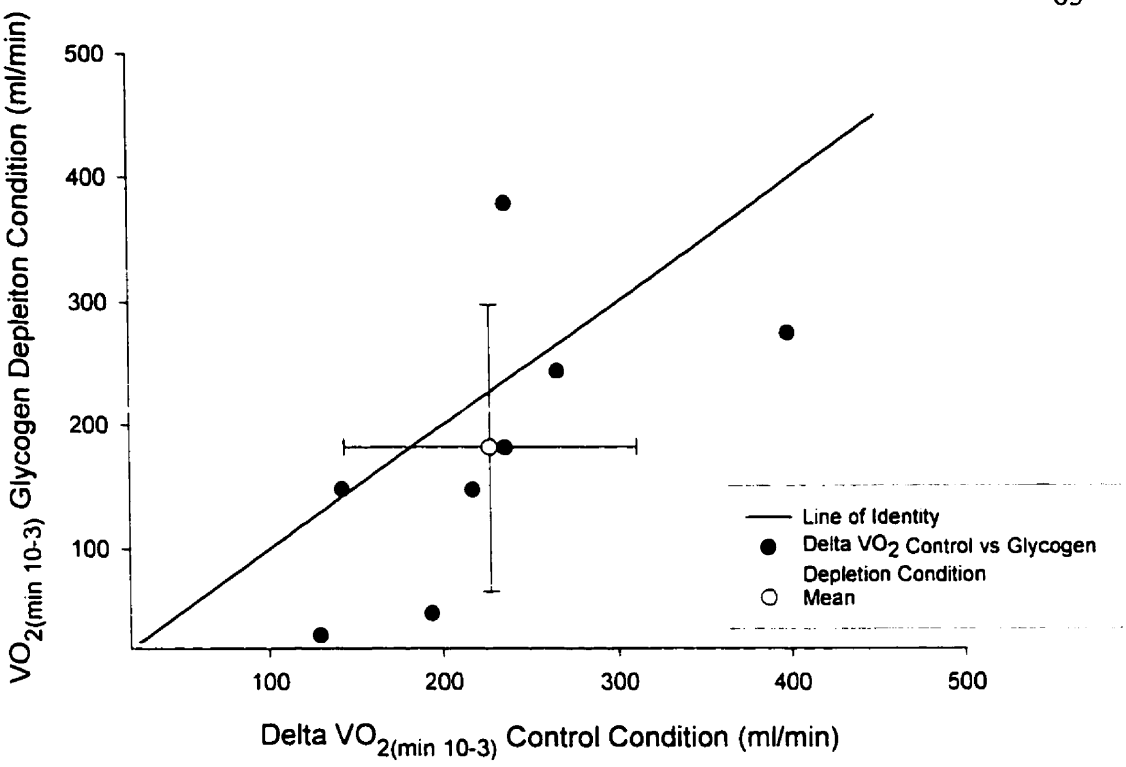


Figure 8. Delta VO_2 (End Exercise VO_2 - VO_2 at min 3) for control condition versus glycogen depletion condition.

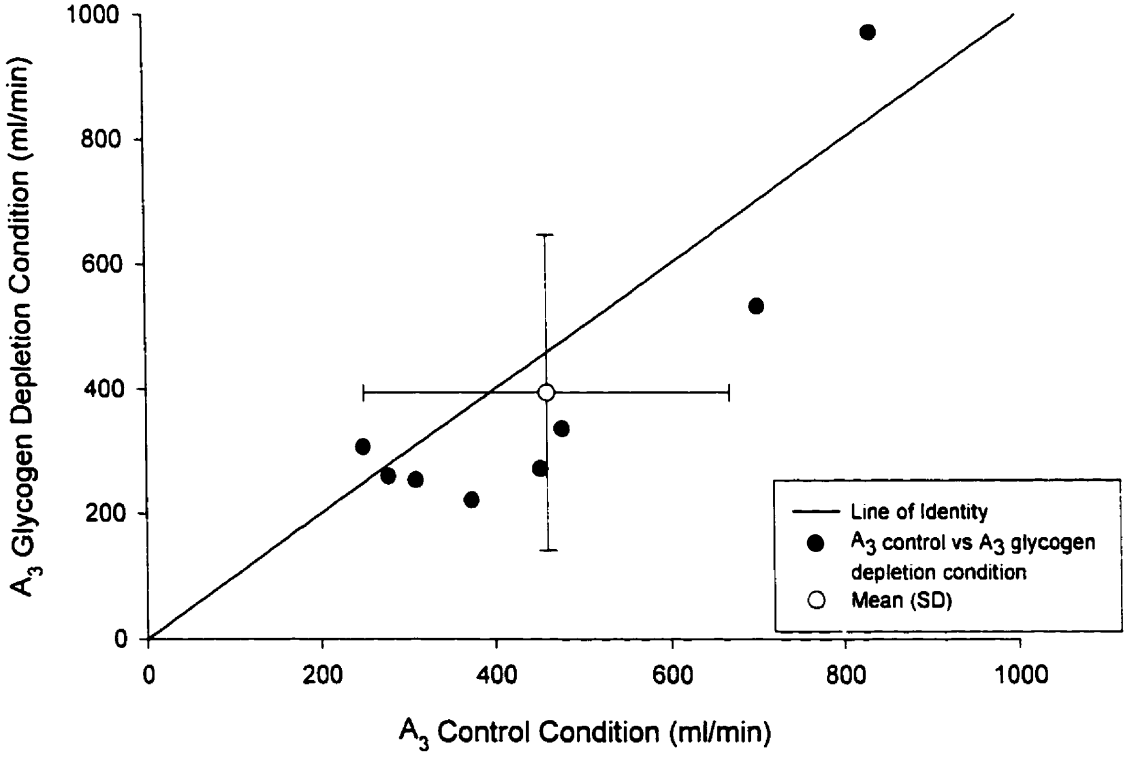


Figure 9. Amplitude of the third component of the model fit (A_3) for control versus glycogen depletion condition.

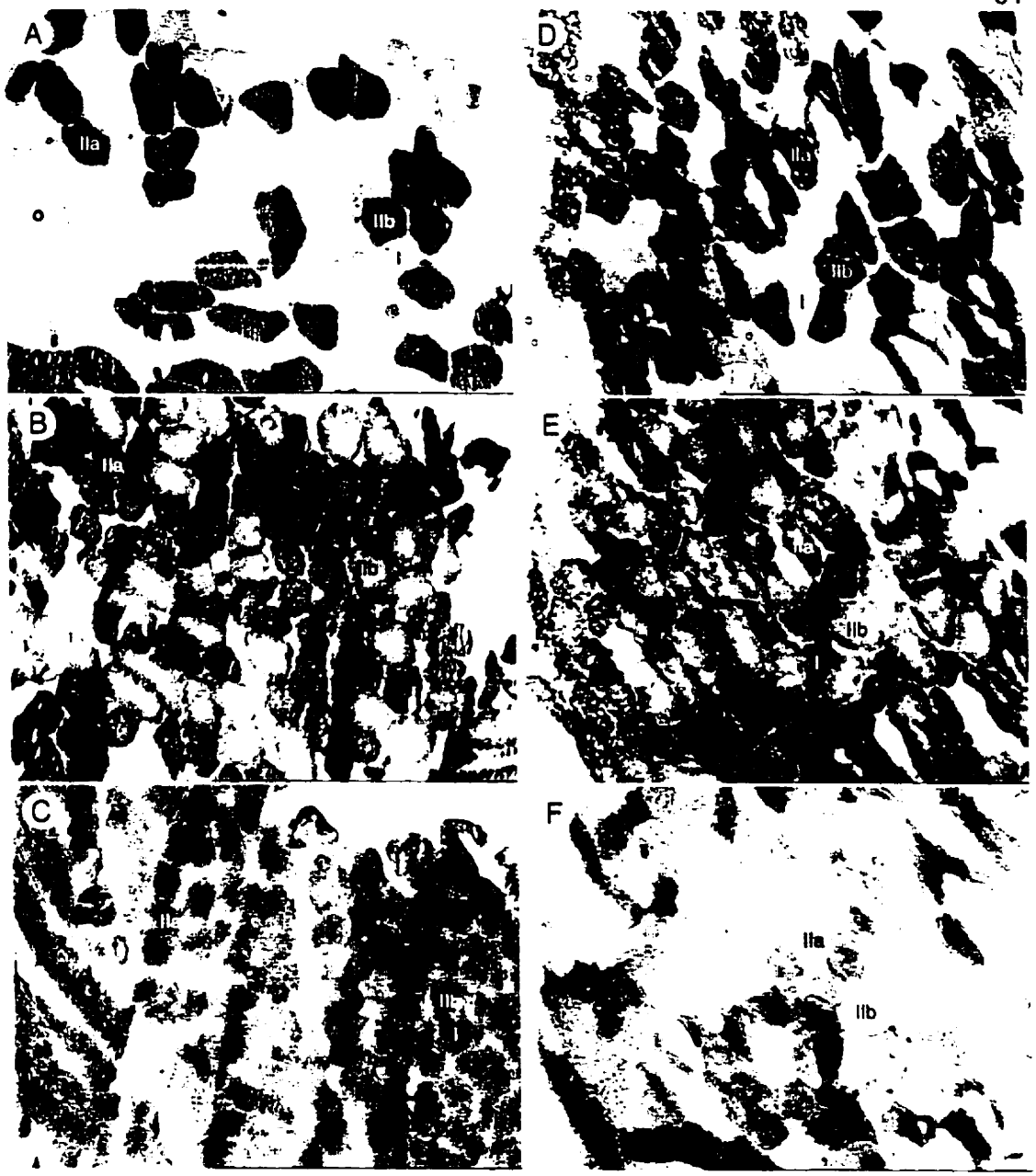


Plate 1. Photomicrographs of vastus lateralis muscle samples at rest (A,B,C) and after 10 1-minute supramaximal exercise bouts (D,E,F) from one representative subject. Serial sections are stained for myosin ATPase activity (A,D), NADH activity (B,E) and glycogen (C,F) with the PAS reaction.

A single type I, IIa and IIb muscle fibre are identified in each figure. At rest (C) both the type II fibres and type I fibres are stained dark for glycogen. After glycogen depletion exercise (F), the type IIb fibres exhibit the greatest glycogen depletion.

Chapter V

Discussion

The current study was undertaken to investigate the role of the contribution of type II muscle fibre recruitment to the $\dot{V}O_2$ slow component. The results of the present study support previous work (e.g. Paterson & Whipp, 1991) in that during heavy intensity exercise, above $T_{\dot{V}E}$, a slow component rise in $\dot{V}O_2$ becomes evident. Further, plasma lactate concentration also increased throughout heavy intensity exercise, which also supported previous research (Roston et al., 1987). However, the results did not support the hypothesis that the magnitude of the $\dot{V}O_2$ slow component would be attenuated following glycogen depletion. Contrary to the hypothesis, there were no changes in the magnitude of the $\dot{V}O_2$ slow component following glycogen depletion. Further, in contrast to the hypothesized speeding of the primary component of $\dot{V}O_2$ kinetics (i.e. phase II) following glycogen depletion, during the on-transient of heavy-intensity exercise, there was a slowing of the phase II $\dot{V}O_2$ kinetics compared to the control condition, as indicated by a larger τ_2 value.

Glycogen Depletion:

The glycogen depletion protocol utilized in this study resulted in a mean muscle glycogen depletion of ~65%. Further, the histochemical analysis showed the type IIb fibres were PAS negative after the glycogen depletion protocol with little change in PAS staining in type I fibres and intermediate staining in type IIa fibres. Thus, the glycogen depletion protocol was effective in reducing overall glycogen levels and selectively reducing glycogen in type II muscle fibres with a greater glycogen depletion in type IIb

than type IIa muscle fibres. These results are in agreement with those of Thompson et al. (1979), who reported a 52% overall depletion of glycogen after 10 supramaximal bouts. The results of the present study are also in agreement with those of Gollnick et al. (1973, 1974) and Vollestad & Blom (1985), in that the supramaximal protocol was effective in reducing the glycogen levels in the type II muscle fibres, evident through a negative PAS stain following the bouts of exercise. Thus, it appears that the glycogen depletion protocol was effective in achieving the goal of reducing glycogen levels in the type II muscle fibres and specifically the type IIb fibres.

Steady State $\dot{V}O_2$

The respiratory exchange ratio (RER) was significantly lower during the steady state of loadless cycling prior to both moderate and heavy intensity exercise in the glycogen depletion condition. This suggested that following the glycogen depletion protocol, there was a shift in substrate utilization with fat oxidation assuming a more important role for energy provision. This is concurrent with the fact that the glycogen depletion protocol significantly lowered mean glycogen levels.

Although there were changes in RER, there were no differences in $\dot{V}O_2$, $\dot{V}CO_2$, and HR in loadless cycling, prior to the onset of exercise between the glycogen depletion and control condition.

$\dot{V}O_2$ on-transient:

There were no differences in the on-kinetics of $\dot{V}O_2$, specifically, the primary phase II component (τ_2), between moderate and heavy intensity exercise. This is in agreement with the findings of Barstow and colleagues (Barstow et al., 1993; Barstow & Mole, 1991). In contrast, both Scheuermann et al. (1998) and Paterson & Whipp (1991)

found a longer τ_2 value for heavy compared to moderate intensity exercise. These differences in the τ_2 values between moderate and heavy intensity exercise may depend on the intensity of the exercise. In the studies by Scheuermann et al. (1998) and Paterson & Whipp (1991), the intensity utilized was Δ 50, while in the present study an intensity of Δ 40 was used. Thus, it could be that the phase II kinetics are slower in heavy compared to moderate intensity exercise at higher exercise intensities.

The cycling efficiency (calculated as end exercise $\dot{V}O_2$ - baseline $\dot{V}O_2$ / Δ WR) was significantly lower during moderate compared to heavy intensity exercise in both the control and glycogen depletion conditions; (11.2 ± 0.8 ml/min/W vs 12.2 ± 0.5 ml/min/W for $<T_{VE}$ and $>T_{VE}$ exercise respectively in the control condition and 11.4 ± 0.4 ml/min/W vs 12.1 ± 0.7 ml/min/W for $<T_{VE}$ and $>T_{VE}$ exercise respectively in the glycogen depletion condition). However, when cycling efficiency for heavy intensity exercise was estimated from the projected steady state of the phase II $\dot{V}O_2$ response (i.e. $\dot{V}O_{2\,(phase\ II)}$- $\dot{V}O_2$ baseline/ΔWR), cycling efficiency was similar between moderate and heavy intensity exercise for both treatment conditions (11.2 ± 0.8 ml/min/W vs 11.3 ± 0.5 ml/min/W for $<T_{VE}$ and $>T_{VE}$ respectively for the control condition). Thus, the decreased cycling efficiency during heavy compared to moderate intensity exercise appears to be due to the appearance of the $\dot{V}O_2$ slow component.

The results of the present study demonstrated that there was no difference in the on-transient kinetics during moderate intensity ($<T_{VE}$) exercise between control and glycogen depletion conditions. However, during heavy intensity ($>T_{VE}$) exercise, there was a small (3.5 s) but significant slowing of the phase II $\dot{V}O_2$ response, (τ_2) following

the glycogen depletion protocol. It was hypothesized that by reducing the glycogen content of the type II muscle fibres the function of the type II fibres would be impaired thus requiring a greater reliance of type I muscle fibres during heavy intensity exercise. Type II muscle fibres have been shown to be less efficient and to have slower kinetics for $\dot{V}O_2$ (Crow & Kushmerick, 1982). Also, Barstow and colleagues (1996) have observed that individuals with a greater percentage of type I muscle fibres had a faster $\dot{V}O_2$ on-response. Finally, Segal and Brooks (1979), observed that there was a greater initial $\dot{V}O_2$ response at the onset of exercise following glycogen depletion, implying that the $\dot{V}O_2$ on-transient kinetics were faster following glycogen depletion. These authors did not quantify this greater response nor did they elaborate as to the significance of this finding. Thus, it was hypothesized that if type II fibre recruitment was impaired, a greater reliance on type I fibres might speed the phase II $\dot{V}O_2$ response at the start of exercise. The present findings did not support this hypothesis.

Although the slowing of τ_2 following glycogen depletion seems small (3.5 s), each subject demonstrated a larger τ_2 value after glycogen depletion (Appendices II.3 and II.4). Further, a 95% confidence interval around the estimate of τ was performed, where the confidence interval refers to a statistical expression for error in estimating τ (Lamarra, 1990). Using τ as the parameter, a 95% confidence interval of $\pm$ 1.8 s was determined, implying that 95 times out of 100, the estimate is within $\pm$ 1.8 s of the true value of τ.

A slowing of the $\dot{V}O_2$ on-response has been shown in studies by Cerretelli and colleagues (Cerretelli et al., 1979). These authors demonstrated a relationship between $\dot{V}O_2$ on-kinetics and early blood lactate accumulation (Cerretelli et al., 1977; Cerretelli et

al., 1979; di Prampero et al., 1989). Cerretelli and coworkers (1979) suggested that a slower $\dot{V}O_2$ on response could be a consequence of increased ATP production through anaerobic glycolysis. Anaerobic glycolysis would be associated with lactate production and increased in muscle and blood [La^-]. Also, the rate of PCr decrease would be attenuated resulting in a slowing of the $\dot{V}O_2$ on-kinetics (Cerretelli et al., 1979; di Prampero et al., 1989). In the present study, although the blood [La^-] appeared to be higher in the glycogen depletion condition than control condition of the present study, there was no difference between conditions. Thus, the slowing of the $\dot{V}O_2$ on-kinetics in this study is not consistent with the early lactate accumulation theory. Furthermore, Scheuermann et al., (1998) showed that carbonic anhydrase inhibition with acetazolamide, a condition known to reduce the plasma [La^-] was not associated with faster $\dot{V}O_2$ on-kinetics (Scheuermann et al., 1998). Thus the early lactate accumulation theory does not agree with the present findings, as there was a slowing of the $\dot{V}O_2$ on-kinetics without any difference in blood [La^-] between control and glycogen depletion conditions.

In contrast to these studies by Cerretelli and colleagues (Cerretelli et al., 1979), Gerbino and colleagues (1996) demonstrated a speeding of the $\dot{V}O_2$ on-kinetics during heavy intensity exercise when the exercise bout was preceded by an initial bout of heavy intensity exercise. The authors suggested that the lactic acidemia present in the muscle prior to the second bout of heavy intensity exercise would enhance muscle blood flow at the start of the second exercise bout and improve O_2 delivery by increasing the O_2 diffusion gradient between the capillary and mitochondria (through enhanced O_2 off-loading from hemoglobin via the Bohr shift), which would accelerate the $\dot{V}O_2$ on-

kinetics. From the present study, it does not seem likely that the slowed τ_2 during heavy intensity exercise in the glycogen deplete state was due to elevated plasma [La^-].

Another explanation for the slowed $\dot{V}O_2$ on-response following the glycogen depletion protocol may be related to substrate availability. During loadless cycling the RER value in the glycogen depletion condition was significantly lower than in the control condition, implying a greater use of fat as a substrate for metabolism. This is in agreement with the 65% lower mean glycogen levels following the glycogen depletion protocol. Thus, the glycogen depletion protocol lowered glycogen concentration enough to influence substrate use in subsequent exercise. The rate of glycogenolysis has been shown to be related to the glycogen concentration in muscle (Hargreaves et al., 1995). Thus, it would be expected that the lowered glycogen concentration with the glycogen depletion condition would be associated with a lower rate of glycogenolysis, and with lower pyruvate (and lactate) accumulation. The lower rate of pyruvate production might slow the activation of the enzyme pyruvate dehydrogenase (PDH) which would subsequently slow the activation of oxidative phosphorylation (Howlett et al., 1999; Putman et al., 1993).

Pyruvate dehydrogenase catalyzes the reaction:

[Pyruvate + NAD^+ + CoA $\rightarrow$ acetyl CoA + NADH + H^+ + CO_2].

The PDH reaction is highly regulated in order to control carbohydrate metabolism. The regulation occurs by way of dephosphorylation and phosphorylation of PDH to active and inactive forms respectively. The dephosphorylated form of PDH is the active form (PDH_a). Phosphorylation occurs through the enzyme PDH kinase, while dephosphorylation occurs via the enzyme PDH phosphatase. PDH_a is activated when

excess acetyl CoA is in the matrix, which indicates an abundance of substrate for the Kreb's cycle (Houston, 1995).

Peters and colleagues (1998) investigated the role of PDH at the onset of exercise through increasing PDH kinase activity, which reduced PDH activation, through manipulation of diet. Subjects consumed a low carbohydrate diet (5% of energy from carbohydrate, 63% from fat and 33% from protein) for 6 days. This diet was successful in reducing the amount of PDH in its active form, which in turn decreased carbohydrate metabolism. Thus, after a low-carbohydrate diet, substrate use shifted to an increased reliance on fat and ketone metabolism. This study demonstrated that skeletal muscle PDH activity can be decreased through low levels of carbohydrate substrate availability. Further, Putman and colleagues (1993), had subjects exercise to exhaustion at 75% $V O_{2max}$ to deplete muscle glycogen levels and then consume either a low or high carbohydrate diet for 3 days; (low carbohydrate diet (LCD): 46% protein, 51% fat and 3% carbohydrate and high carbohydrate diet (HCD): 10% protein, 4% fat and 86% carbohydrate). In the LCD condition, glycogen content was significantly reduced compared to the HCD condition. Both pyruvate and lactate content were lower after a LCD compared to a HCD, and resting muscle acetyl-CoA and acetylcarnitine content were both higher after a LCD than after a HCD. As well, resting PDH activity was significantly lower in the LCD condition. Thus, Putman and colleagues (1993) were successful in causing carbohydrate deprivation, which decreased carbohydrate availability and increased fat utilization. As a result, pyruvate content decreased and PDH activity was reduced.

Several recent studies have shown the importance of PDH activity at the onset of exercise in relating to the O_2 deficit and the rate of oxidative phosphorylation, which could influence the $\dot{V}O_2$ kinetics at the onset of exercise. Dichloroacetate (DCA) infusion has been shown to increase the activation of PDH to its active form by inhibiting PDH kinase (Constantin-Teodosiu et al., 1999; Howlett et al., 1999; Timmons et al., 1998; Timmons et al., 1996). Acetyl CoA and acetylcarnitine concentrations have also been observed to be increased at the onset of exercise after DCA infusion (Howlett et al., 1999). Thus, DCA infusion appears to increase the amount of available substrate for oxidative phosphorylation when exercise begins. An improvement in PDH activation and substrate availability at the onset of exercise has been suggested to improve that rate at which oxidative metabolism of carbohydrate is activated at the start of exercise (Howlett et al., 1999). Timmons and colleagues (1998) suggested that DCA activated PDH in resting skeletal muscle, which maximized the flux of pyruvate through the pyruvate dehydrogenase complex immediately at the onset of exercise, which provides better matching between pyruvate production and oxidation. A faster activation of oxidative phosphorylation this could presumably speed the $\dot{V}O_2$ on-response. Timmons and colleagues (1998) also investigated the role of PDH activation in the O_2 deficit. The authors suggested that the rate of PDH activation appears to be an important determinant of the O_2 deficit, as the O_2 deficit is determined in part by a lag in the onset of oxidative substrate delivery to the Kreb's cycle and thus the O_2 deficit can be reduced by DCA infusion as it increases available substrate levels (Timmons et al., 1998).

Thus, it appears that PDH activation is an important factor in initiating oxidative phosphorylation at the onset of exercise. By increasing both substrate availability and

PDH activation prior to the onset of exercise, PCr degradation is reduced, less glycogen in degraded and there is a smaller O_2 deficit, implying that $\dot{V}O_2$ adapts at a faster rate following the start of exercise. In the present study, glycogen availability was reduced following the glycogen depletion protocol, which would presumably decrease pyruvate content and decrease PDH activity (Putman et al., 1993). If PDH activation and substrate availability can speed the onset of oxidative phosphorylation and $\dot{V}O_2$ kinetics, then perhaps lack of available substrate and reduced activation of PDH could be responsible for the slowed $\dot{V}O_2$ on-kinetics during heavy intensity exercise in the glycogen depletion condition of the current study.

Plasma Lactate:

There were no differences in the plasma [La^-] at any time between control and glycogen depletion conditions during either moderate or heavy intensity exercise. The similar plasma [La^-] between conditions was surprising. Considering the significant degree of glycogen depletion and the lower RER at the onset of both moderate and heavy intensity exercise in the glycogen depletion condition, it was expected that plasma [La^-] would have been reduced during exercise in the glycogen depletion condition. Segal & Brooks (1979) observed that following glycogen depletion, blood lactate concentration was significantly reduced compared to the control condition. One difference between the present study and that of Segal & Brooks (1979) was the timing of the glycogen depletion exercise. In the study by Segal & Brooks the glycogen depletion protocol was performed approximately 12 hours prior to the constant-load exercise tests, while in the present study, only 1 hour elapsed between the glycogen depletion protocol and the constant-load exercise tests. It is likely that in the present study, there was insufficient time for lactate

produced during the 10-1 min bouts of high intensity exercise to be completely metabolized. Thus, the plasma (and muscle) lactate levels in the glycogen depletion condition were probably elevated due to the previous glycogen depletion protocol, and thus in the glycogen depletion condition, the plasma [La^-] and changes in plasma [La^-] would overestimate the amount of glycolysis and muscle lactate production.

$\dot{V}O_2$ Slow Component:

In the present study, no correlation was found between the percentage of type I or type II muscle fibres and the magnitude of the $\dot{V}O_2$ slow component in either the control or glycogen depletion condition. This is contrary to the findings of Barstow and colleagues (1996), who found an inverse relationship between the percentage of type I muscle fibres and the magnitude of the $\dot{V}O_2$ slow component. Possible reasons why this relationship may not have been observed in the present study include a) the number of subjects in whom muscle histochemistry was performed was smaller than in the study by Barstow (n=5 vs n=9), thus reducing the power of this relationship; b) the range of percent type I muscle fibres in this study was smaller (36-48%) compared to that in the study of Barstow and associates (18-67%). Thus, while the correlations were fairly high ($0.5 < r < 0.9$), they were not statistically significant ($P > 0.05$).

Contrary to the hypothesis, the magnitude and time course of the $\dot{V}O_2$ slow component were not influenced by the glycogen depletion protocol. The physiological mechanism responsible for the slow component has received much attention recently. Several theories have been proposed to explain the slow component. A relationship between the magnitude and time course of the slow component and the increase in plasma [La-] has been reported (Roston et al., 1987). It was suggested that the increase

in plasma lactic acidemia would facilitate O_2 off-loading from hemoglobin thus providing more O_2 and allowing $\dot{V}O_2$ to increase (Wasserman, 1994). However, several recent studies have shown that this relationship was merely associative and was not "cause and effect" (Gaesser et al., 1994; Heck et al., 1998; Poole et al., 1991; Scheuermann et al., 1998). In the present study, there was an increase in both plasma [La^-], and $\dot{V}O_2$, with no differences between conditions. Our results do not prove or disprove the lactate theory. However, if as earlier suggested, the lactate values in the glycogen depletion condition were elevated due to the previous supramaximal exercise bouts, then perhaps there was less lactate actually produced in the heavy intensity exercise following glycogen depletion than in the control condition. Thus, even with less lactate produced in the glycogen depletion condition, there was no difference in the slow component between conditions. This result would support the work of Scheuermann et al. (1998).

It has also been suggested that the $\dot{V}O_2$ slow component occurs as a consequence of increased cardiac and ventilatory work during heavy intensity exercise (Gaesser, 1994; Hagberg et al., 1978). It has been suggested that the cardiac and ventilatory work could account for between 14-30% of the slow component (Gaesser & Poole, 1996). In the present study, the glycogen depletion protocol did not have any effect on HR or $\dot{V}_E$ during loadless cycling at end-exercise. Thus, if these factors do contribute to the $\dot{V}O_2$ slow component, their contribution would be similar in both the control and glycogen depletion conditions.

Elevated muscle temperature could contribute to a higher exercise $\dot{V}O_2$ via the Q_{10} effect (Casaburi et al., 1987; Koga et al., 1997). While temperature was not measured in the current study, it is possible that muscle temperature was elevated as a

consequence of the glycogen depletion protocol and remained higher during the constant-load tests. If this was the case, however, it did not have an effect on the $\dot{V}O_2$ during moderate or heavy intensity exercise or on the $\dot{V}O_2$ slow component. Several studies have reported an increase in muscle temperature does not seem to be a cause of the $\dot{V}O_2$ slow component (Casaburi et al., 1987; Koga et al., 1997).

The hypothesis investigated in the current study was that the $\dot{V}O_2$ slow component is related to the recruitment of type II muscle fibres. Type II muscle fibres have been shown to be less efficient (Kushmerick et al., 1992) and to posses slower kinetics of O_2 consumption when activated (Crow & Kushmerick, 1982). It is difficult to manipulate muscle fibre recruitment acutely during exercise. We postulated that by manipulating the substrate level within the muscle fibre (specifically glycogen content), that muscle fibre recruitment would be affected. We hypothesized that by reducing the glycogen content in the type II muscle fibres, that type II fibre activation would be reduced and that the $\dot{V}O_2$ slow component would be attenuated.

The importance of type II muscle fibre recruitment as a mechanism for the $\dot{V}O_2$ slow component has received support in the literature (Barstow, 1994; Barstow et al., 1996; Coyle et al., 1992; Poole, 1994; Poole et al., 1991; Poole et al., 1994a; Whipp, 1994; Willis & Jackman, 1994). Reasons why the recruitment of type II muscle fibres may contribute to the $\dot{V}O_2$ slow component include: a) type II muscle fibres have a longer time constant for the rise in $\dot{V}O_2$ than type I muscle fibres (Crow & Kushmerick, 1982); b) type II muscle fibres are energetically less efficient than type I fibres in that the high energy phosphate produced per oxygen molecule consumed (P/O ratio) is less than

in type I muscle fibres (Barstow et al., 1996; Kushmerick et al., 1992)l; and c) type II muscle fibres may preferentially utilize the α-glycerophosphate shuttle, thus requiring more O_2 to produce the same amount of ATP. However, the results of the present study demonstrate that there was no difference in the magnitude or time course of the $\dot{V}O_2$ slow component between conditions in spite of a 65% reduction in total muscle glycogen content, which was greatest in the type II fibres (type IIb> type IIa> type I). While there was no difference in the magnitude of the $\dot{V}O_2$ slow component between control and glycogen depletion conditions, expressed as either A_3 or $\Delta\dot{V}O_2$ (Figures 8 and 9), there appeared to be a trend towards a smaller magnitude of the slow component after glycogen depletion, with the exception of one subject. Based on the sample in the present study, in order to find a significant relationship between glycogen depletion and the slow component (A_3), 25 subjects would be required. However, if subject 3881 was considered an outlier from the rest of the data, then the trend does become significant. The A_3 value for this subject was greater than ± 2 SD away from the mean of the remaining subjects. If this individual is excluded from the analysis of A_3, then the trend for a decrease in the magnitude of the $\dot{V}O_2$ slow component becomes significant ($P<$ 0.03), thus suggesting that glycogen depletion is associated with a smaller magnitude of the slow component. When the magnitude of the slow component was expressed as $\Delta\dot{V}O_{2(10\text{-}3min)}$, there was no difference between the control and glycogen depletion conditions. In order to establish a significant difference between conditions, based on the present sample, 31 subjects would be required. However, if subject 3881 is removed from the analysis, then the relationship for glycogen depletion and $\Delta\dot{V}O_{2(10\text{-}3min)}$ is also significant ($P< 0.01$), that is, glycogen depletion is associated with a smaller slow

component. However, for this variable, subject 3881 was between ± 1 SD and ± 2 SD away from the mean.

Assuming that glycogen depletion did impair type II muscle fibre recruitment, the findings of the present study suggest that type II fibre recruitment may not be the only factor contributing to the $\dot{V}O_2$ slow component. However, it is also possible that type II fibre recruitment was not affected by substrate depletion, or that the exercise duration was too short for an effect to be seen.

Several other recent studies have also cast doubt on the recruitment of type II muscle fibres as the mechanism for the slow component. Zoladz and colleagues (1995) and Barstow and colleagues (1996) both attempted to manipulate the contribution of the different fibre types to power output through altering cycling cadence during heavy intensity exercise. Both investigations determined that there was no change in the magnitude or onset of the slow component in relation to pedalling rate. Further, Bell and colleagues (1998) looked at the magnitude of the slow component in older adults, who may experience a preferential loss of type II muscle fibres with ageing. The study showed that older adults did have a smaller slow component compared to younger adults, but this appeared to be due to the lower absolute workrates of the older adults. Unfortunately in the study by Bell and colleagues (1998), muscle biopsies were not performed to determine if, in fact, the older adults did have a reduced proportion of type II muscle fibres. The results of all of these studies seem to indicate that the recruitment of type II muscle fibres may not be the mechanism for the $\dot{V}O_2$ slow component.

There is strong evidence that the origin of the majority of the slow component (~86%) is in the exercising muscles (Poole et al., 1991). Thus, the slow component

must be related to some peripheral level mechanism, but from the present study, this does not appear to be related to the recruitment of the type II muscle fibres. It could be that the slow component is not due to just one factor, but that several of the potential factors (i.e. ventilatory and cardiac muscle work, increased muscle temperature, recruitment of type II muscle fibres) all contribute to the $\dot{V}O_2$ slow component. Thus, it is difficult to determine one underlying cause, as the slow component may be a result of several factors acting simultaneously.

In summary, the glycogen depletion protocol resulted in significantly lower glycogen levels, which was most evident in the type II muscle fibres (specifically the type IIb muscle fibres). The glycogen depletion had no effect on the magnitude or time course of the $\dot{V}O_2$ slow component during heavy intensity exercise, but caused a small but significant slowing of the phase II $\dot{V}O_2$ on-kinetics. That the $\dot{V}O_2$ slow component was not effected by type II muscle fibre glycogen depletion suggests that substrate availability and/or type II fibre recruitment may not be related to the development of the slow component. The slower phase II $\dot{V}O_2$ on-kinetics in the glycogen depletion condition may be related to a lack of substrate availability for PDH activation, which slowed the onset of oxidative phosphorylation. Thus, the contribution of the recruitment of type II muscle fibres to the aetiology of the slow component is still unclear.

Chapter VI

Conclusions and Limitations

6.1 Conclusions

The purpose of the present study was to investigate the theory that the $\dot{V}O_2$ slow component is due to the recruitment of lower-efficiency type II muscle fibres. This was examined by investigating the effects of type II muscle fibre glycogen depletion on the $\dot{V}O_2$ slow component. It was hypothesized that glycogen depletion would impair type II muscle fibre recruitment such that the slow component would be attenuated.

The findings of the present study were:

1. The glycogen depletion protocol was effective in depleting overall mean glycogen levels, with glycogen depletion in type IIb fibres > type IIa > type I.
2. There was no difference in the primary phase II $\dot{V}O_2$ on-response (τ_2) for moderate intensity exercise between control and glycogen depletion conditions.
3. The τ_2 of the primary $\dot{V}O_2$ on-response was significantly slower during heavy intensity exercise after glycogen depletion.
4. There was no significant correlation between the percentage of type I or type II muscle fibres and the magnitude of the $\dot{V}O_2$ slow component.
5. There was no difference in the magnitude or time course of the $\dot{V}O_2$ slow component between control and glycogen depletion conditions. However, when one subject, an apparent outlier, was removed from the analysis, there was a significant difference in the magnitude of the $\dot{V}O_2$ slow component (A_3) between the control and glycogen depletion conditions.

6.2 Limitations

There are several factors that may have influenced the present study. The fitness levels and training regimens of the participants may have influenced their $\dot{V}O_2$ response as well as their ability to perform the supramaximal bouts of exercise. Plasma [La^-] was confounded by the prior high intensity bouts of exercise before the constant-load tests. The high plasma [La^-] may not have returned to baseline by the onset of moderate intensity exercise, thus confounding the [La^-] in subsequent exercise. If this experiment was to be repeated, the glycogen depletion protocol should be performed in the evening. Subjects would then return home and return to the laboratory in the morning, without consuming any food, to perform the constant-load exercise tests. In this protocol, [La^-] would have certainly returned to baseline before the constant-load exercise tests. A major limitation of the current study was not knowing whether muscle fibre recruitment was actually effected by glycogen depletion. A possible technique for investigating muscle fibre recruitment in a future study would be to utilize integrated electromyogram (iEMG) during heavy intensity exercise.

APPENDICES

The UNIVERSITY *of* WESTERN ONTARIO

Vice-President (Research)
Ethics Review Board Dental Sciences Building

REVIEW BOARD FOR HEALTH SCIENCES RESEARCH INVOLVING HUMAN SUBJECTS
1998-99 CERTIFICATION OF APPROVAL OF HUMAN RESEARCH

ALL HEALTH SCIENCES RESEARCH INVOLVING HUMAN SUBJECTS AT THE UNIVERSITY OF WESTERN ONTARIO IS CARRIED OUT IN COMPLIANCE WITH THE MEDICAL RESEARCH COUNCIL OF CANADA "GUIDELINES ON RESEARCH INVOLVING HUMAN SUBJECT."

1998-99 REVIEW BOARD MEMBERSHIP

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16) Mrs. R. Yohnicki, Administrative Officer

Alternates are appointed for each member.

THE REVIEW BOARD HAS EXAMINED THE RESEARCH PROJECT ENTITLED:
"The effects of type II muscle fibre glycogen depletion on the slow component of oxygen uptake."

REVIEW NO: 6833

AS SUBMITTED BY: Dr. D.H. Paterson - Kinesiology, Thames Hall

AND CONSIDERS IT TO BE ACCEPTABLE ON ETHICAL GROUNDS FOR RESEARCH INVOLVING HUMAN SUBJECTS UNDER CONDITIONS OF THE UNIVERSITY'S POLICY ON RESEARCH INVOLVING HUMAN SUBJECTS.

APPROVAL DATE: 21 January 1999 (UWO Protocol, Letter of Information & Consent)

AGENCY: NSERC

AGENCY TITLE:

/Bessie Borwein

Bessie Borwein, Chairman

c.c. Hospital Administration

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LETTER OF INFORMATION

The Effects of Type II Muscle Fibre Glycogen Depletion on the Slow Component of Oxygen Uptake

Principal Investigator: Don H. Paterson, PhD.

You are being asked to participate in a research project that is examining the effects of depletion (using up) of your muscle glycogen (the muscle's main fuel during exercise) on exercise responses during exercise of different intensities. Participation involves a visit to the laboratory on 5 different occasions, separated by approximately 1 week. The first visit will take about 30 min to 1 hour, while the other 4 visits will take approximately 5 hours. During this 5 hours, however, there are 2 hours of rest, during which you may read, do homework, etc. You will be required to abstain from exercise and any caffeine-containing food or beverages 24 hours before testing.

The first visit will consist of a progressive exercise test on an exercise bicycle, to voluntary fatigue, where the intensity of exercise will increase until you wish to stop, or until you are unable to continue because of fatigue.

On two of the remaining visits, you will undergo glycogen depletion. On the other two visits, you will not be glycogen deplete, and these trials will act as your control. The glycogen depletion protocol consists of 10 one-minute bouts of cycle ergometer exercise at 130% of your VO_2 max (thus, this is very high intensity exercise). Between each 1-minute bout, you will have 5 minutes of rest. Following the glycogen depletion protocol, you will wait 1 hour, during which time you cannot eat anything, and can consume only water. You are free to read, do homework etc. during this time. After 1-hour recovery, you will be asked to perform a series of exercise tests on the same exercise bicycle. The total exercise time will be about 45 minutes. The test will begin with 8 minutes of loadless pedaling, where there will be no resistance placed on the pedals. The resistance will then be increased, instantaneously to an intensity similar to a casual bike ride and you will continue pedaling for 8 minutes at this workrate. The resistance will then be removed and you will continue pedaling for an additional 8 minutes. The resistance will be increased again, this time to an intensity of a tough bike ride (like riding up a hill) for 10 minutes. Finally the resistance will be removed for a cool down of 8 minutes. After an hour of rest, you will be asked to perform the same series of exercise tests again. This allows us to get a good picture of how your body responds to the exercise.

In the control condition (where you are not glycogen deplete), you will perform the exact same series of exercise tests, but will not undergo the 10 bouts of very high intensity exercise.

Any intensity of exercise carries a slight risk of heart attack, or may be uncomfortable if you are unfit or not used to exercise. There may be some discomfort during the exercise testing. You may experience an increased awareness of breathing, muscle pain and/or fatigue, increased sweating, general feeling of fatigue, nausea. You will be required to wear a small face mask that covers your nose and mouth (this allows us to measure the amount of air you breathe), during the exercise test, which may offer

some initial discomfort. Muscle fatigue and pain may be experienced for a few days after the exercise test.

On one of the test days when you undergo glycogen depletion, you will be required to have a catheter placed into a vein in the back of your hand. This will be done by a qualified individual. There may be some pain when the catheter is placed into your vein (similar to when you get a needle in your arm), after which you should feel no pain or discomfort. The catheter will remain in your vein during the entire test and will be used to sample blood. The volume of blood taken will amount to no more than 24 ml (approximately 1 ounce).

During one of the glycogen depletion visits, a muscle biopsy will be taken from one thigh muscle at 3 different times during the visit, to monitor the changes that occur inside your muscle (including glycogen levels and lactate). A qualified and experienced research scientist (with physician supervision) performs this procedure under a local anaesthetic. It involves the cutting of a small incision, the insertion of a needle into the muscle and the extraction of a small piece of muscle (approximately 50 mg). Slight soreness may occur within the muscle over the next couple of days. This procedure may involve a slight risk of bruising or infection. Keeping the biopsy site clean can usually prevent infection.

You are encouraged to ask questions regarding the purpose of the study and outcome of your exercise test. Participation in the study is voluntary. You may refuse to participate, or withdraw from the test and study at any time without penalty. Records from the studies are confidential and securely stored. The records are listed according to an identification number rather than your name. Published reports resulting from this study will not identify you by name.

If you have any questions regarding the study, please contact Anne Powell (661-1646), or Dr. Don Paterson (661-1606) at the Centre for Activity and Ageing, St. Joseph's Health Annex, Mount St. Joseph.

CONSENT

The Effects of Type II Muscle Fibre Glycogen Depletion on the Slow Component of Oxygen Uptake

Principal Investigator: Don H. Paterson, PhD.

I have read the accompanying "Letter of Information", and have had the nature of the study and the procedures satisfactorily explained to me. All my questions have been answered to my satisfaction.

I agree to participate in this study.

_____ _____

Name (please print) Signature

_____

Date

Appendix II.1 Summary of Individual $\dot{V}O_2$ Kinetic Parameters for Moderate Intensity Exercise during the Control Condition

Parameter	Subjects							
	3697	3756	3832	3835	3868	3881	3884	3899
A_0	699	908	751	730	861	885	729	759
A_1	601	158	432	184	680	457	359	518
A_2	716	426	808	616	925	1159	639	172
TD_1	0.8	7.6	7.4	6.1	-1.3	-3.6	7.8	8.5
TD_2	31	29	20	27	25	16.2	23.4	32.8
Tau_1	17	2.4	2.3	8.6	14.9	14	4.6	21.3
Tau_2	11	13.6	21.2	16	21	21	45.9	67.2
TLT	30.6	33.9	30.3	36.2	31.9	30	44.8	47.3
TG	1317	584	1240	799	1605	1616	700	690

Appendix II.2 Summary of Individual $\dot{V}O_2$ Kinetic Parameters for Moderate Intensity Exercise (Glycogen Depletion Condition)

Parameter	Subject							
	3697	**3756**	**3832**	**3835**	**3868**	**3881**	**3884**	**3899**
A0	773	942	845	737	684	1020	772	914
A1	330	85	549	90.9	549	583	228	197
A2	917	535	639	715	1088	1097	678	503
TD1	4.6	0.7	4.2	2.8	4.7	2.5	7.9	3.0
TD2	27.8	15.8	24	18.5	20.3	16.6	22.9	13.4
Tau 1	1.1	2.9	12.6	0.33	1.3	13.6	1.1	20.4
Tau 2	16.2	25.8	29.9	24.7	17.6	31.3	19.0	54.1
TLT	33.9	36.3	36.8	38.7	27.2	36.8	33.6	43.5
TG	1248	620.2	1188	806	1637	1679	905.8	736

Appendix II.3 Summary of Individual $\dot{V}O_2$ Kinetic Parameters for Heavy Intensity Exercise (Control Condition)

Parameter	Subject							
	3697	3756	3832	3835	3868	3881	3884	3899
A_0	741	978	783	729	940	947	712	790
A_1	1041	1182	874	194	754	1212	510.9	460
A_2	1840	914	1750	1640	2039	1221	1300	964.1
A_3	309	451	700	278	373	833	249	476.1
TD_1	1.6	6.6	6.2	3.8	2.1	3.3	4.5	-2.1
TD_2	22.9	27.6	14.9	14.9	17.4	18.4	19.3	14.5
TD_3	163	85.1	108	119	126	53.6	122.7	121.5
Tau_1	8.9	11.8	6.2	1.2	5.3	14.7	4.4	16.8
Tau_2	25.9	24.2	25.3	28.1	24.2	14.5	20.9	32.5
Tau_3	131	244.3	142.1	157.7	106	151	111.9	175.9
TLT	60.1	85.5	77.1	70.2	55.7	71.2	56.0	102
TG	3190	2547	3324	2111.2	3165	3266	2061	1900

Appendix II.4 Summary of Individual $\dot{V}O_2$ Kinetic Parameters for Heavy Intensity Exercise (Glycogen Depletion Condition)

Parameter	Subject							
	3697	**3756**	**3832**	**3835**	**3868**	**3881**	**3884**	**3899**
A_0	837	1042	847	722.2	786	1019	744	994
A_1	530	235	1394	951	686.9	892	602	927.4
A_2	2168	1884	1243	879.5	2165	1653	1171	636
A_3	253	271	533.4	258.6	221.5	971	307	336.4
TD_1	4.3	-7.5	-2.8	0.1	3.4	-4.1	4.2	-6.9
TD_2	17.1	9.8	9.5	22.0	16.8	14.0	21.4	16.7
TD_3	133	104	112.2	147.7	117.6	67.6	94	170
Tau_1	2.1	7.7	25.8	24.8	3.7	22.4	8.6	40.5
Tau_2	27.1	28.4	31	32.6	25.4	16.4	20.2	40.4
Tau_3	115.8	95.8	139.1	133.5	117.9	192.3	72.3	175.2
TLT	54.8	52.8	68.3	68.6	48.2	90.7	51.6	96.6
TG	2951	2390	3170.6	2089	3073	3517	2080	1900

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