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# The effect of low and high carbohydrate diets on pulmonary oxygen uptake and muscle deoxygenation kinetics during exercise transitions into the heavy-intensity domain

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A thesis submitted in partial fulfillment of the requirements for the Master of Science degree in Physiology

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The effect of low and high carbohydrate diets on pulmonary oxygen uptake and muscle deoxygenation kinetics during exercise transitions into the heavy-intensity domain

(Thesis format: Integrated Article)

by

John Ross Leckie

Graduate Program in Physiology

A thesis submitted in partial fulfillment  
of the requirements for the degree of  
Master of Science

The School of Graduate and Postdoctoral Studies  
The University of Western Ontario  
London, Ontario, Canada  
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## ABSTRACT

The purpose of this study was to determine the effects of a high carbohydrate (HCHO) and low carbohydrate (LCHO) diet on the adjustment of pulmonary oxygen ( $O_2$ ) uptake ( $\dot{V}O_{2p}$ ) and muscle deoxygenation ([HHb]) during transitions to heavy intensity exercise. Young, healthy, male subjects aged (24 $\pm$ 3) underwent a four day LCHO diet followed immediately by a four day HCHO diet, with each diet preceded by a glycogen depletion protocol. This protocol was designed to alter pyruvate dehydrogenase (PDH) activity. Subjects completed three step transitions to a work rate corresponding to 35% of the difference between their lactate threshold and  $\dot{V}O_{2p\ peak}$  for each condition. On day three of the diets gas exchange measurements were collected using mass spectrometry and [HHb] measures were recorded using near-infrared spectroscopy (NIRS). On day four of the diets muscle biopsies were taken from the vastus lateralis muscle of the quadriceps muscle group at steady-state baseline exercise (20 W) and at 15 s and 360 s during the transition to heavy exercise. These biopsies were frozen for later analysis of: PDH activation, ADP concentration, glycogen content, and phosphocreatine concentration. It was found that the dietary manipulation had a significant effect ( $p < 0.05$ ) on phase II  $\dot{V}O_{2p}$  time constant (LCHO=41.9 s, HCHO=33.7 s) and no effect on the rate of adjustment of [HHb] ( $p > 0.05$ ). This study posits that PDH plays an important role in the adjustment of oxidative metabolism to exercise.

### Keywords:

$O_2$  uptake kinetics; near-infrared spectroscopy; metabolism; dietary intervention

### **CO-AUTHORSHIP STATEMENT**

This study was designed by J.R Leckie and J. M. Kowalchuk with input from the advisory committee (D. H. Paterson). The majority of the data were collected and analyzed by J. R.

Leckie with the assistance of J.P. Nederveen (T. J. Doherty performed all needle biopsy procedures). L.L Spriet and J. Whitfield analyzed the muscle tissue from the needle biopsy procedures. J.R. Leckie wrote the original manuscript for the study and the co-authors provided financial support, lab support and editorial feedback.

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## LIST OF TERMS AND ABBREVIATIONS

ADP – adenosine diphosphate

Amp – amplitude

ATP – adenosine triphosphate

Bsl – baseline

CP- critical power

CI 95 – 95% confidence interval

DCA – dichloroacetate

$\Delta 35$  – work rate corresponding to 35% of the difference between estimated lactate threshold and  $\dot{V}O_{2\max}$

EPI- epinephrine

ETC – electron transport chain

ECG- electrocardiogram

GD- Glycogen depletion

GP- glycogen phosphorylase

[HHb] – deoxyhemoglobin; measure of muscle deoxygenation

[HHb]<sub>bsl</sub> – baseline muscle deoxygenation

[HHb]<sub>ss</sub> – steady state muscle deoxygenation

HR – heart rate

HSL- hormone sensitive lipase

IMTG- intramuscular triglycerides

$\hat{\theta}_L$  – lactate threshold

MRT – mean response time

NIRS – Near-infrared spectroscopy

NE- norepinephrine

PO<sub>2</sub> – partial pressure of oxygen

PDH – pyruvate dehydrogenase

Pi – inorganic phosphate

$\tau$  – time constant; time required to attain 63% of the response

TCAC – tricarboxylic acid cycle

TD – time delay

$\dot{V} \square \text{CO}_2$  – carbon dioxide output

$\dot{V} \square \text{E}$  – ventilation

$\dot{V} \text{O}_{2 \text{ max}}$  – maximal oxygen uptake

$\dot{V} \text{O}_{2 \text{ m}}$  – muscle oxygen uptake

$\dot{V} \text{O}_{2 \text{ p}}$  – pulmonary oxygen uptake

$\dot{V} \text{O}_{2 \text{ bsl}}$  – baseline pulmonary oxygen uptake

$\dot{V} \text{O}_{2 \text{ pss}}$  – steady-state pulmonary oxygen uptake

WR – work rate

$\text{WR}_{\text{max}}$  – maximal work rate attained during ramp incremental test

## Chapter 1

### 1 Review of the Literature

#### 1.1 What are $\dot{V}O_{2p}$ kinetics?

When transitioning from rest to exercise there is an immediate change in the energy requirements of the muscle cell. Not all of the energy required for this exercise can be met immediately through aerobic metabolism and thus anaerobic metabolism must support ATP requirements as the mitochondrial oxidative phosphorylation system adjusts. The assessment of the rate at which oxygen uptake ( $\dot{V}O_2$ ) in the contracting skeletal muscle ( $\dot{V}O_{2m}$ ) adapts in response to a change in metabolic demand describes  $\dot{V}O_2$  kinetics. A slower adjustment of  $\dot{V}O_{2m}$  at exercise onset limits exercise tolerance as increased reliance on anaerobic metabolism generates a buildup of metabolites linked to fatigue (e.g. lactate, inorganic phosphate (Pi), ADP). Thus, the consequences of 'slow' oxygen kinetics can be a debilitating problem in older and diseased populations as they are more susceptible to an early onset of fatigue when performing regular activities.

Due to the ethical and practical complications that are involved in measuring  $\dot{V}O_{2m}$  directly, pulmonary  $\dot{V}O_2$  ( $\dot{V}O_{2p}$ ) is typically recorded. This measurement was proven to reflect the time course and steady-state adjustments of  $O_2$  utilization in the active muscle by Grassi et al. (23). Recent evidence from Krstrup et al. (40) shows that  $\dot{V}O_{2p}$  reflects  $\dot{V}O_{2m}$  in the moderate and heavy intensity domain. In this experiment  $\dot{V}O_{2m}$  was measured by the direct Fick technique and  $\dot{V}O_{2p}$  was measured through indirect calorimetry. The two measurements were not significantly different ( $p>0.05$ ).

### *Characterizing the $\dot{V}O_2$ response*

The  $\dot{V}O_{2p}$  response to a step change in exercise intensity is commonly broken down into three phases. Phase I is described as the cardiodynamic phase, it reflects the time it takes for the blood that was present in the muscle at the onset of exercise to travel to the lungs (42) and any increase in  $\dot{V}O_2$  in this phase is due to increased pulmonary blood flow (2). Phase II is termed the “fundamental” phase. This phase begins when deoxygenated blood from the active muscle returns to the lungs (2). Phase II is the most studied segment of the  $\dot{V}O_2$  response and reflects the rate of adjustment of muscle  $O_2$  uptake and mitochondrial oxidative phosphorylation as a major source of energy production. The phase II  $\dot{V}O_{2p}$  kinetics are described by the time constant ( $\tau$ ) which represents the time taken to reach 63% of the steady-state increase in the  $\dot{V}O_{2p}$  response; a  $\dot{V}O_{2p}$  “steady-state” is achieved in the time equivalent of  $4\tau$ . A greater  $\tau \dot{V}O_{2p}$  reflects a slower adjustment, while a smaller  $\tau \dot{V}O_{2p}$  is reflective of a faster rate of adjustment of  $\dot{V}O_{2p}$  and  $\dot{V}O_{2m}$ .

The behavior of phase III is dependent upon exercise intensity. At an exercise intensity performed below the lactate threshold ( $\hat{\theta}_L$ ) a steady state of  $\dot{V}O_{2p}$  is reached. In exercise performed above the  $\hat{\theta}_L$ , a  $\dot{V}O_2$  slow component is observed both in the  $\dot{V}O_{2p}$  and the  $\dot{V}O_{2m}$  responses. The  $\hat{\theta}_L$  represents a threshold above which lactate begins to accumulate in active muscle and blood as a result of a mismatch between the rate of pyruvate production in glycolysis and its subsequent oxidation in the muscle mitochondria.

The  $\dot{V}O_2$  slow component entails  $\dot{V}O_2$  rising above levels that would be predicted from the sub-lactate threshold  $\dot{V}O_2$  work rate (WR) relationship. It is suggested that the slow

component is related to the recruitment of less efficient type II muscle fibers at higher exercise intensities and the fatiguing of type I muscle fibers that have already been recruited (5). Type II muscle fibers have a higher ATP cost of force production and a higher O<sub>2</sub> cost of ATP turnover (51). Heavy intensity exercise also leads to a decrease in Gibbs free energy which reduces the energy release from ATP hydrolysis.

### *Exercise Intensity Domains*

Exercise intensity is characterized based on common profiles of physiological and metabolic responses observed through a range of WRs that are separated into domains (14). Each exercise intensity domain presents different physiological challenges to the body. Moderate, heavy, and severe are the most common terms used to describe these domains. The moderate intensity exercise domain represents a range of work rates that can be performed below the  $\hat{\theta}_L$ . Within this domain, blood lactate concentration remains low (< 4 mmol/L) and,  $\dot{V}O_2$  is elevated (relative to rest) but maintains “steady-state” conditions (3). The heavy intensity domain represents a range of intensities between  $\hat{\theta}_L$  and critical power (CP), while intensities between CP and  $\dot{V}O_{2\max}$  are defined as severe-intensity. Exercise within the heavy-intensity domain is associated with an elevated blood lactate and the appearance of a  $\dot{V}O_2$  slow component which delays the achievement of a plateau in the response (27). CP corresponds to an intensity above which a plateau in blood lactate and  $\dot{V}O_2$  cannot be established, and there are large disturbances in metabolic stability within the muscle as seen by decreases in muscle glycogen, and phosphocreatine, and increases in  $ADP_{\text{free}}$ ,  $AMP_{\text{free}}$ ,  $IMP_{\text{free}}$ , inorganic phosphate and  $H^+$ , changes which are associated with reduced exercise tolerance and fatigue.

These exercise intensity domains are defined within the limits imposed by  $\hat{\theta}_L$ , CP, and  $\dot{V}O_{2\max}$  which likely vary amongst individuals; however the response profiles within each domain are consistent amongst individuals. Therefore, exercise “intensities” are often prescribed relative to one of these defined intensity domains.

## 1.2 What limits $\dot{V}O_2$ kinetics?

There is a long standing debate in the literature as to what limits the rate of adjustment of  $\dot{V}O_2$  kinetics. The two main camps that have emerged include those who claim that kinetics are limited by convective and or diffusive  $O_2$  delivery (31) and those who claim that they are limited by a sluggishness of metabolic activation (19). The  $O_2$  delivery hypothesis claims that the metabolic response at the onset of exercise is adequate but  $O_2$  delivery is insufficient. The metabolic hypothesis claims that  $O_2$  is available in sufficient amounts and that the activity of rate limiting enzymes or the availability of substrate in the oxidative phosphorylation process is what limits the rate of adjustment of oxidative phosphorylation.

### *$O_2$ delivery*

Many investigations have been conducted to test whether  $O_2$  is a limiting factor in  $\dot{V}O_2$  kinetics. Most have focussed on blood flow manipulation. Studies using a supine vs. upright model have shown increased  $\tau \dot{V}O_{2p}$  in supine but a restoration of  $\tau \dot{V}O_{2p}$  once negative pressure is applied to restore blood flow (32). Arm exercise performed above the heart also points to an  $O_2$  delivery limitation (33). Exercise above the heart reduces the effects of the muscle pump and the driving pressure for blood flow leading to increased  $\tau \dot{V}O_{2p}$ .

Restriction of O<sub>2</sub> supply via a reduction in arterial O<sub>2</sub> content or pressure is another situation where limitations are observed. Ischemia, hypoxia, and beta blockade have all been experimental models to explore this (17, 30). These investigations all yielded slower  $\dot{V}O_2$  kinetics. However, some O<sub>2</sub> delivery limiting interventions including: blood withdrawal (10), hemodilution (6), and lower body positive pressure (68) have failed to report increases in  $\tau \dot{V}O_2$  despite reductions in O<sub>2</sub> delivery. These studies indicate that if oxygen delivery is compromised then kinetics may suffer but do not indicate an O<sub>2</sub> delivery limitation under conditions of upright exercise in young healthy individuals.

If O<sub>2</sub> were a limiting factor then increasing O<sub>2</sub> delivery should speed kinetics. Investigations have shown mixed results. Grassi et. al. conducted two investigations in 1998 with pump perfused dogs in situ in which they increased O<sub>2</sub> delivery (21) and increased O<sub>2</sub> driving pressure (20). Both of these investigations showed no difference in the adjustment of  $\dot{V}O_{2m}$ . Wilkerson et. al. conducted an investigation in humans exercising while breathing a hyperoxic gas mixture (67). This investigation showed no effect of hyperoxia on phase II  $\tau \dot{V}O_2$  under moderate, heavy, and severe exercise. Macdonald et. al. studied humans exercising in the heavy domain and found a speeding of phase 2  $\tau \dot{V}O_{2p}$  with hyperoxia (44). These investigations point to a potential speeding of kinetics with increased O<sub>2</sub> delivery only in the heavy intensity domain.

### *Metabolic hypothesis*

The most convincing piece of evidence for the metabolic theory is that the kinetics of bulk muscle blood flow are faster than those for  $\dot{V}O_2$  (1, 23). It is hard to imagine a slower process being limited by a faster one. Nitric oxide (NO) also provides compelling evidence for a metabolic inertia and against an O<sub>2</sub> delivery limitation (22). NO inhibits cytochrome c oxidase

activity, an enzyme in the electron transport chain, but increases blood flow. Blockade of NO synthase speeds  $\dot{V}O_2$  kinetics (34, 35).

Dichloroacetate (DCA) is a drug commonly used to activate pyruvate dehydrogenase (PDH) which is a rate limiting enzyme that controls the entry of carbohydrate derived substrate into the tricarboxylic acid cycle (TCAC). Howlett et. al. found that DCA use reduced the contribution of substrate level phosphorylation to energy production during exercise at 65%  $\dot{V}O_{2\max}$  (29). This group also saw a faster drop in intracellular  $PO_2$  and decreased glycogenolysis after 10 minutes of exercise. These results would suggest faster activation of oxidative metabolism when PDH activation is increased. Investigations which measured DCA's effect on  $\dot{V}O_2$  kinetics have not shown a speeding of  $\tau \dot{V}O_2$  in the moderate (37), or heavy intensity domain (55).

Priming models have been used as an alternative method to increase PDH activation (24). Priming involves the performance of a prior exercise bout before the bout that is being assessed. The issue with this model is that priming exercise increases PDH activation and increases muscle blood flow, this suggests that microvascular perfusion is elevated following priming exercise. This confounds the ability to determine whether metabolic inertia or  $O_2$  delivery is the limiting factor in the increase in oxidative phosphorylation. Spencer et. al. attempted to resolve this issue by adding hypoxia to the experiment (58). This negated increases in  $O_2$  delivery that are normally provided by priming exercise. These authors illustrated that in hypoxia after priming exercise speeding of kinetics was abolished. This seems to indicate that  $O_2$  delivery plays a role in the speeding of kinetics following priming exercise. It is important to note that PDH was not directly measured in this experiment and that a more severe hypoxia than that used by Spencer et. al. has been shown to reduce PDH activation at 1 minute following exercise onset (48). Taken

as a whole, the evidence suggests that  $\dot{V}O_2$  kinetics can be limited by a number of factors dependent upon the individual and situation.

### **1.3 Near-infrared Spectroscopy**

Near-infrared spectroscopy (NIRS) provides a measure of the amount of  $O_2$  bound to haemoglobin (Hb) and myoglobin (Mb) in the area of interrogation (i.e. beneath the NIRS probe). In this regard, the relative concentrations of bound (oxygenated) and unbound (deoxygenated) Hb and Mb can be used to monitor phenomena related to  $O_2$  delivery and  $O_2$  extraction at the microvascular level. Specifically, deoxygenation ([HHb]) kinetics can be used as a proxy for microvascular  $PO_2$  (12). By comparing the adjustment profile of  $\dot{V}O_{2p}$  with [HHb] the ratio of  $O_2$  extraction to  $O_2$  consumption may be discerned. This [HHb]-to- $\dot{V}O_{2p}$  ratio provides a sense of how  $O_2$  uptake at the cell is supported by  $O_2$  delivery (i.e. blood flow and arterial  $O_2$  content). If blood flow (and thus  $O_2$  delivery) is adequate, then [HHb] should match with  $\dot{V}O_2$  in a normalized model. A ratio above 1.0 during a step change in metabolic demand implies under-perfusion to the active muscle that is being compensated for by increased fractional  $O_2$  extraction. This technique offers valuable insight into the relationship between  $O_2$  delivery and utilization within the active muscle particularly during interventions designed to slow (or speed) the adjustment of  $O_2$  consumption in the active tissue.

### **1.4 Overview of fat metabolism**

There is a large reserve of fat in the muscle in the form of intramuscular triglycerides (IMTG) and in adipose tissue stored as triglycerides (TG). This supply is favored as substrate for energy production when at rest and during moderate exercise. The preferential use of fat in these circumstances defends the limited supplies of carbohydrate in the body.

Intramuscular triglycerides and TG from adipocytes are broken down into glycerol and fatty acids by the enzyme hormone sensitive lipase (HSL). The fatty acids from adipocytes are transported attached to albumin through the blood and across protein transporters, such as fatty acid-translocase, into the muscle cell (9). Inside the muscle cell free fatty acids (FFA) are transported into the mitochondria for oxidation by CPT-1 (45). Once inside the mitochondria beta-oxidation converts fatty acids into acetyl-CoA which enters the citric acid cycle. Beta-oxidation also produces NADH which feeds into the electron transport chain (ETC).

Control sites for fat metabolism may include: FFA delivery to the muscle, FFA movement across the mitochondrial membrane, and HSL activity. At the onset of exercise blood flow increases which increases the delivery of FFA to the muscle. At heavy intensities of exercise blood flow is redistributed toward exercising muscle and away from adipose sites. This reduces the ability to transport FFA and therefore fat oxidation is reduced (54).

CPT-1 is inhibited by malonyl-CoA (8). Malonyl-CoA appears in the first committed step in fatty acid synthesis. During exercise malonyl-CoA concentrations decrease and so does its inhibition of CPT-1(41). Recent research has also discovered a sensitivity of CPT-1 to drops in pH (8). As exercise intensity increases pH falls, this inhibits fatty acid transport during heavy intensity exercise.

Hormone sensitive lipase activity responds to increases in epinephrine (EPI) and norepinephrine (NE) (59), insulin, dietary fat intake, and exercise intensity. At rest EPI and NE concentrations are low which reduces the activity of HSL. At the onset of exercise the concentrations of these catecholamines increase, leading to activation of HSL. Insulin inhibits fat metabolism by deactivating HSL (59). Insulin levels also decrease during exercise, increasing the

activity of HSL. After ingesting a high carbohydrate (HCHO) meal insulin levels rise, inhibiting HSL. Studies have shown that after a high fat diet HSL activity is increased 20-30% during exercise compared to a HCHO diet (62). Muscular HSL has a pH optimum of 7.0 (66). As exercise intensity increases pH is reduced, and muscular HSL activity falls.

### **1.5 Overview of Carbohydrate metabolism**

Carbohydrates are the major substrate in exercise performed above 50-60%  $VO_{2max}$  (54). Carbohydrate is stored as glycogen in the liver and muscle cells. Glycogen is broken down into glucose-1-phosphate units which enter glycolysis. The end product of glycolysis is pyruvate; if the rate of pyruvate production exceeds the rate of its oxidation it is reduced to lactate. Pyruvate is otherwise converted into acetyl-CoA for oxidative metabolism by PDH.

Glucose from the blood enters the cell through GLUT4 transporters and is phosphorylated by hexokinase. Exercise stimulates GLUT4 movement from intracellular sites to the cell membrane (39). As exercise intensity increases redistribution of blood flow to exercising muscle increases. Greater muscle blood flow increases glucose delivery to muscle cells. Carbohydrate availability is crucial as glucose transport relies on facilitated diffusion. If blood glucose is insufficient the concentration gradient for diffusion is compromised and glucose transport from the blood is reduced (65). At rest, high glucose levels increase insulin levels in the blood which increases GLUT4 movement to the cell membrane (28). Liver glucose output responds to insulin and glucagon levels. As blood glucose levels decrease glucagon levels rise and the liver breaks down glycogen to deliver glucose to the blood. If blood glucose levels are high then insulin release facilitates the formation of glycogen within the liver.

Utilization of muscle glycogen is most rapid at exercise onset and increases with intensity. At exercise onset there is a rapid increase in  $\text{Ca}^{2+}$ , EPI, AMP/ADP, and Pi. These metabolites all activate glycogen phosphorylase (GP). This is the rate limiting enzyme in glycogenolysis which commits glucosyl units cleaved from glycogen to glycolysis in the form of glucose-6-phosphate.  $\text{Ca}^{2+}$ , EPI, AMP, and Pi increase with exercise intensity and stimulate further glycogen breakdown. At moderate intensity exercise the levels of these metabolites are not as high and glycogen breakdown is reduced.

The PDH enzyme complex exists in two forms: an inactive phosphorylated form and an active dephosphorylated form. PDH kinase (PDK) catalyzes the phosphorylation of the  $\text{E}_1$  enzyme on the PDH complex while PDH phosphatase (PDP) dephosphorylates it. The activities of PDK and PDP are determined by several allosteric regulators. Increased ratios of ATP/ADP, acetyl CoA/CoASH, and NADH/NAD<sup>+</sup> activate PDK and increased pyruvate inhibits it, while increased sarcoplasmic  $\text{Ca}^{2+}$  activates PDP (60). As  $\text{Ca}^{2+}$ , ADP, and pyruvate levels increase with exercise intensity PDH activity increases.

### **1.6 Diet manipulation, PDH, and $\text{VO}_2$**

Dietary manipulation has been shown to have a direct effect on PDH activity. High fat diets have been shown to increase reliance on fat during submaximal exercise (64), and increased activity of PDK (63). Peters et al. reported a decrease in PDH activity while on a HFAT diet which was attributed to elevated PDK activity (50). A follow up study by the same group showed that these changes occur within 1 day of diet manipulation (49). With increased fat availability there is a decrease in GP activators Pi and AMP and PDH activators such as ADP (16). One theory for the reduction of these substances is due to a boost in redox potential from

NADH production in beta-oxidation (46). An investigation by St. Amand et. al. observed subjects undergoing dietary manipulation with no glycogen depletion and found that at rest PDH activity was subdued but during exercise at 65%  $\dot{V}O_{2\max}$  intercellular pyruvate content was able to deactivate PDK and PDH activity was similar during exercise on a mixed and low CHO diet (61).

### **1.7 Purpose**

The current study aimed to use dietary manipulation as a method of inducing changes in PDH activation and observe what effects it had on  $\dot{V}O_2$  kinetics in the heavy intensity domain. Subjects consumed HCHO and LCHO diets preceded by a glycogen depletion protocol. Gas collections and NIRS data were recorded. It was hypothesized that: 1) phase 2  $\dot{V}O_{2p}$  kinetics would be faster in the HCHO diet, 2) no effect of diet on the rate of  $O_2$  extraction would be seen in the NIRS data.

## Chapter 2

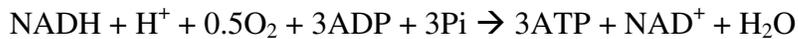
### 2- The effect of low and high carbohydrate diets on pulmonary oxygen uptake and muscle deoxygenation kinetics during exercise transitions into the heavy-intensity domain

#### 2.1 Introduction

Whenever there is a change in the metabolic demand within contracting skeletal muscle, oxygen ( $O_2$ ) consumption ( $\dot{V}O_{2m}$ ) increases in response to the new energy requirement. The adjustment of  $\dot{V}O_2$  following a step-increase in work rate (and thus ATP turnover rate) is not instantaneous but increases towards a new, higher steady-state with a finite (“exponential-like”) time course. Due to the ethical and practical complications that are involved in measuring  $\dot{V}O_{2m}$  directly, pulmonary  $\dot{V}O_2$  ( $\dot{V}O_{2p}$ ) is typically recorded. This measurement was proven to reflect the time course and steady-state adjustments of  $O_2$  utilization in the active muscle by Grassi et. al. (23). The assessment of the speed with which  $\dot{V}O_2$  changes to meet the metabolic requirements of the step change describes  $\dot{V}O_{2p}$  kinetics. The kinetics of  $\dot{V}O_{2p}$  (which reflect the rate of adjustment of oxidative phosphorylation in the active muscle mitochondria) are characterized by three distinct phases: the cardiodynamic phase (phase I) which is representative of a rapid increase in pulmonary circulation, the fundamental phase (phase II) which reflects the increase in  $\dot{V}O_{2m}$  as it adjusts to exercise, and phase III which represents the steady-state  $O_2$  requirement within the moderate-intensity (exercise below lactate threshold( $\hat{\theta}_L$ )) domain or the  $\dot{V}O_{2p}$  slow component in the heavy-intensity (exercise between  $\hat{\theta}_L$  and critical power (CP)) domain. The slow component is defined as  $\dot{V}O_2$  rising above levels that would be predicted from the sub-lactate threshold  $\dot{V}O_2$ -work rate relationship. The phase II  $\dot{V}O_{2p}$  response is described

by the time constant ( $\tau \dot{V}O_{2p}$ ) which represents the time it takes to reach 63% of the steady-state increase in the  $\dot{V}O_{2p}$  response; a  $\dot{V}O_{2p}$  “steady-state” is achieved in the time equivalent of  $4\tau$ . A greater  $\tau \dot{V}O_{2p}$  reflects slower adjustment of  $\dot{V}O_{2p}$ , while a smaller  $\tau \dot{V}O_{2p}$  is reflective of a faster rate of adjustment of  $\dot{V}O_{2p}$ .

There is a long standing debate over what limits the rate of adjustment of  $\dot{V}O_{2p}$  when transitioning to a higher metabolic rate. Oxidative phosphorylation is described by the equation:



The flux through this process is determined both by  $\text{O}_2$  and NADH availability which is dependent on oxidative substrate availability for its production. Two main hypotheses have been proposed to explain the slow time course of  $\dot{V}O_{2p}$  relative to the change in ATP requirement: i) inadequate convective and/or diffusive  $\text{O}_2$  delivery to the terminal oxidase of the mitochondrial Electron Transport Chain (ETC) (14) and ii) “sluggish” activation of rate limiting enzymes and metabolic pathways, and delivery of oxidative substrates (which result in the production of NADH) to the mitochondrial Tricarboxylic Acid Cycle (TCAC) and ETC (6).

One method of investigating theory “ii” is to observe the effect of experimental activation of rate limiting enzymes on the rate of adjustment of  $\dot{V}O_2$  (reflected in  $\tau \dot{V}O_{2p}$  parameter). In this regard, pyruvate dehydrogenase (PDH) is a rate-limiting enzyme complex which catalyzes the oxidative decarboxylation of pyruvate to Acetyl CoA, NADH,  $\text{H}^+$  and  $\text{CO}_2$  and thus regulates the entry of carbohydrate (CHO)-derived substrate into the mitochondrial TCAC and ETC. This complex has received much attention as a possible contributor to the sluggishness of metabolic activation. The PDH complex exists in two forms: an inactive phosphorylated form and an active

dephosphorylated form, with its activity at any instance determined by the relative phosphorylation state. The covalent regulation of PDH activity is determined by the relative activities of two regulatory enzymes, PDH kinase (PDK) and PDH phosphatase (PDP). Up regulation of PDK results in phosphorylation and inhibition of the PDH complex (specifically, phosphorylation of the E<sub>1</sub> enzyme), while up regulation of PDP leads to dephosphorylation and activation of the PDH complex. The activity of PDK is increased by higher: ATP/ADP, NADH/NAD<sup>+</sup>, and Acetyl CoA/CoA ratios. It is inhibited by increased intracellular pyruvate and sarcoplasmic Ca<sup>2+</sup> levels. Increased Ca<sup>2+</sup> levels activate PDP (60). Specifically, as Ca<sup>2+</sup>, ADP, and pyruvate levels increase with exercise intensity PDH activity increases.

Pharmacological up-regulation of PDH prior to exercise has been used to investigate the importance of PDH with respect to the rate of adjustment of  $\dot{V}O_2$  (29, 37, 55). Dichloroacetate (DCA) is a drug commonly used to experimentally activate PDH through the inhibition of PDK. Howlett et. al. found that DCA reduced the contribution of substrate-level phosphorylation (as measured by lower lactate accumulation) to energy production during exercise at 65%  $\dot{V}O_{2\text{ peak}}$  (29). This group also saw a reduction in glycogenolysis and inorganic phosphate (Pi) accumulation after 10 minutes of exercise. These results suggested faster activation of oxidative metabolism when PDH activation was increased. Investigations that have measured the effect of DCA administration on  $\dot{V}O_2$  have not shown a decrease in  $\tau \dot{V}O_{2p}$  within the moderate (37), or heavy intensity domains (55) in vivo. These DCA studies have shown an effect of increased PDH activity on oxidative metabolism but that effect has not been powerful enough to argue that PDH activity limits  $\tau \dot{V}O_{2p}$ .

Dietary manipulation has been shown to have a direct effect on PDH activity at rest. Peters et al. reported a decrease in PDH activity at rest while on a low carbohydrate (LCHO) diet which was attributed to elevated PDK activity (50). A follow-up study by the same group showed that PDH responds to dietary manipulation within one day of dietary manipulation (49). An investigation by St. Amand et. al. observed subjects undergoing a LCHO diet and found that at rest PDH activity was depressed but during exercise at 65%  $\dot{V}O_{2\text{ peak}}$ , intracellular muscle pyruvate content inhibited PDK and PDH activity was similar during exercise on a mixed and LCHO diet (61).

Few investigations have measured the effect of the combination of dietary manipulation and muscle glycogen depletion (GD) on  $\tau \dot{V}O_{2p}$  during exercise. Raper et. al. measured the effects of a dietary manipulation and GD protocol on  $\dot{V}O_{2p}$  in the moderate domain. They found that  $\tau \dot{V}O_{2p}$  was increased in the LCHO condition (indicating slower  $\dot{V}O_{2p}$  kinetics) (53). Lima-Silva et. al. measured  $\dot{V}O_{2p}$  after GD and dietary manipulation in the heavy intensity domain and found increased  $\tau \dot{V}O_{2p}$  in the LCHO condition (43). These investigations speculate that through the combination of dietary manipulation and GD the effects on PDH are persistent even during exercise.

Dietary manipulation may also have effects on  $O_2$  delivery. Raper also measured near-infrared-spectroscopy (NIRS) in their investigation. NIRS provides a measure of the amount of  $O_2$  bound to haemoglobin (Hb) in the area of interrogation. In this regard, the relative concentrations of bound (oxygenated) and unbound (deoxygenated) Hb can be used to monitor phenomena related to  $O_2$  delivery and  $O_2$  extraction at the microvascular level. Specifically, deoxygenation ( $[HHb]$ ) kinetics can be used as a proxy for microvascular  $PO_2$  (12). By

comparing the adjustment profile of  $\dot{V}O_{2p}$  with [HHb] the ratio of O<sub>2</sub> extraction to O<sub>2</sub> consumption may be discerned. Raper found that the time constant for [HHb] ( $\tau$ [HHb]) in the LCHO and HCHO condition was the same despite differences in  $\tau \dot{V}O_{2p}$ . This suggests a slowing in microvascular blood flow in the LCHO condition that must be compensated for through increased O<sub>2</sub> extraction.

The purpose of the current investigation was to examine the effects of low and high carbohydrate (HCHO) diets in combination with GD on the adjustment of  $\dot{V}O_{2p}$  and [HHb] in subjects when transitioning from light- to heavy intensity exercise. Dietary intervention and GD were administered in order to manipulate substrate availability and enzyme activity (PDH). The hypotheses being tested were that 1) subjects will display greater phase II  $\tau \dot{V}O_{2p}$  values in the LCHO condition; 2) dietary manipulation would not have an effect on the rate of microvascular blood flow adjustment as indicated by similar [HHb] kinetics.

## **2.2 Methods**

Participants: Healthy male subjects (n, 10; age, 24 ± 3 yrs);  $\dot{V}O_{2 \text{ peak}}$  (3.94 ± 0.47 L/min), volunteered to participate in the study. All subjects were recreationally active, non-smokers, and had no known history of respiratory, cardiovascular, metabolic or musculoskeletal disease and were not taking medications that might have affected the physiological variables under investigation. Subjects were informed of the protocol, and possible risks and discomforts associated with the procedure, both verbally and in writing, before starting data collection. Subjects gave written, informed consent prior to their voluntary participation in the study. All procedures were approved by The University of Western Ontario Health Sciences Research Ethics Board.

Preliminary testing: Subjects reported to the laboratory for a ramp incremental exercise test (25w/min) to volitional fatigue on an electrically-braked cycle ergometer (H-300-R Lode; Lode BV) for determination of  $\dot{V}O_{2p\ peak}$  and estimated  $\hat{\theta}_L$ . The values obtained from this test were used to determine a WR within the heavy-intensity domain of exercise which corresponded to ~ 35% of the difference between the  $\dot{V}O_{2p}$  at the  $\hat{\theta}_L$  and  $\dot{V}O_{2\ peak}$  (i.e.  $\Delta 35$ ). The  $\hat{\theta}_L$  was estimated using standard ventilatory and gas exchange indices (25). It was defined as the  $\dot{V}O_{2p}$  at which 1) pulmonary  $CO_2$  output ( $\dot{V}CO_{2p}$ ) began to increase out of proportion to the increase in  $\dot{V}O_{2p}$  and 2) there was a systematic rise in the ventilatory equivalent for  $\dot{V}O_{2p}$  ( $\dot{V}_E/\dot{V}O_{2p}$ ) and end-tidal  $PO_2$  ( $P_{ET}O_2$ ) without a systematic rise in the ventilatory equivalent for  $\dot{V}CO_{2p}$  ( $\dot{V}_E/\dot{V}CO_{2p}$ ) and  $P_{ET}CO_2$ .

Subjects submitted a two-day diet record (one weekday, one weekend day) which was used to analyze their “normal” mixed diet composition and to estimate their daily caloric intake. Food records were analyzed using Diet Analysis 9.0 software. This program was also used to design individualized LCHO and HCHO diets that were used as the dietary intervention for this study; diets were eucaloric relative to their “normal” mixed diet submitted in the food records. The LCHO diet was aimed to contain 10%, 70%, and 20% of carbohydrates, fat, and protein respectively. The HCHO diet was aimed to contain 80%, 10%, and 10% of carbohydrates, fat, and protein respectively. Similar diets have been shown in the past to affect PDH activity (38).

Glycogen depletion and dietary intervention: A GD protocol was completed by subjects on their second visit to the laboratory. The protocol consisted of 60 min cycle ergometer exercise at an intensity equivalent to 70%  $\dot{V}O_{2\ peak}$ , which was followed immediately by 5 repeated cycling bouts at a WR corresponding to 110% of the peak WR achieved during the initial

incremental exercise test. Each cycling interval bout lasted 1 min and was separated by 4 min of loadless cycling. Variations of this protocol have previously lowered muscle glycogen content by 55-90% (11).

Following the GD protocol subjects began the LCHO diet. Each dietary phase lasted four days. On day three of the diet subjects performed two step-transitions to  $\Delta 35$  exercise, with each separated by at least 1 hour of resting recovery. The  $\Delta 35$  constant-load exercise test began with six minute baseline cycling at 20 W, followed by a step-increase to a WR corresponding to  $\Delta 35$  which lasted eight minutes. During these exercise tests pulmonary gas exchange was measured breath-by-breath and local muscle [HHb] was measured continuously in the vastus lateralis muscle of the quadriceps muscle group using NIRS. Subjects were instructed to refrain from strenuous exercise in the preceding 24 hours before a test. On day four of the diets subjects performed a  $\Delta 35$  exercise bout with the collection of muscle biopsies. During the biopsy rides subjects had muscle biopsies taken from the vastus lateralis muscle during cycling at three time points: during 6 minute baseline 20W cycling, 15 s after the onset of  $\Delta 35$  exercise, and 8 minutes after the step transition. Biopsies were frozen in liquid nitrogen for later analysis of: glycogen content, ADP concentration, Pcr concentration, and PDH activation.

Following the biopsies on day four of the LCHO diet the GD protocol was repeated. After the second GD protocol a HCHO diet was followed and measurements were repeated in the same timeframes as in the LCHO condition. The order of the diets were not randomized because this order of LCHO followed by HCHO diet, with each preceded by a glycogen depletion protocol, has been shown to significantly lower and then maximize muscle glycogen levels (7). All food was purchased and provided to the subjects along with strict meal plans. If

any portion of the prescribed diet was not consumed, this portion was weighed to establish the exact caloric intake for each subject during each of the dietary phases.

Measurements: Pulmonary gas-exchange measurements were similar to those previously described (57). Briefly, inspired and expired flow rates were measured using a low dead space bidirectional turbine (Alpha Technologies VMM 110) which was calibrated before each test using a 3L syringe. Inspired and expired gases were sampled continuously (50Hz) at the mouth and analyzed for concentrations of O<sub>2</sub>, CO<sub>2</sub>, and N<sub>2</sub> by mass spectrometry (Innovision, Amis 2000, Lindvedvej, Denmark) after calibration with precision-analyzed gas mixtures. Changes in fractional gas concentrations were aligned with inspired and expired gas volumes by measuring the time delay (TD) for a square-wave bolus of gas to travel from the turbine transducers along a capillary sample line to the mass spectrometer. Data were transferred to a computer, which aligned concentrations with volume information to build a profile of each breath. Breath-by-breath alveolar gas exchange was calculated using the algorithms of Beaver et. al. (4).

Heart rate (HR) was monitored continuously by three-lead electrocardiogram (ECG) using PowerLab (ML132/ML880; ADInstruments, Colorado Springs, CO); HR was calculated based upon the R-R interval and was reported on a second-by-second basis. Data were recorded on a separate data collection computer using LabChart v6.1.

Local muscle [HHb] of the quadriceps vastus lateralis muscle was monitored continuously with a frequency-domain multi-distance NIRS system (Oxiplex TS, Model 95205, ISS, Champaign, IL, USA) as described elsewhere (58). The NIRS probe was placed on the belly of the muscle, midway between the lateral epicondyle and greater trochanter of the femur; it was secured in place with an elastic strap tightened to prevent movement and covered with an

optically-dense, black vinyl sheet, thus minimizing the intrusion of extraneous light and loss of NIR light. NIRS measurements were collected continuously for the entire duration of each trial. Briefly, the system was comprised of a single channel consisting of eight laser diodes operating at two wavelengths ( $\lambda = 690$  and  $828$  nm, four at each wavelength) which were pulsed in a rapid succession (110 MHz) and a photomultiplier tube. The lightweight plastic NIRS probe (connected to laser diodes and photomultiplier tube by optical fibers) consisted of two parallel rows of light emitter fibers and one detector fiber bundle; the source-detector separations for this probe were 2.0, 2.5, 3.0, and 3.5 cm for both wavelengths.

The NIRS system was calibrated at the beginning of each testing session following an instrument warm-up period of at least 20 minutes. Calculation of [HHb] reflected continuous measurements of a reduced scattering coefficient ( $\mu_s'$ ) made throughout each testing session (i.e., constant scattering value not assumed). Data were stored online at an output frequency of 25 Hz, but were reduced to 1 s bins for all subsequent analyses within the present study.

Data Analysis: Gas exchange data were edited by removing aberrant data points that lay outside four standard deviations (SD) of the local mean. The data for each transition were linearly interpolated to 1 s intervals and time-aligned such that time zero represented the onset of exercise. Data from both  $\Delta 35$  transitions were ensemble-averaged to yield a single, averaged response for each subject. This transition was further time-averaged into 5 s bins to provide a single time-averaged response for each subject. The phase I-phase II transition was identified as previously described . The on-transient response for  $\dot{V}O_{2p}$  was modeled using the following equation:

$$Y_{(t)} = Y_{bsln} + \text{Amp} [1 - e^{-(t-TD)/\tau}] \quad \text{Eq. 1}$$

Where  $Y_{(t)}$  represents  $\dot{V}O_{2p}$  at any time;  $Y_{bsln}$  is the average  $\dot{V}O_{2p_{ss}}$  measured during the period immediately before the change in WR; Amp (amplitude) is the steady-state increase in  $\dot{V}O_{2p}$  above the baseline  $\dot{V}O_{2p}$  ( $\dot{V}O_{2p_{bsl}}$ );  $\tau$  represents the time required to attain 63% of the steady-state amplitude; and TD is mathematically generated as the point at which the exponential model is predicted to intersect the baseline. Steady-state  $\dot{V}O_{2p_{bsl}}$  was established from data 60 s before the change in WR. Data were modeled from the phase I-phase II transition to the onset of the slow component (determined in a similar fashion to the phase I-phase II transition (25)) using Origin data fitting software (OriginLab). The 95% confidence interval for the estimated  $\tau$  was determined following a preliminary fit with  $Y_{bsln}$ , Amp, and TD constrained to best fit values, with the  $\tau$  allowed to vary. The slow component was modelled using a linear equation over the last 30 s of exercise. End exercise  $\dot{V}O_{2p}$  was measured as the average of the last 15 s of exercise.

The NIRS-derived [HHb] data were time-aligned and ensemble-averaged to 5 s bins to yield a single response for each subject. The [HHb] profile has been described to consist of a time delay at the onset of exercise followed by an “exponential-like” increase in the signal towards a new steady-state exercise value (15). The TD for the [HHb] response was estimated for each subject using second-by-second data and corresponded to the time after the onset of exercise where the [HHb] signal showed a consistent increase above the nadir value, as described previously (24). The [HHb] data were modeled from the end of the TD-HHb to 90 s using a monoexponential function of the form in Eq. 1 to determine  $\tau$ [HHb]. This model was chosen because it offered the best consistent fits for the transition. Baseline [HHb] was determined for each trial as the mean value in the 60 s before a transition. Mean response time ( $MRT = [HHb]_{TD} + \tau$ [HHb]) was calculated to describe the overall time course for muscle [HHb]. End-exercise

data was calculated as the average of the last 30 s of exercise. The steady-state value for [HHb] was determined as the end point for the fitting of the monoexponential function ( $[\text{HHb}]_{\text{ss}}$ ).

The second-by-second [HHb] and  $\dot{V}O_{2p}$  data were normalized for each subject (0%, representing the 20 W baseline value, and 100%, representing the phase II value). The normalized phase II  $\dot{V}O_{2p}$  were then modeled using the parameter values for individual subjects. The normalized adjustment of muscle  $O_2$  utilization was shifted toward the start of each step transition by a time corresponding to the estimated phase II TD for each transition, thereby making the normalized  $\dot{V}O_{2p}$  at the immediate onset of the transition equal to “zero”. [HHb] data was not modeled; it was derived from the raw second-by-second signal. An  $[\text{HHb}]/\dot{V}O_{2p}$  ratio was calculated with a value of 1.0 corresponding to a match between [HHb] and  $\dot{V}O_{2p}$ . An overshoot in the  $[\text{HHb}]/\dot{V}O_{2p}$  response profile was estimated by integrating the area bounded by the  $[\text{HHb}]/\dot{V}O_{2p}$  profile and a ratio value equal to 1.0. The start point was selected to be 20 s to begin the analysis. During the adjustment within the initial ~20 s, the blood flow response and  $O_2$  delivery is adequate to sustain early increases in  $\dot{V}O_{2p}$  and therefore, the  $[\text{HHb}]/\dot{V}O_{2p}$  as described was not used to attempt to characterize this period of the response. The end point of the analysis was selected to be 120 s, meant to reflect the time point at which the  $[\text{HHb}]/\dot{V}O_{2p}$  ratio reached a steady-state of 1.0 in all subjects.

The on-transient HR response was modeled from the onset of exercise to the end of phase II of  $\dot{V}O_{2p}$  using Eq. 1. This strategy offered the best consistent fits for the transition.  $\text{HR}_{\text{bsl}}$  was calculated from the last 30 s of 20 W exercise. End exercise HR was calculated from the last 30 s of the entire HR response.

Statistics: Statistical analyses were performed using SPSS 20. Differences between HCHO and LCHO diets with respect to parameter estimates of:  $\dot{V}O_2$ , HR, and [HHb] were analyzed using repeated measured ANOVA (diet condition as main effect). Statistical significance was accepted at  $p < 0.05$ . All data are presented as mean  $\pm$  SD.

### 2.3 Results

Physical characteristics of the subjects and aerobic parameters measured during the ramp incremental exercise test are presented in Table 1. Subjects were able to complete the entire glycogen depletion protocol prior to the LCHO diet. None of the subjects were able to complete the full one hour of cycling at 70%  $\dot{V}O_{2\text{ peak}}$  before the HCHO diet (35 minutes  $\pm$  5 minutes), all were able to complete the five one-minute sprint interval component of the glycogen depletion protocol before the HCHO diet.

Diets had an average daily caloric intake of  $2810 \pm 324$ ,  $2834 \pm 318$ , and  $2791 \pm 308$  kcal for mixed, LCHO, and HCHO respectively. The relative carbohydrate, fat, and protein content of the diets consumed during the study were, respectively: 10%  $\pm$  2, 64%  $\pm$  3, and 27%  $\pm$  4 for the LCHO, and 76%  $\pm$  3, 11%  $\pm$  2, and 16%  $\pm$  2 for the HCHO.

As expected, the WRs used in the present study represented an intensity within the heavy domain of exercise as evidenced by an end-exercise  $\dot{V}O_{2p}$  that was greater than the  $\dot{V}O_{2p}$  at  $\hat{\theta}_L$  and a  $\dot{V}O_{2p}$  profile which exhibited a visually identifiable  $\dot{V}O_{2p}$  slow component in 9 of 10 subjects.

The group mean response profile for the calculated respiratory exchange ratio ( $RER = \dot{V}CO_{2p} / \dot{V}O_{2p}$ ) for the LCHO and HCHO conditions is presented in Figure 1. The calculated

RER during steady-state baseline cycling at 20 W was significantly higher ( $p < 0.05$ ) in the HCHO ( $0.97 \pm 0.18$ ) compared to the LCHO ( $0.8 \pm 0.07$ ) diet; RER remained elevated in HCHO compared to LCHO throughout the transition to  $\Delta 35$  exercise (Figure 1). This data is indicative of a lesser oxidation of CHO in the LCHO condition compared to the HCHO condition.

The group mean response for  $\dot{V}CO_{2p}$  is presented in Figure 2.  $\dot{V}CO_{2p}$  was significantly lower ( $p < 0.05$ ) in the LCHO ( $0.79 \pm 0.16$ ) condition compared with the HCHO ( $0.90 \pm 0.19$ ) condition during baseline 20 W exercise.

The group means response profile for  $\dot{V}O_{2p}$  in the LCHO and HCHO conditions is presented in Figure 3A, while the  $\dot{V}O_{2p}$  response profile for a representative subject during the HCHO condition, along with the model-derived line-of-best fit and residuals (difference between actual data and the model-derived line-of-best fit) is presented in Figure 3B. The summary of the parameter estimates for the  $\dot{V}O_{2p}$  kinetics response for the dietary conditions is presented in Table 2. Statistical analysis showed that all variables with the exception of  $\dot{V}O_{2p}$  baseline, slow component amplitude, and TD were significantly greater in the LCHO condition ( $p < 0.05$ ) including: amplitude, phase II  $\dot{V}O_{2p}$ ,  $\tau \dot{V}O_{2p}$ , end-exercise  $\dot{V}O_{2p}$ , and  $\dot{V}O_{2p}$  gain ( $\dot{V}O_{2p}$  gain =  $\Delta \dot{V}O_{2p} / \Delta WR$ ) ( $p < 0.05$ ).  $\dot{V}O_{2p}$   $_{bsl}$  and slow component amplitude were not statistically different ( $p > 0.05$ ). TD was significantly greater in the HCHO condition ( $p < 0.05$ ). Figure 4 illustrates the individual  $\tau \dot{V}O_{2p}$  for each subject in the LCHO and HCHO diet, along with the group mean values for each diet; the  $\tau \dot{V}O_{2p}$  was greater in LCHO than in HCHO in 9 of the 10 subjects.

Parameter estimates for the [HHb] response kinetics for transitions to  $\Delta 35$  during the LCHO and HCHO conditions are presented in Table 3. The group means response profile for [HHb] for LCHO and HCHO conditions is presented in Figure 5A, while the [HHb] response profile for a representative subject along with the model-derived line-of-best fit and residuals during the HCHO condition is presented in Figure 5B. Baseline [HHb] was the only parameter which showed a significant difference between LCHO ( $24.55 \pm \text{SD } \mu\text{M}$ ) and HCHO ( $21.73 \pm \text{SD } \mu\text{M}$ ) diets ( $p < 0.05$ ). Parameters of [HHb] for amplitude, steady state,  $\tau$ [HHb], TD, MRT, and end-exercise were not statistically significant ( $p < 0.05$ ) between conditions.

The [HHb]/  $\dot{V}O_{2p}$  ratio for the LCHO and HCHO diets are presented in Figure 6A and B respectively. The [HHb]/  $\dot{V}O_{2p}$  ratio displayed a greater overshoot in the LCHO (1.27) condition compared to the HCHO (1.09) condition ( $p < 0.05$ ). All subjects displayed an overshoot across both conditions.

The parameter estimates for HR kinetics during the transition to heavy-intensity exercise are presented in Table 4. Parameters of baseline,  $\tau$ HR, and end-exercise were not statistically significant ( $p > 0.05$ ). Amplitude of the HR response was greater ( $p < 0.05$ ) in the LCHO ( $63.4 \pm 10.4$ ) condition than that HCHO ( $57.9 \pm 12.1$ ) condition. Group mean HR data is presented in Figure 7a while data for a representative subject along with the model-derived line-of-best fit and residuals during the HCHO condition is presented in Figure 7b.

Table 1. Physical characteristics and response to ramp incremental test

Age (yr)	Body mass (kg)	Height (cm)	$\dot{V}O_{2p \text{ peak}}$ (L/min)	$\dot{V}O_{2p \text{ peak}}$ (ml/kg/min)	$\dot{V}O_{2p}$ at $\hat{\theta}_L$ (L/min)	Estimated $\dot{V}O_{2p}$ at $\Delta 35$ (L/min)	WR at $\Delta 35$ (W)
24 ± 3	81 ± 12	177 ± 5	3.9 ± 0.5	33.1 ± 21.9	2.2 ± 0.3	2.8 ± 0.4	204 ± 31

Values are expressed as mean ± SD.  $\dot{V}O_{2p \text{ peak}}$ , peak oxygen uptake;  $\hat{\theta}_L$ , estimated lactate threshold;  $\Delta 35$ , 35% $\Delta$ ; WR, work rate.

Table 2. Summary of parameter estimates for  $\dot{V}O_{2p}$  on-transients to heavy-intensity exercise during the low carbohydrate (LCHO) and high carbohydrate (HCHO) diets.

	LCHO diet	HCHO diet
$\dot{V}O_{2p}$ Baseline (L/min)	1.0 ± 0.1	0.9 ± 0.2
$\dot{V}O_{2p}$ Amplitude (L/min)	1.9 ± 0.4	1.7 ± 0.4*
End-exercise $\dot{V}O_{2p}$ (L/min)	3.2 ± 0.5	3 ± 0.5*
Phase II $\dot{V}O_{2p}$ (L/min)	2.8 ± 0.4	2.6 ± 0.4*
Phase II $\tau\dot{V}O_{2p}$ (s)	41.9 ± 11.5	33.9 ± 7.3*
$\dot{V}O_{2p}$ TD (s)	1.6 ± 7.8	9 ± 7.2*
Phase II $\dot{V}O_{2p}$ gain (ml/min/W)	10.1 ± 1.1	9.4 ± 1.3*
$\dot{V}O_{2p}$ Amp <sub>sc</sub> (L)	0.3 ± 0.1	0.3 ± 0.1
95% CI (s)	5.5 ± 2.1	3.9 ± 1.5

Values are expressed as means ± SD.  $\tau\dot{V}O_{2p}$ , time constant for phase II  $\dot{V}O_{2p}$ ; TD, time delay;  $\dot{V}O_{2p}$  gain,  $\Delta\dot{V}O_{2p}/\Delta WR$ ; Amp<sub>sc</sub>, slow component amplitude; CI, confidence interval for phase II  $\dot{V}O_{2p}$ .

\*Significant difference (P<0.05) between the LCHO and HCHO diets.

Table 3. Parameter estimates for NIRS-derived deoxygenation concentration [HHb] changes during the transition to heavy-intensity exercise in the LCHO and HCHO diets

	LCHO	HCHO
[HHb] baseline ( $\mu\text{M}$ )	$24.6 \pm 6.4$	$21.7 \pm 5.5^*$
[HHb] amplitude ( $\mu\text{M}$ )	$12.5 \pm 6.7$	$9.9 \pm 3.6$
[HHb] steady state ( $\mu\text{M}$ )	$37 \pm 12.7$	$31.6 \pm 8.6$
$\tau$ [HHB] (s)	$12.9 \pm 3.4$	$10.6 \pm 1.9$
CTD [HHb] (s)	$5.8 \pm 2.6$	$5.7 \pm 1.8$
MRT (s)	$18.7 \pm 3.4$	$16.3 \pm 2.1$
95% CI (s)	$2.3 \pm 0.8$	$2.5 \pm 1.5$
End exercise ( $\mu\text{M}$ )	$39.1 \pm 12.8$	$34.2 \pm 9.1$

Values are expressed as means  $\pm$  SD.  $\tau$ [HHb], time constant for the adjustment of [HHb]; CTD [HHb], calculated time delay for [HHb]; MRT, mean response time; CI, confidence interval for  $\tau$ [HHb].

\*Significant difference ( $P < 0.05$ ) between LCHO and HCHO diets

Table 4. Parameter estimates for heart rate (HR) kinetics during the transition to heavy-intensity exercise in the LCHO and HCHO diets.

	LCHO	HCHO
Baseline HR (beats/min)	89 ± 12.2	91.8 ± 7.9
HR Amplitude (beats/min)	63.4 ± 10.4	57.9 ± 12.1*
τHR (s)	42.3 ± 23.5	39.9 ± 14.6
End exercise HR (beats/min)	166.3 ± 13.9	160.1 ± 12.3
95% CI (s)	7.3 ± 8.6	7.45 ± 7.5

Values are expressed as means ± SD. τHR, time constant for HR adjustment; CI, confidence interval for τHR.

\*Significant difference (P<0.05) between the LCHO and HCHO diets

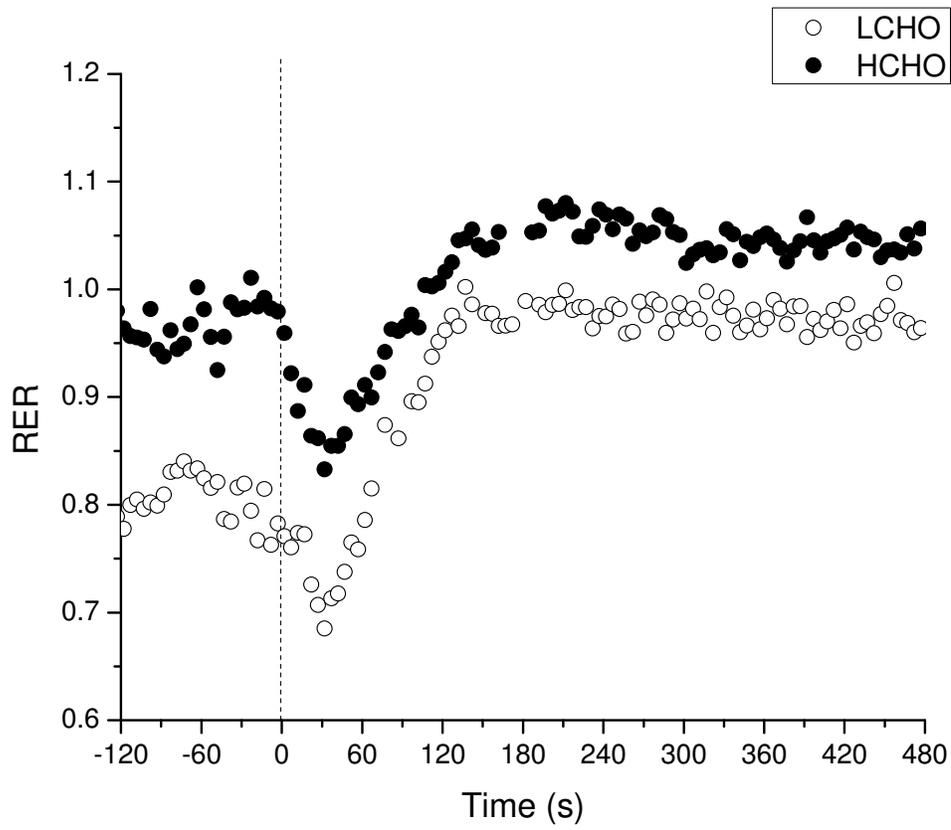


Figure 1. Group mean respiratory exchange ratio ( $RER=VCO_2/VO_2$ ) response during baseline and heavy-intensity exercise; open circles, LCHO diet; closed circles, HCHO diet. Dashed line represents the onset of step-change in WR.

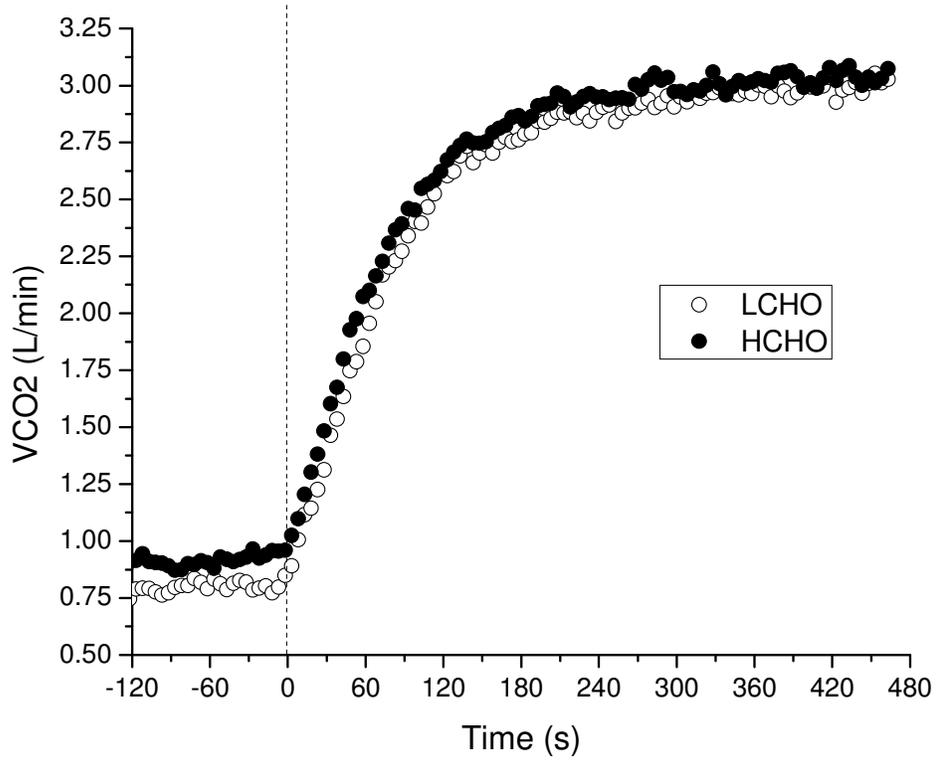


Figure 2. Second-by-second group mean VCO<sub>2</sub> response during the transition to heavy-intensity exercise; open circles, LCHO diet; closed circles, HCHO diet. Dashed line represents the onset of the step-change in WR.

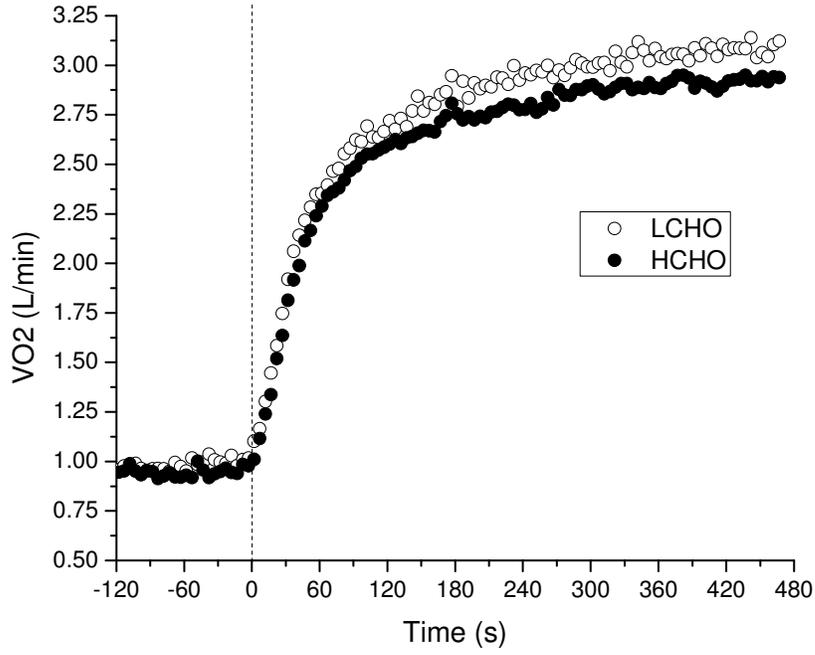


Figure 3a. Second-by-second group mean VO<sub>2</sub> response during the transition to heavy-intensity exercise; open circles, LCHO diet; closed circles, HCHO diet. Dashed line represents the onset of the step-change in WR.

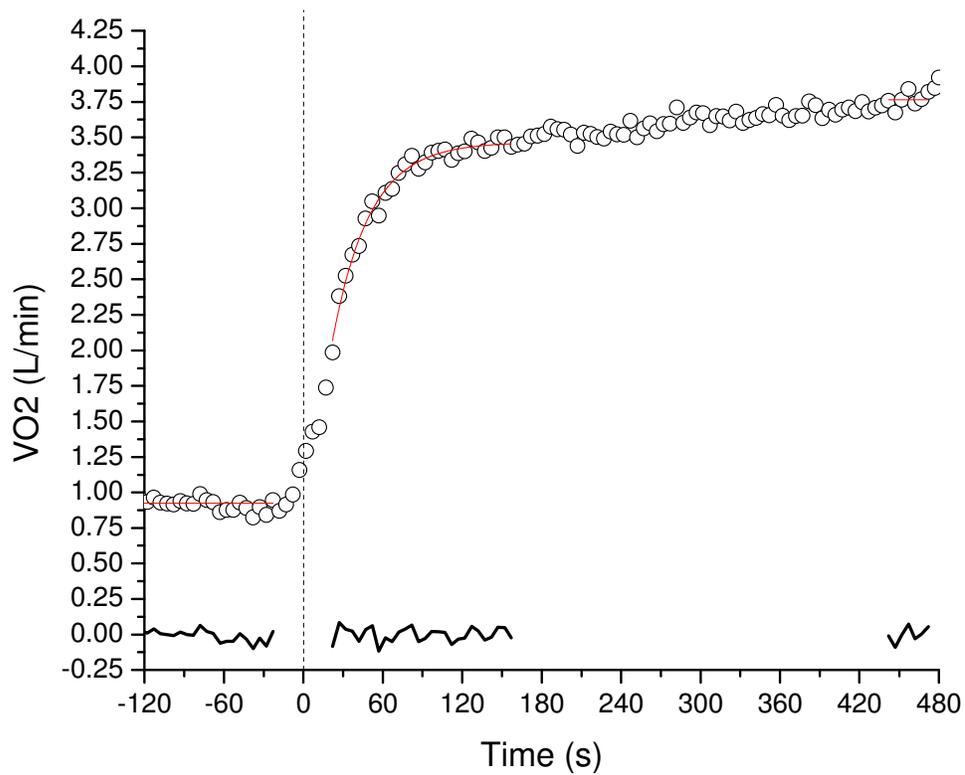


Figure 3b. Second-by-second  $VO_{2p}$  response for a representative subject during the transition to heavy-intensity exercise in the HCHO condition. Red lines represent the modelled line of best fit. Black lines represent residuals. Dashed line represents the onset of the step-change in WR.

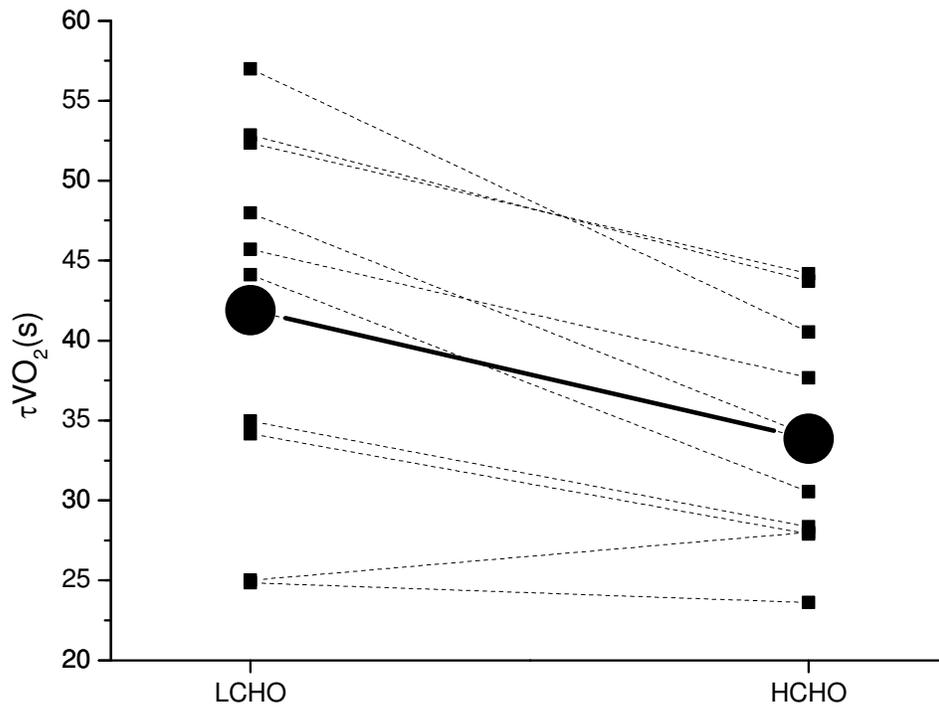


Figure 4. Individual and group mean VO<sub>2</sub> time constants ( $\tau$ VO<sub>2</sub>) for the LCHO and HCHO diets. Dotted lines connect individual data. Solid line connects group mean data.

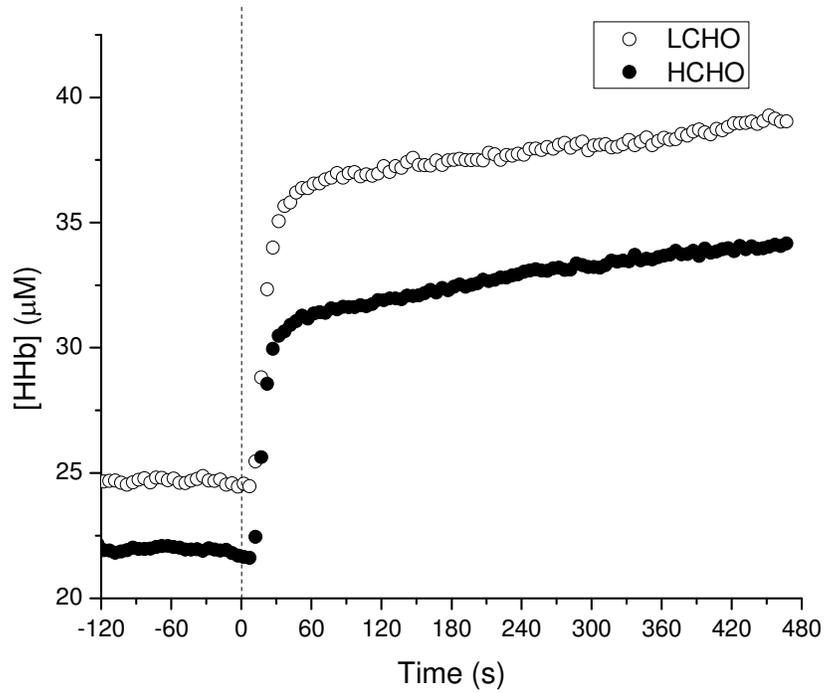


Figure 5a. The group mean [HHb] response during the transition to heavy-intensity exercise; open circles, LCHO diet; closed circles, HCHO diet. Dashed line represents onset of the step-change in WR.

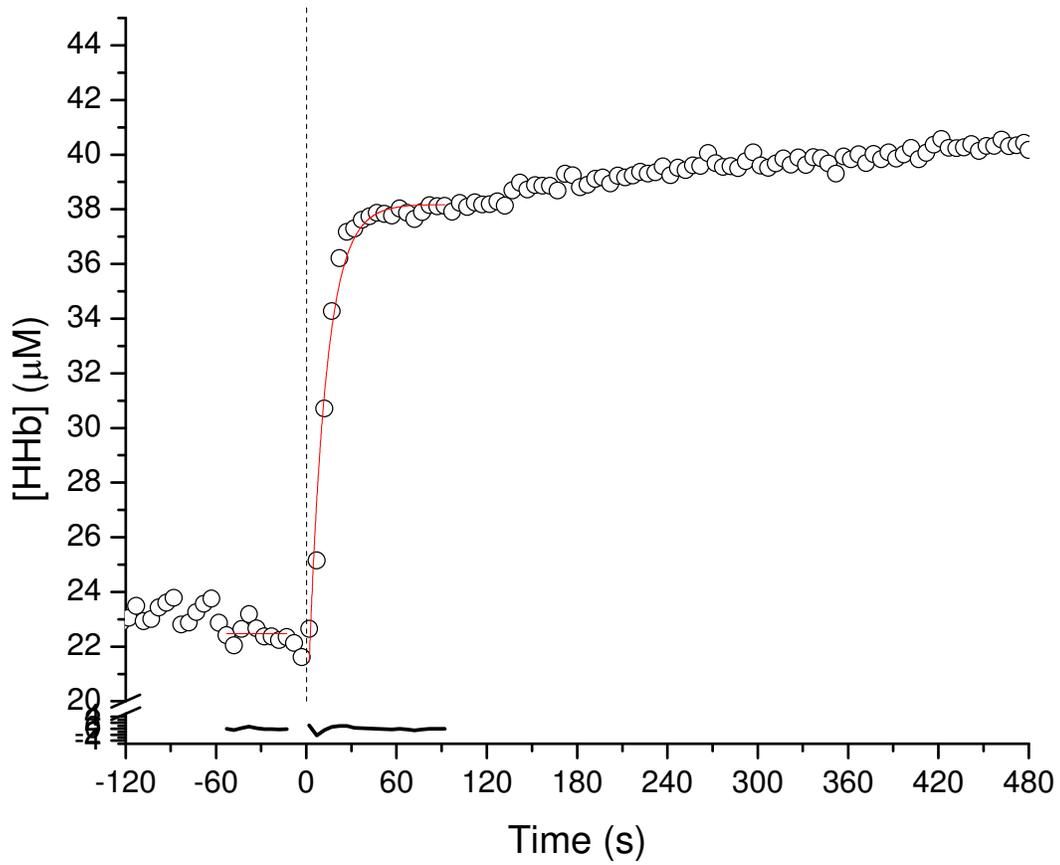


Figure 5b. The [HHb] response during the transition to heavy-intensity exercise in a representative subject during the HCHO condition. Red line represents the modelled line of best fit. Black line represents the residuals. Dashed line represents the onset of the step-change in WR.

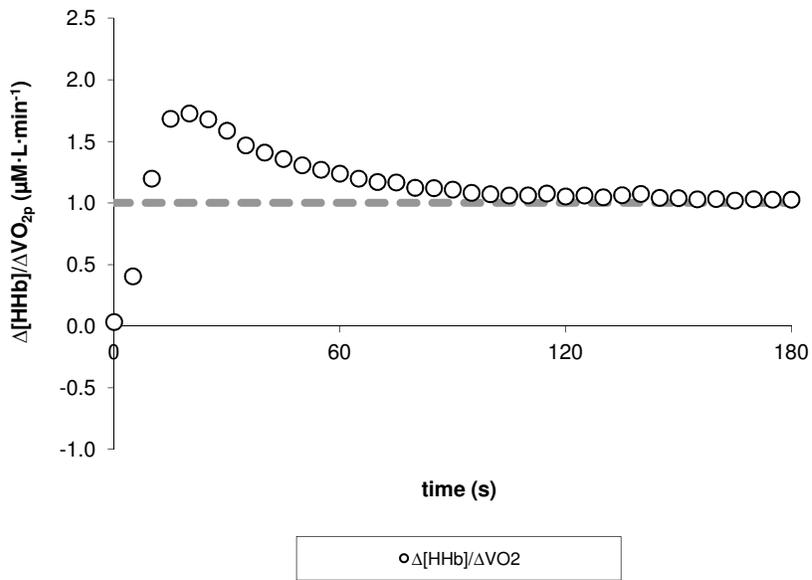


Figure 6a. Group mean profiles for the relative adjustment of  $[\text{HHb}]/\dot{\text{V}}\text{O}_{2p}$  in the initial 120 s of heavy intensity exercise transitions, shown in the LCHO condition.

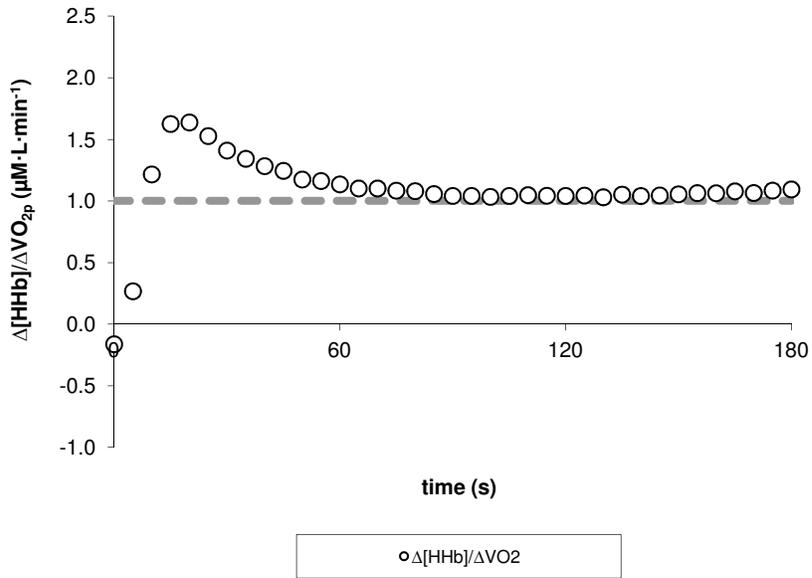


Figure 6b. Group mean profiles for the relative adjustment of  $[\text{HHb}]/\dot{\text{V}}\text{O}_{2p}$  in the initial 120 s of heavy intensity exercise transitions, shown in the HCHO condition.

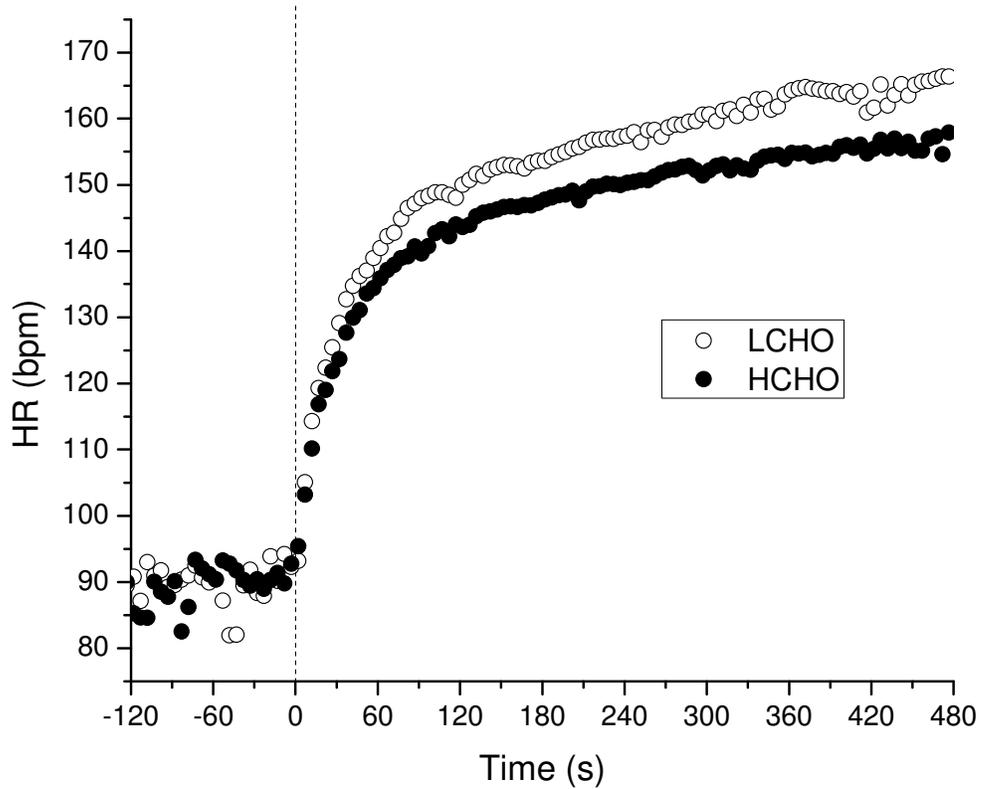


Figure 7a. Group mean beat-by-beat mean heart rate (HR) response during the transition to heavy-intensity exercise; open circles, LCHO diet; closed circles, HCHO diet. Dashed line represents the onset of the step-change in WR.

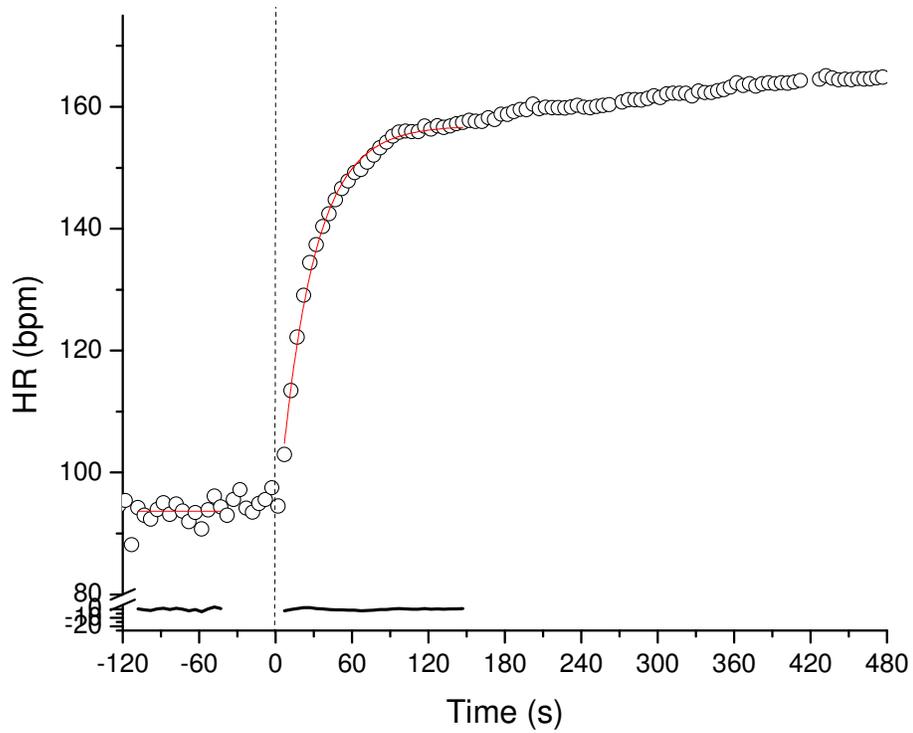


Figure 7b. Beat-by-beat heart rate (HR) response during the transition to heavy-intensity exercise in a representative subject in the HCHO condition. Red line represents the modelled line of best fit. Black line represent the residuals. Dashed line represents the onset of the step-change in WR.

## 2.4 Discussion

This study examined  $\dot{V}O_{2p}$  and muscle [HHb] kinetics during transitions to heavy-intensity exercise during LCHO and HCHO dietary interventions combined with a GD protocol in an attempt to manipulate muscle substrate availability and the level of PDH activation. The major findings of this study were as follows: 1) the kinetics of  $\dot{V}O_{2p}$  during the transition to heavy-intensity exercise were slower during the LCHO condition (greater  $\tau \dot{V}O_{2p}$ ) compared to the HCHO condition, suggesting that the activation of muscle oxidative phosphorylation was slowed on the LCHO diet; 2) despite slower  $\dot{V}O_{2p}$  kinetics in the LCHO condition, the adjustment of [HHb] was not different between diets, suggesting that the adjustment of muscle microvascular blood flow was attenuated in the LCHO condition compared to the HCHO condition.

It appears that the dietary manipulation and GD protocol that was administered was effective in influencing substrate availability and utilization. Although muscle glycogen data was not available at the time of writing, previous studies using a similar GD and dietary intervention observed a 55-90% decrease in glycogen levels following GD (11). In addition, the resting RER data are significantly ( $p < 0.05$ ) different between conditions. This suggests differences in substrate utilization. The combined GD and dietary manipulation protocol was effective in altering  $\dot{V}O_{2p}$  kinetics as demonstrated by the greater  $\tau \dot{V}O_{2p}$  in LCHO diet compared to the HCHO diet. This is in agreement with Lima-Silva et. al.. They measured  $\dot{V}O_{2p}$  in subjects exercising at  $\Delta 75$  until exhaustion after consuming a LCHO diet for 48 hours and completing a GD protocol (43). They observed a much greater  $\tau \dot{V}O_{2p}$  in the LCHO condition compared to the

HCHO condition (33 vs. 48 s). Raper et. al. also observed increased  $\tau \dot{V}O_{2p}$  after dietary manipulation and GD in the LCHO condition during moderate exercise (40s vs. 32 s) (53).

Slowed  $\dot{V}O_{2p}$  kinetics reflect a slower adjustment of oxidative metabolism at the onset of exercise. This slowed adjustment could be caused by limitations in  $O_2$  delivery or the inadequate provision of substrate for the ETC through the activity of rate-limiting metabolic enzymes. As PDH controls the entry of carbohydrate derived substrate into the TCAC and eventually the ETC it plays a large role in the initiation of oxidative metabolism. Fat metabolism is not activated as quickly as CHO metabolism at the onset of exercise. The ability to quickly utilize CHO-derived substrate allows for a faster adjustment to the step-transition in WR.

It is reasonable to assume that the intervention in the present study altered PDH activity. Peters et. al. investigated the effect of dietary manipulation on PDK activity and found that PDK was elevated during a high fat diet (50). St. Amand et. al. and Constantin-Teodosiu et. al. also reported reduced PDH activity at rest following a LCHO diet (13, 61). The LCHO diets in these and the present study potentially cause increased rates of beta-oxidation which increased the levels of NADH within the cell (62). LCHO diets also lead to reduced intracellular pyruvate. These changes lead to a greater activation of PDK and therefore reduced PDH activity (49, 50, 64). Dietary manipulation in the absence of GD has not been shown in the past to alter PDH activity during exercise. In St. Amand et. al. and Constantin-Teodosiu et. al. PDH activity in subjects during the LCHO condition was shown to match that of mixed diet subjects at exercise (13, 61). During exercise pyruvate levels in the cell increase which leads to the deactivation of PDK and the activation of PDH. In our investigation this may not have occurred in the LCHO condition as GD could have led to reduced intracellular pyruvate levels and PDH activity during

exercise. In the HCHO condition pyruvate levels were potentially restored after depletion which could have led to elevated PDH activity.

Muscle biopsy samples were not analyzed at the time of writing. It is expected that biopsy data will confirm a number of ideas speculated upon in this paper. The LCHO condition will presumably show: lower muscle glycogen content, greater phosphocreatine breakdown, a higher Pi content, a lower lactate level, a greater estimated ADP, and reduced PDH activation throughout exercise. A lower rate of PDH activation at 15 s is also expected to be shown in the LCHO condition. These results would mirror the changes seen by Putman et. al. during a similar investigation (52). These changes are reflective of a slower rate of muscle oxidative phosphorylation, consistent with a slower rate of activation of PDH as well as a reduced rate of O<sub>2</sub> delivery.

The NIRS-derived [HHb] data provides insight into what effects the dietary manipulation had on O<sub>2</sub> delivery. In the present study, despite a significant difference in cellular O<sub>2</sub> demand (evidenced by differences in  $\tau \dot{V}O_{2p}$ ) there was no significant difference in  $\tau$ [HHb] between conditions suggesting that the local microvascular blood flow response was attenuated in the LCHO condition. This also was evident in the higher [HHb]/VO<sub>2</sub> ratio in the LCHO diet. This indicates a slower adjustment of microvascular blood flow and a greater reliance on O<sub>2</sub> extraction from the microvasculature in the LCHO condition. Studies have shown that consumption of a HFAT meal leads to impaired endothelial dependent vasodilation (EDV) through a number of mechanisms including: eNOS inhibition, insulin resistance, and increased oxidative stress (18, 36, 56). These findings agree with the present data, however exercise has been reported to counteract the effects of a HFAT meal through anti-oxidant production and increased shear stress (47). Padilla et. al. measured EDV after a high fat meal at rest and during

exercise (47). They found that after a high fat meal and exercise EDV was greater than after a mixed meal without exercise. This investigation studied humans exercising for 45 minutes one hour prior to the measurement of EDV. Our investigation did not display an exercise effect on restoring blood flow following the ingestion of high fat meals. There are a few reasons for the apparent discrepancy in our results. 1) The timing of their measurement was different as their investigation was concerned with the effect on EDV one hour following exercise (it has been shown that endothelial function increases maximally one hour after exercise (26)). The present investigation was interested in measuring the effects of a HFAT diet on blood flow during the initial minute of exercise. 2) The longer duration of the exercise in Padilla's investigation would also lead to more prolonged sheer stress and anti-oxidant production. 3) Lastly, our fat feeding was prolonged instead of a single meal (47).

## **2.5 Conclusion**

A 4-day dietary manipulation and GD protocol was effective in influencing substrate utilization and  $\tau \dot{V}O_{2p}$  during heavy intensity exercise. During the LCHO condition  $\tau \dot{V}O_{2p}$  was significantly higher ( $p < 0.05$ ). It is suggested that two factors contributed to the slower adjustment of  $\dot{V}O_{2p}$  to exercise in the LCHO condition: 1) a reduced activation of PDH, 2) a slower adjustment of microvascular blood flow. This study provides evidence of PDH's important role in determining the speed of adjustment of  $\dot{V}O_{2p}$  to exercise. It is also suggests a potential contribution of reduced  $O_2$  delivery to an increase in  $\tau \dot{V}O_{2p}$  during exercise on a LCHO diet.

## **2.6 Future directions and limitations**

The direct measure of conduit artery blood flow and administration of Doppler ultrasound for the assessment of endothelial dependent vasodilation would assist in making more conclusive statements on the effect of the current protocol on blood flow adjustments. Near-infrared spectroscopy is a useful tool but does not provide the certainty of the measures above.

Therefore, future research would further the present study if it gathered information on blood flow through conduit artery measurements or the use of Doppler ultrasonography.

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# Appendix A: Ethics Approval Notice



**Western  
Research**

**Use of Human Participants - Ethics Approval Notice**

Research Ethics

**Principal Investigator:**Dr. John Kowalchuk  
**File Number:**6085  
**Review Level:**Full Board  
**Approved Local Adult Participants:**20  
**Approved Local Minor Participants:**0  
**Protocol Title:**Adaptation of pulmonary O2 uptake and muscle pyruvate dehydrogenase (PDH) activity during the transitions from the lower and upper regions of the moderate-intensity domain (REB #16003)  
**Department & Institution:**Health Sciences\Kinesiology,Western University  
**Sponsor:**  
**Ethics Approval Date:**March 07, 2013  
**Ethics Expiry Date:**December 31, 2014

**Documents Reviewed & Approved & Documents Received for Information:**

Document Name	Comments	Version Date
Revised Western University Protocol	Revised compensation	2013/02/23
Revised Letter of Information & Consent		2013/02/23
Other	Response Letter	2013/02/23

This is to notify you that the University of Western Ontario Health Sciences Research Ethics Board (HSREB) which is organized and operates according to the Tri-Council Policy Statement: Ethical Conduct of Research Involving Humans and the Health Canada/CH Good Clinical Practice Practices: Consolidated Guidelines; and the applicable laws and regulations of Ontario has reviewed and granted approval to the above referenced study on the approval date noted above. The membership of this HSREB also complies with the membership requirements for REB's as defined in Division 5 of the Food and Drug Regulations.

The ethics approval for this study shall remain valid until the expiry date noted above assuming timely and acceptable responses to the HSREB's periodic requests for surveillance and monitoring information. If you require an updated approval notice prior to that time you must request it using the University of Western Ontario Updated Approval Request form.

Member of the HSREB that are named as investigators in research studies, or declare a conflict of interest, do not participate in discussions related to, nor vote on, such studies when they are presented to the HSREB.

The Chair of the HSREB is Dr. Joseph Gilbert. The HSREB is registered with the U.S. Department of Health & Human Services under the IRR registration number IRR 00000940



# Curriculum Vitae

**Name:** John Leckie

**Post-secondary Education and Degrees:** The University of Western Ontario  
London, Ontario, Canada  
2007-2011 B.A.

The University of Western Ontario  
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2011-2013 M.Sc.

**Related Work Experience:** Teaching Assistant  
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