Effect of high-fat and high-carbohydrate diets on pulmonary O₂ uptake kinetics during the transition to moderate-intensity exercise

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Raper JA, Love LK, Paterson DH, Peters SJ, Heigenhauser GJF, Kowalchuk JM. Effect of high-fat and high-carbohydrate diets on pulmonary O₂ uptake kinetics during the transition to moderate-intensity exercise. J Appl Physiol 117: 1371–1379, 2014. First published October 2, 2014; doi:10.1152/japplphysiol.00456.2014.—Mitochondrial pyruvate dehydrogenase (PDH) regulates the delivery of carbohydrate-derived substrate to the mitochondrial tricarboxylic acid cycle and electron transport chain. PDH activity at rest and its activation during exercise is attenuated following high-fat (HFAT) compared with high-carbohydrate (HCHO) diets. Given the reliance on carbohydrate-derived substrate early in transitions to exercise, this study examined the effects of HFAT and HCHO on phase II pulmonary O₂ uptake (VO₂p) kinetics during transitions into the moderate-intensity (MOD) exercise domain. Eight active adult men underwent dietary manipulations consisting of 6 days of HFAT (73% fat, 22% protein, 5% carbohydrate) followed immediately by 6 days of HCHO (10% fat, 10% protein, 80% carbohydrate); each dietary phase was preceded by a glycogen depletion protocol. Participants performed three MOD transitions from a 20 W cycling baseline to work rate equivalent to 80% of estimated lactate threshold on days 5 and 6 of each diet. Steady-state VO₂p was greater (P < 0.05), and respiratory exchange ratio and carbohydrate oxidation rates were lower (P < 0.05) during HFAT. The phase II VO₂p time constant (τVO₂p) [HFAT 40 ± 16, HCHO 32 ± 19 s (mean ± SD)] and VO₂p gain (HFAT 10.3 ± 0.8, HCHO 9.4 ± 0.7 ml·min⁻¹·W⁻¹) were greater (P < 0.05) in HFAT. The overall adjustment (effective time constant) of muscle deoxygenation (Δ[Hb]) was not different between diets (HFAT 24 ± 4 s, HCHO 23 ± 4 s), which coupled with a slower rVO₂, indicates a slowed microvascular blood flow response. These results suggest that the slower VO₂p kinetics associated with HFAT are consistent with inhibition and slower activation of PDH, a lower rate of pyruvate production, and/or attenuated microvascular blood flow and O₂ delivery.

O₂ uptake kinetics; high-fat diet; high-carbohydrate diet; muscle deoxygenation; near-infrared spectroscopy; substrate oxidation

DURING STEP-TRANSITIONS IN WORK RATE (WR) initiated either from rest or light-intensity exercise, to heavier exercise intensities requiring a greater rate of ATP turnover, pulmonary O₂ uptake (VO₂p), and muscle O₂ utilization (VO₂m) increase with a finite time course toward a new, higher steady-state level (14, 34). The delay in attaining a steady state for VO₂p and VO₂m (and mitochondrial oxidative phosphorylation) is considered to reflect a metabolic capacitance (represented by total creatine) and resistance, which are inherent to the energy-producing systems (mitochondria) (24, 12). More specifically, this delay has been attributed to a sluggish activation of rate-limiting enzymes and metabolic pathways responsible for providing oxidizable substrates (other than O₂) to the mitochondrial tricarboxylic acid (TCA) cycle and electron transport chain (ETC) to a slow adjustment of microvascular blood flow and O₂ delivery to the exercising muscle, or to a combination of both factors [for reviews see (31, 33, 45)]. The consequence of a delay in meeting the increased ATP demands at the onset of exercise through oxidative phosphorylation is that there is greater reliance on substrate-level phosphorylation with a consequent fall in metabolic stability (15) within the active muscle as evidenced by an intensity-dependent increase in O₂ deficit and breakdown of phosphocreatine (PCr) and glycogen; accumulation of lactate−, hydrogen ions (H⁺), ADPₚ, AMPₚ, free inosine 5′-monophosphate (IMPₚ), and inorganic phosphate (Pi₂); and fall in the Gibbs free energy release associated with ATP hydrolysis.

The mitochondrial pyruvate dehydrogenase (PDH) complex catalyzes the rate-limiting, irreversible oxidative decarboxylation of pyruvate to acetyl CoA and therefore, regulates the entry of carbohydrate-derived substrate into the mitochondria for provision of acetyl CoA and reducing equivalents (NADH; FADH₂) to the TCA cycle and ETC, respectively (37). The activity of PDH is determined in part by covalent phosphorylation/dephosphorylation, and thus by the activities of the associated PDH kinase (PDK; phosphorylation/inhibition) and PDH phosphatase (dephosphorylation/activation) enzymes (37). For example, when exercise was initiated from a baseline of elevated PDH activity either by means of dichloroacetate (DCA; an inhibitor of PDK) infusion or subsequent to a priming bout of heavy-intensity exercise, the reliance on substrate-level phosphorylation was reduced (16, 44) and, in the case of priming exercise, the adjustment of VO₂p was faster (16). Alternatively, when the activation of PDH was attenuated [via hyperventilation-induced hypocapnic alkalosis (22)], physiological and metabolic responses consistent with a slower activation of oxidative phosphorylation were observed (6, 22).

It is established that PDH activation and whole-body rates of carbohydrate and fat oxidation during exercise are dependent on the availability of carbohydrate and fat within the muscle [for review see (38)]. Previous studies examining increased lipid (or decreased carbohydrate) availability following high-fat (low-carbohydrate) diets or intralipid infusions alone or in
combination with prior glycogen depletion (GD) have shown that PDH kinase was upregulated and steady-state PDH activity was downregulated (30, 32, 46), whereas increased carbohydrate availability (or reduced fatty acid availability) resulted in greater PDH activation (48).

Additionally, a high-fat meal was found to impair flow-mediated dilation (47), a consequence of impaired endothelial function (1). Given the importance of the vascular endothelium in blood flow regulation (7), dietary-induced endothelial dysfunction may limit the adjustment of microvascular blood flow and O₂ delivery at the onset of exercise, and thus possibly impact the activation of mitochondrial oxidative phosphorylation. Muscle deoxygenation as measured using near-infrared spectroscopy (NIRS) reflects the dynamic relationship between local muscle O₂ delivery and muscle O₂ utilization (9), and when combined with measures of VO₂p kinetics (reflecting muscle O₂ utilization), can provide information on the dynamics of microvascular blood flow during transitions to exercise.

Therefore, given the importance of carbohydrates in energy production early in exercise and the possible role of PDH in limiting the provision of carbohydrate-derived substrate and thus the activation of mitochondrial oxidative phosphorylation, the primary purpose of this study was to examine the effects of a high fat/low carbohydrate (HFAT) and high carbohydrate/low fat (HCHO) dietary intervention on the adjustment of phase II VO₂p (reflecting muscle O₂ utilization) during the transition to exercise. In addition, given the possible effects of diet on blood flow regulation, a secondary purpose was to examine the effects of diet on muscle deoxygenation and thus the dynamic relationship between local microvascular blood flow (and O₂ delivery) and muscle O₂ utilization. It was hypothesized that 1) phase II VO₂p kinetics would be slower during HFAT compared with HCHO, reflecting a slower activation of mitochondrial PDH and thus oxidative phosphorylation, and 2) muscle deoxygenation kinetics would be faster during HFAT, reflecting attenuated muscle microvascular blood flow and O₂ delivery dynamics, and an attenuated muscle microvascular blood flow-to-muscle O₂ utilization relationship, which also may contribute to slowed VO₂p during transitions to exercise.

METHODS

Subjects. Eight male subjects volunteered for this study. All subjects were healthy nonsmokers and had no known respiratory, cardiovascular, or metabolic disease. The protocol and procedures associated with this study were approved by The University of Western Ontario Research Ethics Board for Health Sciences Research Involving Human Subjects, in accordance with the Declaration of Helsinki.

Study design. During the initial visit to the laboratory subjects performed a ramp incremental (RI) test (25 W/min) to volitional fatigue on a cycle ergometer (H-300-R; Lode, Groningen, Netherlands) for determination of VO₂max and estimated lactate threshold (LT). Subjects were instructed to keep a 2-day dietary record of exact amounts of food and liquids consumed. The dietary records were analyzed for nutrient and energy intake using a commercially available computer software program (Diet Analysis Plus 8.0.1; Thomson-Nelson, Scarborough, ON, Canada), and individualized isocaloric diets (relative to the normal mixed diet) were designed and given to each subject. The dietary intervention protocols were modified on the basis of previous studies [e.g., (29, 32)] for the HFAT (75% fat, ~20% protein, ~5% carbohydrate) and HCHO (~10% fat, ~10% protein, ~80% carbohydrate) conditions.

The dietary intervention protocol began with the HFAT diet and was followed immediately by the HCHO diet with each dietary phase lasting 6 days. Before the start of each diet, subjects performed a GD protocol that consisted of 60 min of constant-load leg cycling exercise at a WR corresponding to ~70% VO₂max. This was followed by five repeated bouts of cycling at a WR corresponding to ~110% of the peak WR achieved during the RI test with each bout lasting 1 min and separated by 4 min of cycling at a light intensity WR (~20 W). Variations of this protocol have been shown previously to be effective in lowering muscle glycogen content by approximately 55–90% (5, 32). This GD protocol was included to amplify the shift in fuel selection for oxidation by maximally decreasing carbohydrate availability for oxidation during HFAT (thereby increasing fat oxidation) and maximally increasing carbohydrate availability during HCHO through glycogen supercompensation (thereby increasing carbohydrate oxidation).

Moderate-intensity exercise testing. On days 5 and 6 of each of the HFAT and HCHO diets, subjects reported to the laboratory having been advised to eat their final meal at least 2 h prior to arriving at the laboratory, and to not engage in strenuous physical activity or consume alcohol or caffeinated beverages during the previous 24-h period. Subjects then performed a series of constant-load, step exercise transitions on an electronically braked cycle ergometer. Each transition began with 6 min of baseline cycling at 20 W followed by an instantaneous increase in WR to an intensity equivalent to ~80% LT lasting 8 min. Three repetitions of this protocol were repeated on each of days 5 and 6 of the HFAT and HCHO diets, with each repetition separated by ~30 min of resting recovery to allow physiological and metabolic variables to return back toward pretransition, baseline levels.

On day 5 of each of the dietary phases a percutaneous Teflon catheter (Angiocath, 21 gauge) was inserted into a dorsal hand vein for blood sampling. The venous blood was arterialized by wrapping the hand and forearm with a heating pad, with additional heating provided by a tungsten heating lamp. Blood samples (~5 ml) were drawn at rest, during 20 W of baseline cycling, and at specific times (1, 2, 3, 4, 6, and 8 min) during the transition to the moderate intensity (MOD) WR. One portion of whole blood (100 μl) was deproteinized 1:4 with 500 μl of 0.5 M perchloric acid (PCA), placed on ice for 5 min, mixed and neutralized with 125 μl of 1 M K₂CO₃. The neutralized sample was centrifuged for 5 min and the PCA extract was removed, placed into a labeled Eppendorf tube, and stored at ~80°C for later analysis of β-hydroxybutyrate, glucose, and lactate as previously described (4). Another portion of whole blood (~3 ml) was centrifuged for 5 min, and the supernatant transferred to a labeled Eppendorf tube and stored at ~80°C for later analysis of plasma concentrations of free fatty acids (FFA) (NEFA C test kit; Wako Chemicals, Richmond VA). The remaining plasma portion of the blood sample was stored at ~80°C for later analysis of plasma concentrations of insulin (Coat-a-Count insulin test kit; Diagnostics Products, Los Angeles, CA).

Measurements. Gas-exchange measurements were similar to those previously described (36). Briefly, inspired and expired flow rates were measured using a low dead space (90 ml) bidirectional turbine (VMM 110; Alpha Technologies). Inspired and expired gases were sampled continuously (every 20 ms) at the mouth and analyzed for concentrations of O₂, CO₂, and N₂ by mass spectrometry (Amis 2000; Innovision, Odense, Denmark). Data collected every 20 ms 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 outlined by Beaver et al. (2).

Heart rate (HR) was monitored continuously by electrocardiogram (ECG) using PowerLab ML132/ML880 (ADInstruments, Colorado Springs, CO) and using a three-lead ECG arrangement. Data were recorded on a separate data collection computer using LabChart v4.2 (ADInstruments).
Local muscle oxygenation and deoxygenation profiles of the vastus lateralis were made using NIRS (NIRO 300; Hamamatsu Photonics, Hamamatsu, Japan). Optodes were placed on the belly of the muscle midway between the lateral epicondyle and greater trochanter of the femur using an interoptode spacing of 5 cm. The optodes were housed in an optically dense plastic holder thus ensuring that the position of the optodes, relative to the skin, was fixed and invariant. The thigh, with the attached optodes and covering, was wrapped with an elastic bandage to minimize movement of the optodes while still permitting freedom of movement for cycling.

The physical principles of tissue spectroscopy and the manner in which these are applied were described in detail by Elwell (10). Briefly, one fiber optic bundle carried the near infrared light produced by the laser diodes to the tissue of interest while a second fiber optic bundle returned the transmitted light from the tissue to a photon detector (photomultiplier tube) in the spectrometer. The intensity of the incident and transmitted light were recorded continuously at 2 Hz and, along with the specific extinction coefficients and optical path length, used for online estimation and display of the concentration changes of oxy-(Δ[O₂Hb]), deoxy-(Δ[HHb]), and total (Δ[HbTOT]) hemoglobin-myglobin relative to the steady-state, resting baseline; subjects rested quietly for at least 5 min to ensure steady-state conditions before the NIRS unit was initialized and the signals set to zero. Because of the uncertainty of the actual path length traveled by the photons in muscle tissue and the stability of the path length during the transition to exercise, values are reported in arbitrary units (au). In this study, changes in [HHb] from a zero baseline prior to the onset of exercise are reported as a change (Δ) in au.

**Data analysis.** Gas exchange data for each trial were edited by removing aberrant data points that lay outside 4 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 each transition were ensemble-averaged to yield a single average 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 on-transient HR response was modeled from the onset of exercise to end-exercise (480 s), with the time delay fixed to the onset of the exercise transition (i.e., TD = 0).

The NIRS-derived Δ[HHb] data were time-aligned and ensemble-averaged to 5-s bins to yield a single response for each subject. The Δ[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 toward a new steady-state exercise value (16). The time delay for the Δ[HHb] response (TD-Δ[HHb]) was estimated for each subject using the second-by-second data (i.e., data before they were ensemble-averaged into 5-s bins) and corresponded to the time after the onset of exercise where the Δ[HHb] signal showed a consistent increase above the baseline or nadir value, as described previously (16). Determination of the TD-Δ[HHb] was made on individual trials and averaged to yield a single value for each individual. The Δ[HHb] data were modeled from the end of the TDA[HHb] to the end of exercise using an exponential model as described in equation 1. The τΔ[HHb] described the time course for the increase in Δ[HHb], whereas the overall change of the effective time constant for Δ[HHb] (τΔ[HHb] = TD[AHHb] + τΔ[HHb]) described the overall time course of the Δ[HHb] from the onset of exercise.

The Δ[O₂Hb] and Δ[HbTOT] signals did not approximate an exponential response and thus were not modeled during the exercise transition. The Δ[O₂Hb] and Δ[HbTOT] values were reported as the average responses calculated during the steady state at baseline (30 s to 0 s) and end-exercise (450 s to 480 s).

**Statistics.** Differences in dependent variables and model parameter estimates between HFAT and HCHO diets were analyzed using paired t-tests and repeated measures ANOVA. Statistical analyses were performed using SPSS (v. 17; SPSS, Chicago, IL). Statistical significance was accepted at P < 0.05, with significant interactions and main effects analyzed using Tukey’s LSD post hoc test. All data are presented as means ± SD.

### RESULTS

**Subject characteristics and glycerol depletion protocol.** Subject characteristics and results of the initial RI test are presented in Table 1. Body mass was lower (P < 0.05) during the HFAT (82 ± 8 kg) diet compared with either the normal mixed (84 ± 8 kg) or HCHO (84 ± 8 kg) diets despite there being no differences in caloric intake among the diets. Analysis of the dietary composition confirmed differences (P < 0.05) between the HCHO and HFAT diets (Table 2).

**Table 1. Physical characteristics and response to initial ramp incremental test**

<table>
<thead>
<tr>
<th>Age, yr</th>
<th>Body Mass, kg</th>
<th>Height, cm</th>
<th>VO₂peak, liter/min</th>
<th>VO₂peak, ml·kg⁻¹·min⁻¹</th>
<th>0% 80% VO₂peak, liter/min</th>
<th>0% 80% VO₂peak, W</th>
<th>WRmax, W</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 ± 1</td>
<td>84 ± 8</td>
<td>181 ± 6</td>
<td>3.77 ± 0.37</td>
<td>45 ± 4</td>
<td>2.11 ± 0.29</td>
<td>1.69 ± 0.23</td>
<td>93 ± 20</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SD. VO₂peak, peak oxygen uptake; 0% 80% VO₂peak, estimated lactate threshold; WRmax, final work rate at the point of fatigue during the ramp incremental test.
During the GD protocol, WRs corresponding to the heavy intensity, constant-load exercise bout at 70% \( \dot{V}O_{2\text{peak}} \) and to the severe-intensity, intermittent exercise bouts at 110% \( WR_{\text{max}} \) were 189 ± 24 W and 369 ± 42 W, respectively. Subjects successfully completed the entire GD protocol before the start of the HFAT diet, but were able to complete only 33 ± 2 min of the heavy intensity constant-load exercise bout prior to the start of the HCHO diet, and although they were able to complete all five of the severe-intensity interval exercise bouts, they were unable to maintain the required WR (due either to a decrease in cadence or to a decrease in imposed load) despite strong verbal encouragement.

**Substrate oxidation rates and blood metabolites.** Calculated respiratory exchange ratio (RER) and fat and carbohydrate oxidation rates for steady-state baseline and the steady state of the moderate-intensity exercise transition are presented in Fig. 1. The steady-state RER was lower (\( P < 0.001 \)) during the HFAT than in the HCHO diet at baseline (HFAT 0.82 ± 0.09, HCHO 0.95 ± 0.14) and end-exercise (HFAT 0.83 ± 0.02, HCHO 0.96 ± 0.04). The steady-state fat oxidation rates were higher (\( P < 0.05 \)) in HFAT than in HCHO at baseline (HFAT 0.29 ± 0.14 g/min, HCHO 0.18 ± 0.08 g/min) and at end-exercise (HFAT 0.48 ± 0.09 g/min, HCHO 0.12 ± 0.06 g/min). Also, the steady-state carbohydrate oxidation rates were lower (\( P < 0.001 \)) in HFAT than in HCHO at baseline (HFAT 0.51 ± 0.36 g/min, HCHO 0.95 ± 0.54 g/min) and end-exercise (HFAT 0.99 ± 0.25 g/min, HCHO 1.81 ± 0.42 g/min).

Blood [glucose] was higher (\( P < 0.05 \)) at rest in HCHO (5.02 ± 1.52 mmol/liter) than HFAT (2.74 ± 0.98 mmol/liter), but was similar between conditions during exercise (end-exercise HCHO 4.19 ± 0.93 mmol/liter, end-exercise HFAT 3.59 ± 0.92 mmol/liter). Blood [lactate] was higher (\( P < 0.05 \)) in HCHO than in HFAT from the second minute to end-exercise (HFAT 0.48 ± 0.09 g/min, HCHO 0.12 ± 0.06 g/min). Also, the steady-state carbohydrate oxidation rates were lower (\( P < 0.001 \)) in HFAT than in HCHO at baseline (HFAT 0.51 ± 0.36 g/min, HCHO 0.95 ± 0.54 g/min) and end-exercise (HFAT 0.99 ± 0.25 g/min, HCHO 1.81 ± 0.42 g/min).

Blood [insulin] was higher (\( P < 0.05 \)) in HFAT than in HCHO at baseline (HFAT 0.99 ± 0.25 g/min, HCHO 1.81 ± 0.42 g/min).

Gas exchange (\( \dot{V}O_2, \dot{V}CO_2 \)) kinetics. The pulmonary \( \dot{V}O_2 \) responses during the transitions to MOD exercise are presented in Fig. 2 and the parameter estimates for \( \dot{V}O_2p \) kinetics are presented in Table 3. For steady-state baseline (20 W) and end-exercise (30 W), the phase II \( \dot{V}O_2p \) time constant (\( \tau_\text{VO}_2p \)) was 25% greater \( (P < 0.015) \) in HFAT \( (40 \pm 16 \ s; \ Ch_95\%, 3.6 \ s) \) than in HCHO \( (32 \pm 19 \ s; \ Ch_95\%, 2.5 \ s, \ Fig. \ 3) \).

Baseline \( \dot{V}CO_2p \) (HFAT 0.80 ± 0.13 liter/min, HCHO 0.83 ± 0.15 liter/min, \( P < 0.105 \)) and end-exercise \( \dot{V}CO_2p \) (HFAT 1.41 ± 0.20 liter/min, HCHO 1.45 ± 0.24 liter/min, \( P < 0.048 \)) were lower, and the rate of adjustment of \( \dot{V}CO_2p \) during the exercise transition (\( \tau_\text{VCO}_2p \)) HFAT 105 ± 42 s, \( \tau_\text{VCO}_2p \) HCHO 79 ± 42 s, \( P < 0.040 \)) was greater in HFAT than in HCHO.

**HR kinetics.** The HR responses to MOD exercise are presented in Table 4. Baseline (\( P < 0.024 \)) and end-exercise HRs (\( P < 0.003 \)) were ~10 beats per minute higher in HFAT than in HCHO, with no difference in HR amplitude between diets. The \( \tau\text{HR} \) was greater (\( P < 0.014 \)) in the HFAT \( (31 \pm 10 \ s) \) than in the HCHO diet \( (24 \pm 8 \ s) \).

NIRS-derived deoxy-(\( \Delta[Hb] \)), oxy-(\( \Delta[O_2Hb] \)), and total (\( \Delta[HbT] \)) hemoglobin concentration change. Parameter estimates for \( \Delta[Hb] \) exercise response are presented in Table 5. The delay prior to a sustained increase in the \( \Delta[Hb] \) (TD\( \Delta[Hb] \)) was ~12 s, with no difference between condi-
The major new findings presented in this study are 1) during the transition to MOD exercise, the time constants for the phase II $\dot{V}_O_2p$ ($\tau_{\dot{V}_O_2p}$) and $\dot{V}_CO_2p$ ($\tau_{\dot{V}_CO_2p}$) responses were ~25% and ~30% greater, respectively, in the HFAT compared with the HCHO diet; 2) the $\dot{V}_O_2p$ gain (i.e., $\Delta \dot{V}_O_2p/\Delta W_R$), a reflection of the $O_2$ cost of exercise and related inversely to exercise efficiency, was greater during the HFAT compared with the HCHO diet; 3) muscle deoxygenation kinetics (i.e., MRT$[\Delta[Hb]]$) were similar between diets despite slower $\dot{V}_O_2p$ kinetics in the HFAT condition; and 4) steady-state baseline and end-exercise HRs and HR kinetics ($\tau_{HR}$) during the transition to exercise were greater in the HFAT diet.

Effectiveness of the dietary-GD intervention. The sequence of a 6-day HFAT followed by a 6-day HCHO dietary intervention used in the present study, with each preceded by a GD exercise protocol, has been shown previously to minimize (HFAT) and then to maximize (HCHO) muscle carbohydrate (glycogen supercompensation) availability [as modified from Bergström et al. (5)]. Variations of this protocol were previously shown to be effective in lowering muscle glycogen content by approximately 55–90% (5, 32), and simultaneously to downregulate and attenuate the exercise-induced activation of PDH in HFAT compared with HCHO (8, 29, 32). Although muscle glycogen content was not measured in the present study, the inclusion of heavy- and severe-intensity exercise bouts immediately before starting each of the dietary protocols, the extremes in fat/carbohydrate composition of the diets...
(HFAT ~75% fat, ~5% carbohydrate; HCHO ~10% fat, ~80% carbohydrate), and the duration of the dietary intervention (6 days) ensured that muscle glycogen content and carbohydrate availability were lower [and muscle triglyceride levels were higher (21, 41)] in the HFAT than in the HCHO portions of the protocol. The effectiveness of the dietary intervention is supported by the following: 1) steady-state RER and \( \Delta V_{CO_2p} \) were higher (by ~16% and ~3%, respectively), and absolute \( V_{O_2p} \) and \( V_{CO_2p} \) gain were lower (by ~11% and ~9%, respectively) in HCHO; 2) steady-state fat oxidation rates were higher (by approximately twofold to fourfold) in HFAT, whereas carbohydrate oxidation rates were higher (by approximately twofold) in HCHO, which is in accordance with previous studies showing similar changes (8, 42); 3) blood [glucose] was higher in HCHO, while blood [FFA] and [\( \beta \)-hydroxybutyrate] were elevated in HFAT; and 4) steady-state HR was higher (by ~11%) in HFAT perhaps to enhance bulk \( O_2 \) delivery to muscle in support of the increased \( O_2 \) requirement of fatty acid oxidation. Thus the dietary-induced effects on fat and carbohydrate oxidation rates suggests that reduced availability of carbohydrate during HFAT, and greater carbohydrate availability during HCHO, resulted in adaptations within muscle, which shifted the substrate being preferentially oxidized during exercise.

**Effect of diet on \( V_{O_2p} \), HR, and muscle deoxygenation kinetics.** The main novel finding of the present study was that phase II \( V_{O_2p} \) kinetics were slowed (\( P < 0.015 \)) in the HFAT compared with the HCHO diet during the transition to MOD exercise, reflecting a slower activation and adjustment of muscle \( O_2 \) utilization and mitochondrial oxidative phosphorylation in the HFAT diet. In addition, HR kinetics (\( \tau \)HR), a measure of central blood flow (cardiac output) adjustment, also was slower (\( P < 0.014 \)) in HFAT than in HCHO diets. In a previous study, Hughson and Kowalchuk (19) examined the relationship between \( V_{CO_2p} \) and ventilation after a 72-h period of HFAT or HCHO dietary restrictions in females, and although it was not a focus of their study, they reported no differences in \( \tau V_{O_2p} \) between diets. However, in that study, only a single exercise transition was performed within each of the dietary conditions, and gas exchange variables were calculated less frequently (15-s intervals during the first 5 min of the test, 30-s intervals during the final 5 min of the 10-min exercise protocol), which would reduce the confidence of estimating the kinetic parameters for the gas exchange variables. Additionally, participants were all females and there was no control for their menstrual cycle phase; the prediet GD protocol was less severe, being performed at a lower exercise intensity and without inclusion of severe-intensity exercise bouts; the prescribed diets were not monitored or analyzed for caloric intake and dietary composition, and the diets were each separated by a 5-day period of consuming a balanced, mixed diet.

Molé and Hoffmann (25) proposed that the initial changes in substrate flux and fat and carbohydrate oxidation rates would determine the phase II dynamics of muscle \( V_{O_2p} \). Using assumptions related to control and time course of activation of metabolic flux rates, the authors used computer modeling to deconstruct the phase II and III \( V_{O_2p} \) profiles into individual components related to rate of activation of fat and carbohydrate oxidation-dependent components in relation to RER during exercise. The major finding of that paper, as related to our present study, was that the time constant for fat oxidation (\( \tau \)FAT) was independent of RER, whereas the time constant for carbohydrate oxidation (\( \tau \)CHO) was inversely related to RER. We further analyzed their data [presented in Table 4 on p. 2,101 in Molé and Hoffmann (25)] by calculating an overall mean response time (MRT) for \( V_{O_2p} \) (calculated as a weighted sum of the asymptotes and time constant; the reported time delay was ignored for simplicity) for each of the fat and carbohydrate components [similar to that described in Hughson et al. (18)]. According to this calculation, the overall MRT was inversely related to RER (i.e., MRT of ~15 s and ~60 s at an RER of 0.97 and 0.78, respectively), a trend similar to that
reported in the present study. In their discussion, Molé and Hoffmann state that while their results were “encouraging, [they] must reserve judgment until a more complete assessment of dietary effects can be made and other experimental tests of the model are undertaken and reported” [p. 2,103 (25)]. Our current study, which specifically altered dietary intake and muscle substrate availability, extends the findings of Molé and Hoffmann (25) by including a more rigorous estimation of VO2p kinetics during defined dietary and muscle substrate interventions.

The slower VO2 adjustment in HFAT compared with HCHO may be attributed in part to an inhibition and attenuated activation of the mitochondrial PDH complex. Previous studies reported that after 3–6 days of a HFAT diet, or after 30 min of intralipid infusion to raise plasma [FFA], PDH activity was lower at rest (28, 30, 32, 39, 46) and during exercise (32), and the rate of PDH activation during the transition to exercise was slowed (32). Also, the resting PDH kinase (PDK) activity responsible for catalyzing the covalent phosphorylation and thus inhibition of the PDH complex, was elevated early (within 1–3 days) on a HFAT diet in humans (30, 29, 46), and within 24 h of fasting in rats (28). Additionally, both PDK mRNA and mitochondrial PDK protein content were elevated (within 1 day) on the HFAT diet (28, 29). Thus a fat-induced inhibition of PDH activity may delay the activation of PDH during transitions to exercise and attenuate the delivery of carbohydrate-derived substrate (acetyl CoA, reducing equivalents) to the mitochondrial TCA and ETC pathways.

In the present study, each of the diets was preceded by a heavy/severe-intensity GD protocol to lower existing muscle glycogen levels. By design, carbohydrate ingestion in HFAT compared with HCHO was minimized, thereby ensuring that muscle glycogen content would remain lower during the HFAT than during the HCHO phase of the diet. It was expected that establishing and maintaining low muscle glycogen levels in combination with an HFAT diet would reduce the rate of muscle glycogenolysis and attenuate muscle pyruvate production and oxidation during a subsequent bout of exercise. Putman and coworkers (32) reported that a combined GD and HFAT dietary protocol was associated with reduced PDH activity at rest, and at the onset of exercise, glycogen breakdown, pyruvate formation, and PDH activation were lower in HFAT than HCHO. In contrast, St. Amand and coworkers (39) reported that HFAT alone (i.e., without prior GD) was associated with a lower PDH activity at rest, but during MOD exercise, muscle PDH activation was not different between the HFAT and higher carbohydrate diet, presumably because of higher rates of glycogen breakdown and pyruvate production. Importantly, pyruvate is both a substrate for the PDH-catalyzed reaction and an allosteric inhibitor of the PDH kinase, therefore a low rate of pyruvate formation during exercise in HFAT (a consequence of a low resting muscle glycogen content) would prevent or slow activation of the PDH complex through its combined effects as a substrate for the PDH-catalyzed reaction and as an allosteric inhibitor of PDH kinase (32).

Pharmacological activation of PDH before exercise with DCA administration (35, 44) was shown to reduce glycogen and PCR breakdown, reduce muscle lactate accumulation, reduce substrate-level phosphorylation (44), and increase the rate of fall of muscle intracellular P02 (17). Also performing a prior bout of heavy-intensity exercise to prime the muscle (16) was associated with a higher pretransition level of PDH activation and faster VO2p kinetics during the transition to exercise (16). Taken together, these responses are consistent with a faster activation of mitochondrial oxidative phosphorylation. Given the regulatory (rate-limiting) role played by PDH in providing carbohydrate-derived substrate to the mitochondrial oxidative pathways and the reported downregulation of PDH activity in high-fat, low-carbohydrate conditions, it is not unreasonable to suggest that inhibition of PDH activation at the onset of exercise may impose a metabolic limitation and constrain the activation of oxidative phosphorylation that may contribute to the slower VO2p kinetics during the HFAT diet in the present study. However, a measurable speeding of VO2p kinetics following DCA-induced PDH activation has not always been observed (13, 35). Therefore, although PDH can impose a limitation under some circumstances or in some individuals, in normal circumstances it may not always be limiting for the adjustment of oxidative phosphorylation and VO2p kinetics.

In the present study, NIRS was used to measure oxygenation and deoxygenation changes within the vastus lateralis muscle. Increases in muscle deoxygenation (i.e., Δ[HbO2]) are related in part to muscle fractional O2 extraction and thus the ratio of local muscle microvascular blood flow-to-O2 utilization (QO2r-to-VO2m), and when used in combination with phase II VO2p kinetics, the Δ[HbO2] provides an estimate of local microvascular blood flow changes during the exercise transition (11). In the present study, the overall Δ[HbO2] kinetics as defined by the τΔ[HbO2] was similar between dietary conditions despite the slower VO2p kinetics in the HFAT condition. Combined, these data suggest possible differences in microvascular blood flow between dietary conditions, with the adjustment of microvascular blood flow being attenuated and/or slowed relative to the increase in muscle O2 utilization in the HFAT than in the HCHO diet.

It was previously demonstrated that high fat content in the diet or meals, or elevated intralipid-infusion-induced plasma FFA levels were associated with the development of insulin resistance, endothelial dysfunction, impaired endothelial nitric oxide production, and reduced microvascular perfusion (23, 26, 43). However, studies have shown that exercise/contraction-induced increases in blood flow are intact in models that display endothelial dysfunction (20, 49), indicating that exercise/contraction follows endothelium-independent mechanisms for increasing blood flow. In particular, St.-Pierre et al. (40) showed that contraction-mediated increases in microvascular blood flow were not altered by high fat feeding in rats. However, the similar kinetics of muscle deoxygenation in the two dietary conditions despite a slower rate of adjustment of muscle O2 utilization may reflect an impaired adjustment of muscle microvascular blood flow in the HFAT condition (i.e., the slow microvascular blood flow and O2 delivery limits the activation of muscle O2 utilization) (3). Alternatively, a slower muscle blood flow response in HFAT may simply be consequent to the slower adjustment of muscle O2 utilization (i.e., consistent with a metabolic limitation). Although these data cannot distinguish between the two mechanisms, in either case, during the exercise transient, a given change in WR (and presumably ATP utilization) is associated with a lower VO2m and higher HHb-to-VO2m in HFAT, implicating a lower microvascular blood flow response in the HFAT condition.
Conclusion. This study demonstrated that a GD plus 6-day HFAT dietary intervention followed by an HCHO dietary intervention was effective in altering fuel selection during exercise, with carbohydrate oxidation rate being lower and fat oxidation rate being greater in the HFAT than in the HCHO condition. Additionally, the steady-state baseline and end-MOD exercise VO_{2p} and HR, and the increase in VO_{2p} gain (ΔVO_{2p}/ΔWR) were greater in the HFAT than in the HCHO condition, consistent with the higher O_{2} cost of fat compared with carbohydrate oxidation, whereas the higher VO_{2p} gain in HFAT suggests that exercise efficiency was lower compared with the HCHO condition. The novel findings of the present study were that the phase II pulmonary VO_{2p} time constant (τVO_{2p}) was ~25% greater in the HFAT (40 s) compared with the HCHO condition (32 s), suggesting that the adjustment of muscle O_{2} utilization and mitochondrial oxidative phosphorylation were slowed during the transition to MOD exercise during the HFAT compared with the HCHO diet. In spite of slower VO_{2p} kinetics in the HFAT condition, muscle deoxygenation kinetics were similar in the two dietary conditions, suggesting that the adjustment of microvascular blood flow during the exercise transition was lower and/or slower during the HFAT diet. Therefore, the slower VO_{2p} kinetics during the transition to MOD exercise in the HFAT compared with the HCHO diet may reflect a downregulation of PDH activity at baseline and delayed activation during the exercise transition, and/or a slower rate of glycogenolysis and pyruvate production, which would attenuate the delivery of carbohydrate-derived substrate [i.e., acetyl-CoA; reducing equivalents (NADH; FADH_{2})] into the mitochondrial TCA cycle and ETC pathways. A slower adjustment of microvascular blood flow could slow O_{2} diffusion from the red blood cell to the mitochondria, thereby limiting availability of O_{2} at the mitochondrial terminal oxidase. Thus attenuated deliveries of oxidative substrate required in oxidative phosphorylation (O_{2}, NADH, H^{+}) in the HFAT compared with the HCHO condition could contribute to a slower VO_{2mn} and thus slow the adjustment of pulmonary VO_{2p} during the transition to MOD exercise.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES


