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## Decreased PDH activation and glycogenolysis during exercise following fat adaptation with carbohydrate restoration

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**Stellingwerff, Trent, Lawrence L. Spriet, Matthew J. Watt, Nicholas E. Kimber, Mark Hargreaves, John A. Hawley, and Louise M. Burke.** Decreased PDH activation and glycogenolysis during exercise following fat adaptation with carbohydrate restoration. *Am J Physiol Endocrinol Metab* 290: E380–E388, 2006. First published September 27, 2005; doi:10.1152/ajpendo.00268.2005.—Five days of a high-fat diet while training, followed by 1 day of carbohydrate (CHO) restoration, increases rates of whole body fat oxidation and decreases CHO oxidation during aerobic cycling. The mechanisms responsible for these shifts in fuel oxidation are unknown but involve up- and downregulation of key regulatory enzymes in the pathways of skeletal muscle fat and CHO metabolism, respectively. This study measured muscle PDH and HSL activities before and after 20 min of cycling at 70%  $\dot{V}O_{2\text{ peak}}$  and 1 min of sprinting at 150% peak power output (PPO). Estimations of muscle glycogenolysis were made during the initial minute of exercise at 70%  $\dot{V}O_{2\text{ peak}}$  and during the 1-min sprint. Seven male cyclists undertook this exercise protocol on two occasions. For 5 days, subjects consumed in random order either a high-CHO (HCHO) diet (10.3 g·kg<sup>-1</sup>·day<sup>-1</sup> CHO, or ~70% of total energy intake) or an isoenergetic high-fat (FAT-adapt) diet (4.6 g·kg<sup>-1</sup>·day<sup>-1</sup> FAT, or 67% of total energy) while undertaking supervised aerobic endurance training. On day 6 for both treatments, subjects ingested an HCHO diet and rested before their experimental trials on day 7. This CHO restoration resulted in similar resting glycogen contents (FAT-adapt 873 ± 121 vs. HCHO 868 ± 120 μmol glucosyl units/g dry wt). However, the respiratory exchange ratio was lower during cycling at 70%  $\dot{V}O_{2\text{ peak}}$  in the FAT-adapt trial, which resulted in an ~45% increase and an ~30% decrease in fat and CHO oxidation, respectively. PDH activity was lower at rest and throughout exercise at 70%  $\dot{V}O_{2\text{ peak}}$  (1.69 ± 0.25 vs. 2.39 ± 0.19 mmol·kg wet wt<sup>-1</sup>·min<sup>-1</sup>) and the 1-min sprint in the FAT-adapt vs. the HCHO trial. Estimates of glycogenolysis during the 1st min of exercise at 70%  $\dot{V}O_{2\text{ peak}}$  and the 1-min sprint were also lower after FAT-adapt (9.1 ± 1.1 vs. 13.4 ± 2.1 and 37.3 ± 5.1 vs. 50.5 ± 2.7 glucosyl units·kg dry wt<sup>-1</sup>·min<sup>-1</sup>). HSL activity was ~20% higher ( $P = 0.12$ ) during exercise at 70%  $\dot{V}O_{2\text{ peak}}$  after FAT-adapt. Results indicate that previously reported decreases in whole body CHO oxidation and increases in fat oxidation after the FAT-adapt protocol are a function of metabolic changes within skeletal muscle. The metabolic signals responsible for the shift in muscle substrate use during cycling at 70%  $\dot{V}O_{2\text{ peak}}$  remain unclear, but lower accumulation of free ADP and AMP after the FAT-adapt trial may be responsible for the decreased glycogenolysis and PDH activation during sprinting.

skeletal muscle metabolism; pyruvate dehydrogenase; substrate phosphorylation; fat oxidation; carbohydrate oxidation

THERE HAS BEEN MUCH INTEREST in the metabolic adaptations that occur in skeletal muscle following a high-fat diet to increase rates of whole body fat oxidation and decrease rates of carbohydrate (CHO) oxidation. It is well established that consuming a high-fat diet for >3 days decreases muscle glycogen utilization (11, 21, 30), elevates plasma free fatty acid (FFA) concentrations (16, 19, 31), and elevates FFA uptake by the muscle (12, 16, 19) during steady-state exercise, compared with an isoenergetic high-CHO diet.

Intriguingly, a series of recent studies has shown that increases in whole body fat oxidation induced by a high-fat diet persist even after 1 day of CHO restoration. This CHO restoration fully normalized glycogen levels and, most likely, liver stores, (3, 5, 6, 38) and abolished the increased plasma FFA concentrations reported after chronic high-fat diets (3, 5, 6). The residual effect of the high-fat diet in these studies may be due to the well-trained status of the subjects coupled with the maintenance of a high volume of aerobic exercise during the 5-day high-fat diet protocol (6, 11). The potential mechanisms responsible for these shifts in fuel utilization are equivocal but would be expected to involve either an up- and/or downregulation of key regulatory enzymes in the pathways of skeletal muscle fat and CHO metabolism, respectively (for reviews see Refs. 4 and 28).

Therefore, the primary aim of this study was to compare the effects of a 5-day high-fat diet with 1 day of CHO restoration (FAT-adapt) to a 6-day isoenergetic high-CHO diet (HCHO) on the regulation of key enzymes involved in skeletal muscle CHO and fat metabolism. Measurements of pyruvate dehydrogenase (PDH) and hormone-sensitive lipase (HSL) activities were made during 20 min of cycling at 70% peak  $\dot{V}O_{2\text{ peak}}$  consumption ( $\dot{V}O_{2\text{ peak}}$ ) and 1 min of sprinting at 150% peak power output (PPO). Estimations of muscle glycogenolysis [estimate of flux through glycogen phosphorylase (Phos)] were also made during the initial minute of exercise at 70%  $\dot{V}O_{2\text{ peak}}$  and during the 1-min sprint. We hypothesized that a 5-day high-fat diet, followed by 1 day of CHO restoration, would result in decreased muscle glycogenolysis at the onset of exercise, decreased PDH activity, and increased HSL activity during exercise at 70%  $\dot{V}O_{2\text{ peak}}$ . A second hypothesis was that decreased pyruvate contents and reduced accumulations of free ADP and AMP after FAT-adapt would explain the enzymatic changes of estimated glycogenolysis (Phos flux) and PDH activity.

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We also estimated skeletal muscle substrate phosphorylation (anaerobic ATP provision) during the initial minute of cycling at 70%  $\dot{V}O_{2\text{ peak}}$  and the 1-min sprint. We hypothesized that a reduction in muscle glycogenolysis following the high-fat diet protocol would also translate into decreases in substrate phosphorylation at the onset of exercise and during the 1-min sprint. To our knowledge, no previous studies have utilized this unique short-term high-fat diet with CHO restoration in conjunction with maintained aerobic exercise training in well-trained subjects to examine the accompanying changes in skeletal muscle metabolism during exercise.

## METHODS

**Subjects and preliminary testing.** Seven endurance-trained male cyclists or triathletes ( $30.0 \pm 0.7$  yr;  $72.7 \pm 2.9$  kg;  $\dot{V}O_{2\text{ peak}}$   $60.7 \pm 2.6$  ml·kg<sup>-1</sup>·min<sup>-1</sup>; sustained PPO  $334 \pm 17$  W, means  $\pm$  SE) participated in this study, which was approved by the Ethics Committee of the Australian Institute of Sport. All subjects were fully informed about the possible risks of all procedures before providing their written consent.

Before the experimental trials, each subject undertook an incremental cycling test to exhaustion on an electronically braked cycle ergometer (Lode, Groningen, The Netherlands). The initial testing protocol has been described in detail previously (15). During the maximal test and during the steady-state portion of the experimental trials, subjects inspired air through a mouthpiece and low-resistance turbine ventilometer (Flowtransformer K 520; KL Engineering, Belconnen, Australia) and gas analysis system (O<sub>2</sub> and CO<sub>2</sub> analyzer; AEI Technologies, Pittsburgh, PA) interfaced to a computer, which calculated the instantaneous rates of O<sub>2</sub> consumption ( $\dot{V}O_2$ ), CO<sub>2</sub> production ( $\dot{V}CO_2$ ), minute ventilation ( $V_{\text{ESTPD}}$ ), and the respiratory exchange ratio (RER) every 30 s from conventional equations. Before each maximal test and all experimental trials, the analyzers were

calibrated with commercially available gases of known O<sub>2</sub> and CO<sub>2</sub> content.  $\dot{V}O_{2\text{ peak}}$  was defined as the highest O<sub>2</sub> uptake a subject attained during any 60 s of the test. PPO was calculated from the last completed work rate plus the fraction of time spent in the final noncompleted work rate multiplied by 25 W. These values were used to determine the power output corresponding to ~70% of each subject's  $\dot{V}O_{2\text{ peak}}$  (63% of PPO) and 150% PPO to be used in the subsequently described experimental trials.

**Study design.** Each subject undertook two 7-day trials in a randomized, crossover design separated by a 2-wk washout period (Fig. 1A). As in our previous studies (3, 5, 6), treatments consisted of supervised training while undertaking either a 5-day adaptation to a high-fat diet followed by a 1-day CHO restoration (FAT-adapt trial) or a 6-day high-CHO diet (HCHO trial). On *day 1* of each trial, subjects reported to the laboratory after a 12- to 14-h overnight fast. They undertook the same high-intensity cycling protocol developed as the test protocol to be undertaken on *day 7* of each trial. This exercise block, which served as the initial training session for each trial, was undertaken to test the suitability of power outputs and gearing used in the high-intensity portions of the test protocol and to familiarize the subjects with the time trial (TT) component of the protocol.

On completion of this familiarization session, subjects commenced 5 days of a supervised diet and training program. During the high-fat adaptation (FAT-adapt), subjects were prescribed a high-fat ( $4.6$  g·kg<sup>-1</sup>·day<sup>-1</sup> or 67% energy from fat), low-CHO ( $2.5$  g·kg<sup>-1</sup>·day<sup>-1</sup> CHO, 18% of energy) diet supplying 0.25 MJ/kg body mass (BM) and 2.3 g/kg protein (15% of energy). The fatty acid breakdown of the diet varied little from day to day, and was ~40–50% saturated, 40–45% monounsaturated, and 10% polyunsaturated fatty acids. The control treatment (HCHO) was a protein- and energy-matched diet providing  $10.3$  g·kg<sup>-1</sup>·day<sup>-1</sup> and 70% of energy from CHO and 1.0 g/kg and 15% of energy from fat. Diets were constructed to maximize, or at least match, absorbable energy. Fiber intake was kept to a daily mean intake of ~40 g and matched to within 5–10 g

**A**

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7
<b>DIET</b>	FAT or CHO	FAT or CHO	FAT or CHO	FAT or CHO	FAT or CHO	CHO restoration	Testing Trial (see Fig 1b for details)
<b>TRAINING</b>	Interval Training	3-4 hr long ride	2-3 hr hill ride	Interval Training	3-4 hr long ride	Rest	

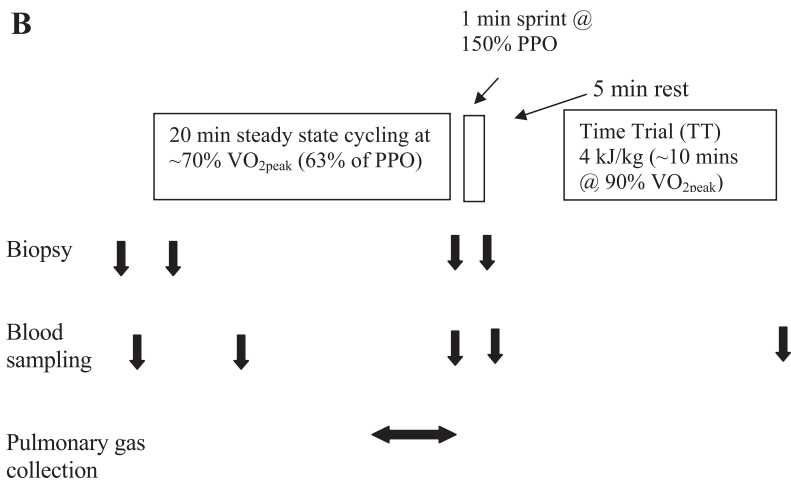


Fig. 1. A: overview of 7-day study design. B: schema of testing protocol on trial days (*day 7*). FAT-adapt, high-fat diet with carbohydrate (CHO) restoration; HCHO, high-CHO diet;  $\dot{V}O_{2\text{ peak}}$ ; maximal oxygen uptake.

each day between dietary treatments. All meals and snacks were supplied to subjects, with diets being individualized for food preferences as well as BM. Food for each 24-h period was provided to subjects in preprepared packages, and food diaries were checked on a daily basis to check compliance with the designed diets.

Training programs were individualized for each subject according to their fitness level and current training load. Subjects were required to undertake a second training session on *day 1* and on *days 2–5* complete sessions involving long slow distance (LSD), LSD with hills, interval training and LSD respectively (Fig. 1A). On *day 6* of both trials, subjects refrained from all exercise and consumed a HCHO diet providing 0.25 MJ and 10.7 g CHO/kg BM. Each subject completed an identical training program during both trials, keeping logs of the volume, intensity, and duration of each training session.

**Test day protocol.** On the morning of *day 7*, subjects returned to the laboratory after a 12- to 14-h overnight fast to undertake the high-intensity exercise protocol practiced on *day 1*. On arrival in the laboratory, a catheter (Terumo 20G, Tokyo, Japan) was inserted into the antecubital vein for blood sampling. A basal blood sample was collected from the catheter, which was kept patent by saline. A single leg was then prepared for muscle biopsies, as four incisions were made 2–3 cm apart along the vastus lateralis muscle such that four biopsies were sampled per trial. A resting muscle sample was then taken using the percutaneous biopsy technique with suction and was rapidly frozen in liquid N<sub>2</sub>.

Subjects began the first part of the exercise protocol (Fig. 1B), consisting of 20 min of steady-state cycling at 70%  $\dot{V}O_{2\text{ peak}}$  (63% PPO; 211 ± 11 W). After 1 min, cycling was suspended, and a biopsy was performed while subjects remained seated on the bike. The total time elapsed from cessation of cycling, biopsy procedure, and resumption of exercise was ~40 s. After this second biopsy, subjects resumed cycling to finish the 20-min ride at 70%  $\dot{V}O_{2\text{ peak}}$ , after which another biopsy was immediately performed. Blood samples were taken at 5 and 20 min, and pulmonary gas samples were collected from 15 to 20 min to confirm the previously reported substrate oxidation shifts (3, 5, 6).

After completing the 20-min submaximal ride, subjects rested for 1 min (inclusive of the 3rd biopsy) and then performed a 60-s all-out sprint at 150% PPO (502 ± 25 W), followed by the immediate collection of a blood sample and the fourth and final muscle biopsy. A standardized rest period of 5 min was undertaken while the Lode bike was changed to linear mode so that subjects could undertake a subsequent time trial (TT). Subjects were instructed to complete a set amount of work (4 kJ/kg BM) “as fast as possible” and were provided with feedback in the form of the elapsed percentage of the work completed. The same researcher supervised each TT, and subjects were given the results of their TT performances only after the entire study was completed.

**Blood sampling and analyses.** Eight milliliters of blood were collected at each sampling time, of which 4 ml were placed in a tube containing fluoride heparin and spun. The plasma was stored at –80°C and later analyzed for plasma glucose and lactate concentrations using an automated method (EML-105; Radiometer, Copenhagen, Denmark). Insulin concentrations were determined using a solid-phase, two-site chemiluminescent immunometric assay (Immulin Diagnostic Products, Los Angeles, CA). A further aliquot of blood was added to preservative consisting of EGTA and reduced glutathione in normal saline, mixed gently, and spun in a centrifuge. The plasma was later analyzed for free fatty acid (FFA) concentration using an enzymatic colorimetric method (NEFA C test kit; Waco Chemical, Tokyo, Japan) and catecholamines (epinephrine and norepinephrine) using an enzymatic immunoassay (2-Cat Labor Diagnostika Nord, Nordhorn, Germany).

**Muscle sampling and analyses.** All muscle biopsies were rapidly frozen in liquid N<sub>2</sub> within seconds of the designated time point for collection. A small piece of wet muscle (10–15 mg) was chipped from each biopsy (under liquid N<sub>2</sub>) for the measurement of PDHa (PDH

fraction in the active form), as previously described (31). The remainder of the biopsy was freeze-dried, powdered, and dissected free of blood, connective tissue, and fat before being weighed into three separate aliquots for analyses of selected muscle metabolites, glycogen contents, and HSL activity.

One aliquot of freeze-dried muscle (~10 mg) was extracted with 0.5 M perchloric acid (PCA) containing 1 mM EDTA and neutralized with 2.2 M KHCO<sub>3</sub>. This extract was used for the measurement of creatine (Cr), phosphocreatine (PCr), ATP, lactate, and glucose 6-phosphate (G-6-P) by enzymatic spectrophotometric assays (1, 14) and acetyl-CoA and acetylcarnitine with radiometric measurements (7). Pyruvate was analyzed fluorometrically (25). Muscle glycogen content was measured from a second aliquot of freeze-dried muscle (~4–6 mg). A third aliquot of powdered muscle was used for the determination of HSL activity (22) as modified by Watt et al. (42). All HSL measurements were made in triplicate, and the mean of these values is reported. This assay measures the activity of HSL against a triolein substrate and not total HSL content. It also measures the covalent effects on HSL and does not allow for the quantification of possible allosteric effects. All muscle measurements were normalized to the highest total Cr measured among the eight biopsies from each subject.

**Calculations.** Free ADP (ADP<sub>f</sub>) and AMP (AMP<sub>f</sub>) contents were calculated by assuming equilibrium of the creatine kinase and adenylate kinase reactions (10). Specifically, ADP<sub>f</sub> was calculated using the measured ATP, Cr, and PCr values, an estimated H<sup>+</sup> concentration, and the creatine kinase equilibrium constant of 1.66 × 10<sup>9</sup>. The H<sup>+</sup> concentration was estimated from the measured lactate and pyruvate contents as described by Sahlin et al. (32). AMP<sub>f</sub> was calculated from the estimated ADP<sub>f</sub> and measured ATP content using the adenylate kinase equilibrium constant of 1.05. Free inorganic phosphate (P<sub>if</sub>) was calculated by adding the estimated resting free phosphate of 10.8 mmol/kg dry mass (dm) (10) to the difference in PCr content (Δ[PCr]) minus the accumulation of G-6-P between rest and the given exercise time points. Substrate level phosphorylation and total glycogenolysis were calculated for the exercise transitions from rest to 1 min of cycling at 70%  $\dot{V}O_{2\text{ peak}}$  and also from the end of 20 min of cycling at 70%  $\dot{V}O_{2\text{ peak}}$  to 1 min of 150% PPO sprint. Substrate level phosphorylation was calculated using the following equation:

$$\text{ATP provision rate} = 1.5 \Delta[\text{lactate} + \text{pyruvate}] + \Delta[\text{PCr}],$$

where Δ is the difference between rest and 1 min values, and square brackets indicate concentration (35). Estimates of muscle glycogenolysis (calculated in 6 carbon glucosyl units) were derived two different ways: 1) from the accumulation of muscle G-6-P, pyruvate, and lactate plus the flux of pyruvate through PDH (the 1-min PDH value – rest and the 21-min PDH value – the 20-min value) and 2) also accounting for estimated lactate efflux during the initial minute of exercise (20–30% of total muscle lactate accumulation).

Whole body rates of carbohydrate and fat oxidation (g/min) were calculated from the respiratory data collected during the last few minutes of the 20-min exercise bout at 70%  $\dot{V}O_{2\text{ peak}}$ . The calculations were made from  $\dot{V}CO_2$  and  $\dot{V}O_2$  measurements, assuming a nonprotein RER value, according to the following equations (26).

$$\text{CHO oxidation} = 4.585 \dot{V}CO_2 - 3.226 \dot{V}O_2$$

$$\text{fat oxidation} = 1.695 \dot{V}O_2 - 1.701 \dot{V}CO_2$$

**Statistical analyses.** All data are presented as means ± SE. A two-way repeated-measures ANOVA (treatment × time) was used to determine significant differences between treatments during the steady-state 70%  $\dot{V}O_{2\text{ peak}}$  cycling portion of the trial. When a significant *F*-ratio was obtained, post hoc analysis was completed using a Student-Newman-Keuls test. All postsprint and post-TT blood and muscle measurements, glycogen utilization, and TT

performance and any net calculated differences between selected time points between the FAT-adapt and HCHO trials were compared using a paired dependent-samples *t*-test. Statistical significance was accepted at  $P < 0.05$ .

## RESULTS

**Subject diet and training compliance.** Feedback from food and training logs showed that all subjects complied well with the dietary and training interventions required for this study. After the completion of the study, dietary analysis was performed. During *days 1–5* on the FAT diet, subjects consumed on average  $18,189 \pm 704$  kJ (18% carbohydrate, 67% fat, 15% protein), and during the CHO diet they consumed  $18,215 \pm 703$  kJ (70% carbohydrate, 15% fat, 15% protein). After both diets, on *day 6* (carbohydrate restoration), subjects consumed  $18,483 \pm 715$  kJ (71% carbohydrate, 15% fat, 14% protein). During the 5-day FAT dietary intervention, subjects trained by cycling  $293.4 \pm 19.9$  km over  $10.5 \pm 0.7$  h, and during the CHO diet the total cycling training volume was remarkably similar, as subjects rode  $292.6 \pm 16.6$  km over  $10.5 \pm 0.7$  h. Subjects did not train on the day of CHO restoration (*day 6*).

**Respiratory measurements.** Due to technical problems, respiratory data were collected from only three subjects. Nevertheless, substrate shifts were pronounced and similar to those of previous studies (3, 5, 6) in that, after the 5-day FAT-adapt diet with carbohydrate restoration, the RER decreased (FAT-adapt  $0.85 \pm 0.02$  vs. HCHO  $0.91 \pm 0.01$ ;  $P = 0.08$ ) during the 20-min cycle ride at  $70\% \dot{V}O_{2\text{ peak}}$  compared with the HCHO diet. There was an  $\sim 45\%$  increase in fat oxidation (FAT-adapt  $0.80 \pm 0.19$  vs. HCHO  $0.43 \pm 0.05$  g/min) and a concomitant  $\sim 30\%$  decrease in carbohydrate oxidation (FAT-adapt  $1.97 \pm 0.30$  vs. HCHO  $2.81 \pm 0.08$  g/min) as a result of the RER shift.

**Blood measurements.** There was no difference between the FAT-adapt and HCHO diets for lactate, glucose, insulin, FFA, epinephrine, and norepinephrine concentrations at any time points during the 20-min ride at  $70\% \dot{V}O_{2\text{ peak}}$  (Table 1). Plasma lactate, glucose, epinephrine, and norepinephrine were

increased during the 20-min ride ( $P < 0.05$ , main effect), and plasma FFA was decreased ( $P < 0.05$ ) by the 20-min mark at  $70\% \dot{V}O_{2\text{ peak}}$ . Concentrations of glucose and lactate were significantly increased after the sprint at 150% PPO and post-TT, but were not different between trials.

**PDH activity.** At rest, PDHa was 56% lower following the FAT-adapt ( $0.39 \pm 0.10$  vs. HCHO  $0.82 \pm 0.18$  mmol·kg wet wt<sup>-1</sup>·min<sup>-1</sup>; Fig. 2). During the onset of exercise, PDHa increased rapidly and similarly in both trials ( $\Delta$ increase from rest to 1 min: FAT-adapt  $1.20 \pm 0.29$  vs. HCHO  $1.59 \pm 0.25$  mmol·kg wet wt<sup>-1</sup>·min<sup>-1</sup>) but continued to be 29% lower throughout the  $70\% \dot{V}O_{2\text{ peak}}$  cycling exercise during the FAT-adapt compared with HCHO [FAT-adapt  $1.69 \pm 0.25$  vs. HCHO  $2.39 \pm 0.19$  mmol·kg wet wt<sup>-1</sup>·min<sup>-1</sup> average of 1- and 20-min values (significant trial effect;  $P = 0.003$ )]. After a 1-min rest and a subsequent 1-min sprint, PDHa increased rapidly and similarly in both trials but again remained lower ( $P < 0.05$ ) in the FAT-adapt trial (Fig. 2). However, the increase in PDH activation ( $\sim 35\%$ ) during the 1-min sprint was not different between dietary treatments ( $\Delta$ increase during the 1-min sprint: FAT-adapt  $0.98 \pm 0.45$  vs. HCHO  $1.29 \pm 0.46$  mmol·kg wet wt<sup>-1</sup>·min<sup>-1</sup>).

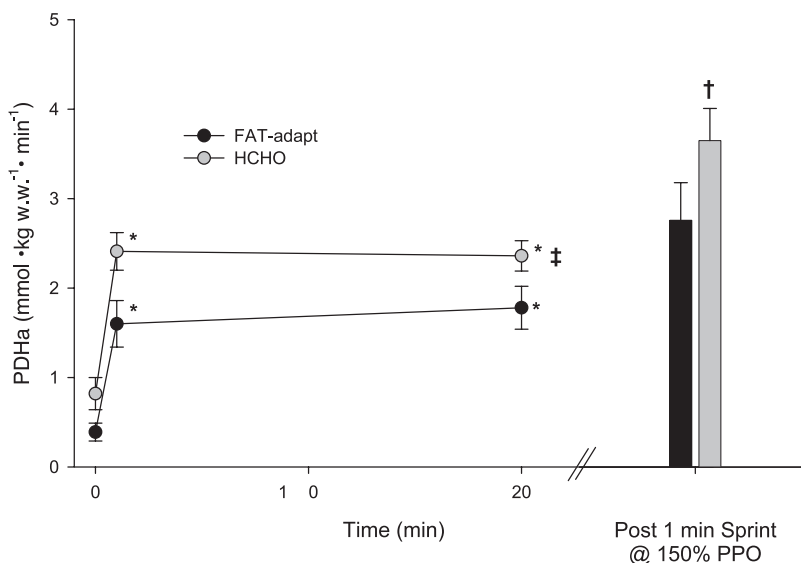
**HSL activity.** HSL activity was 30% higher after the 5-day high-fat diet with carbohydrate restoration compared with the HCHO, although this did not reach statistical significance (FAT-adapt  $3.47 \pm 0.73$  vs. HCHO  $2.38 \pm 0.43$  mmol·kg dry wt<sup>-1</sup>·min<sup>-1</sup>,  $P = 0.09$ ). The increase in HSL activity at the onset of exercise was not different between trials ( $\Delta$ increase from rest to 1 min: FAT-adapt  $0.91 \pm 0.28$  vs. HCHO  $0.93 \pm 0.57$  mmol·kg dry wt<sup>-1</sup>·min<sup>-1</sup>), such that HSL activity remained  $\sim 20\%$  higher ( $P = 0.12$ ) after the FAT-adapt diet compared with HCHO (FAT-adapt  $5.32 \pm 0.79$  vs. HCHO  $4.26 \pm 0.28$  mmol·kg dry wt<sup>-1</sup>·min<sup>-1</sup> average for 1 and 20 min). HSL rapidly decreased ( $P < 0.05$ ) during the 1-min 150% PPO sprint, and there were no differences between treatments (FAT-adapt  $3.38 \pm 0.41$  vs. HCHO  $3.98 \pm 0.26$  mmol·kg dry wt<sup>-1</sup>·min<sup>-1</sup> after the 1-min sprint).

Table 1. Plasma measurements at rest and during 20 min of cycling at  $\sim 70\% \dot{V}O_{2\text{ peak}}$  followed by a 1-min sprint at 150% of PPO and a TT on day 7, after 5 days of FAT-adapt or HCHO

Parameter	Condition	Time, min				
		Rest	70% $\dot{V}O_{2\text{ peak}}$		150% PPO	
			5 min	20 min	1 min	Post-TT
		2-Way RM ANOVA			Paired <i>t</i> -test	
Lactate, mM	FAT-adapt	2.3±0.5	4.2±0.3*	4.1±0.3*	14.1±1.1	16.2±0.9
	HCHO	1.8±0.1	5.0±0.5*	4.7±0.6*	13.9±0.4	16.2±1.1
Glucose, mM	FAT-adapt	5.9±0.1	5.9±0.1	6.0±0.4	7.5±0.3	8.7±0.8
	HCHO	5.2±0.3	5.8±0.1	6.0±0.2	7.3±0.3	8.6±0.7
Insulin, pM	FAT-adapt	33.6±3.1	39.6±3.9	31.1±3.9	ND	ND
	HCHO	30.0±5.3	32.4±2.4	30.1±5.2	ND	ND
FFA, mM	FAT-adapt	0.23±0.05	0.24±0.06	0.17±0.04*†	ND	ND
	HCHO	0.23±0.03	0.19±0.02	0.16±0.02*†	ND	ND
Epinephrine, ng/ml	FAT-adapt	1.3±0.5	2.9±0.8	6.8±1.4*†	ND	ND
	HCHO	1.8±0.7	3.2±1.1	7.4±2.5*†	ND	ND
Norepinephrine, ng/ml	FAT-adapt	7.4±4.6	14.6±5.7*	24.2±2.4*	ND	ND
	HCHO	2.9±0.7	15.0±3.8*	19.6±6.0*	ND	ND

Values are means  $\pm$  SE;  $n = 7$ . FAT-adapt, adaptation to high-fat diet and 1 day of carbohydrate (CHO) restoration; HCHO, high-CHO diet; PPO, peak power output; TT, time trial; FFA, free fatty acid; ND, no determination. \*Significantly different from 0 min ( $P < 0.05$ ); †significantly different from preceding time point ( $P < 0.05$ ).

Fig. 2. Pyruvate dehydrogenase activity in the active form (PDHa) at rest and during 20 min of cycling at ~70%  $\dot{V}O_{2\text{ peak}}$ , followed by a 1-min sprint at 150% of peak power output (PPO) on day 7, after either 5 days of FAT-adapt or HCHO. Values are means  $\pm$  SE;  $n = 7$ . \*Significantly different from 0 min ( $P < 0.05$ ); ‡significant trial effect of HCHO being higher than FAT-adapt ( $P < 0.05$ ); †significantly different from corresponding time point for FAT-adapt ( $P < 0.05$ ).



**High-energy phosphates.** PCr was similar at rest and decreased ( $P < 0.05$ ) to the same extent during 20 min at 70%  $\dot{V}O_{2\text{ peak}}$  and after the sprint at 150% PPO in both trials (Table 2). The muscle content of ATP was unaffected by prior dietary treatment or exercise (Table 2).  $ADP_f$  and  $AMP_f$  were not different at rest and increased similarly between trials throughout the 20-min exercise period (Table 2). After the 1-min 150% PPO sprint, calculated  $ADP_f$  and  $AMP_f$  were greater ( $P < 0.05$ ) during the HCHO trial compared with FAT-adapt. Calculated  $P_{if}$  increased ( $P < 0.05$ ) similarly during exercise for both dietary treatments (Table 2).

**Glycogen, glycolytic metabolites, and estimated glycogenolysis.** There was no difference in preexperimental trial glycogen contents following 1 day of carbohydrate restoration between dietary treatments (FAT-adapt  $873 \pm 36$  vs. HCHO  $868 \pm 31$  mmol glucosyl units/kg dry wt; Table 3). Resting muscle G-6-P and glucose content were similar and increased to the same extent during steady-state exercise (Table 3). There was a significantly higher G-6-P content postsprint after the

HCHO diet. Pyruvate and lactate contents at rest, during exercise at 70%  $\dot{V}O_{2\text{ peak}}$ , and after the 150% PPO sprint were not different between treatments (Table 3). The net differences in the accumulations of G-6-P, pyruvate, and lactate, coupled with the PDHa differences, during the 1st min of exercise at 70%  $\dot{V}O_{2\text{ peak}}$  resulted in a higher ( $P < 0.05$ ) estimated glycogenolysis in the HCHO vs. FAT-adapt trial (FAT-adapt  $9.1 \pm 1.1$  vs. HCHO  $13.4 \pm 2.1$  glucosyl units · kg dry wt<sup>-1</sup> · min<sup>-1</sup>; Fig. 3). Estimated glycogenolysis remained higher in the HCHO trial during the 1-min sprint (FAT-adapt  $37.3 \pm 5.1$  vs. HCHO  $50.5 \pm 2.7$  glucosyl units · kg dry wt<sup>-1</sup> · min<sup>-1</sup>; Fig. 3). These differences between treatments for estimated glycogenolysis remained when the estimated lactate efflux that may have occurred was also added (Fig. 3).

**Acetylated metabolites.** Acetyl-CoA and acetylcarnitine were similar at rest and at 1 min into steady-state exercise, and both dietary treatments had parallel increases ( $P < 0.05$ ) after 20 min of submaximal exercise and the 1-min sprint (Table 3).

Table 2. High-energy phosphate contents at rest and during 20 min of cycling at ~70%  $\dot{V}O_{2\text{ peak}}$ , followed by a 1-min sprint at 150% of PPO on day 7, after 5 days of FAT-adapt or HCHO

Measure	Condition	Time, min			
		Rest	70% $\dot{V}O_{2\text{ peak}}$		150% PPO
			1 min	20 min	1 min
		2-Way RM ANOVA			
		Paired <i>t</i> -test			
PCr	FAT-adapt	73.6 $\pm$ 2.0	64.2 $\pm$ 2.1*	48.6 $\pm$ 5.8*†	28.1 $\pm$ 6.2
	HCHO	73.1 $\pm$ 1.2	59.4 $\pm$ 4.1*	54.0 $\pm$ 4.6*†	19.5 $\pm$ 2.5
ATP	FAT-adapt	22.93 $\pm$ 0.41	23.70 $\pm$ 1.84	23.22 $\pm$ 0.94	22.84 $\pm$ 1.30
	HCHO	24.41 $\pm$ 1.07	24.89 $\pm$ 1.51	25.47 $\pm$ 1.82	21.48 $\pm$ 1.58
$ADP_f$	FAT-adapt	78.8 $\pm$ 3.5	112.8 $\pm$ 15.5*	203.8 $\pm$ 28.9*†	274.7 $\pm$ 23.1
	HCHO	81.3 $\pm$ 3.8	134.1 $\pm$ 20.7*	165.9 $\pm$ 25.0*†	361.9 $\pm$ 31.3‡
$AMP_f$	FAT-adapt	0.26 $\pm$ 0.02	0.55 $\pm$ 0.12	2.08 $\pm$ 0.48*†	3.97 $\pm$ 1.11
	HCHO	0.26 $\pm$ 0.02	0.77 $\pm$ 0.20	1.21 $\pm$ 0.31*†	7.74 $\pm$ 0.93‡
$P_{if}$	FAT-adapt	10.8 $\pm$ 0.0	19.1 $\pm$ 8.4*	31.3 $\pm$ 6.1*†	52.5 $\pm$ 5.2
	HCHO	10.8 $\pm$ 0.0	23.6 $\pm$ 3.8*	31.0 $\pm$ 5.2*†	54.6 $\pm$ 1.5

Values are means  $\pm$  SE;  $n = 7$ . PCr, phosphocreatine; f, free. All values are expressed as mmol/kg dry wt except for  $ADP_f$  and  $AMP_f$ , which are  $\mu\text{mol/kg}$  dry wt. Resting  $P_{if}$  of 10.8 is assumed from Dudley et al. (10). \*Significantly different from 0 min ( $P < 0.05$ ); †significantly different from preceding time point ( $P < 0.05$ ); ‡significantly different from corresponding time point for FAT-adapt ( $P < 0.05$ ).

Table 3. Muscle metabolite contents at rest and during 20 min of cycling at  $\sim 70\%$   $\dot{V}O_{2\text{ peak}}$  followed by a 1-min sprint at 150% of PPO on day 7, after 5 days of FAT-adapt or HCHO

Measure	Condition	Time, min			
		Rest	70% $\dot{V}O_{2\text{ peak}}$		150% of PPO
			1 min	20 min	1 min
		2-Way RM ANOVA			Paired <i>t</i> -test
Glycogen	FAT-adapt	873.1 ± 36.3	ND	ND	731.9 ± 30.1*
	HCHO	867.9 ± 31.3	ND	ND	770.2 ± 42.4*
G-6-P	FAT-adapt	0.96 ± 0.43	4.70 ± 0.34*	3.26 ± 0.47*†	14.97 ± 1.75
	HCHO	1.13 ± 0.03	4.09 ± 0.63*	3.06 ± 0.41*†	23.30 ± 1.87‡
Glucose	FAT-adapt	2.05 ± 0.23	3.23 ± 0.44*	4.59 ± 0.47*	7.47 ± 1.62
	HCHO	2.73 ± 0.20	6.78 ± 2.62*	6.62 ± 1.56*	7.23 ± 0.61
Pyruvate	FAT-adapt	0.17 ± 0.02	0.20 ± 0.03*	0.28 ± 0.04*	0.89 ± 0.28
	HCHO	0.23 ± 0.04	0.35 ± 0.08*	0.29 ± 0.05*	1.30 ± 0.28
Lactate	FAT-adapt	6.7 ± 1.3	12.0 ± 2.4*	20.9 ± 5.0*†	67.2 ± 6.9
	HCHO	6.3 ± 1.4	20.0 ± 3.8*	24.3 ± 6.0*†	78.0 ± 7.2
Acetyl-CoA	FAT-adapt	7.4 ± 1.9	7.0 ± 1.7	20.4 ± 5.7*†	24.0 ± 4.5
	HCHO	3.5 ± 0.6	10.2 ± 1.3	25.4 ± 4.3*†	26.2 ± 4.3
Acetylcarnitine	FAT-adapt	3.5 ± 0.8	4.3 ± 0.8	8.7 ± 1.9*†	12.9 ± 0.9
	HCHO	3.1 ± 1.2	4.0 ± 0.4	9.8 ± 1.0*†	14.1 ± 0.5

Values are means ± SE; *n* = 7. G-6-P, glucose 6-phosphate. All values are expressed as mmol/kg dry wt except for acetyl-CoA, which is  $\mu\text{mol/kg}$  dry wt. \*Significantly different from 0 min ( $P < 0.05$ ); †significantly different from preceding time point ( $P < 0.05$ ); ‡significantly different from corresponding time point for FAT-adapt ( $P < 0.05$ ).

**Estimated substrate phosphorylation during cycling.** During the 1st min of exercise at 70%  $\dot{V}O_{2\text{ peak}}$ , substrate phosphorylation was decreased by 49% ( $P < 0.05$ ) following the FAT-adapt treatment compared with HCHO (Fig. 4). This trend continued when substrate phosphorylation was examined during the 1-min sprint at 150% PPO, as FAT-adapt resulted in a 22% decrease in estimated substrate phosphorylation compared with HCHO (paired *t*-test  $P = 0.167$ ).

**Performance ride.** There were no between-treatment differences in time to complete the 4 kJ/kg BM time trial (FAT-adapt 13.18 ± 0.89 vs. HCHO 13.26 ± 0.95 min), mean power output (FAT-adapt 283 ± 19 vs. HCHO 282 ± 22 W), or the

percent (PPO) sustained throughout the TT (FAT-adapt 84 ± 2 vs. HCHO 84 ± 2%).

## DISCUSSION

The present study compared the effects of either a 5-day high-fat diet with a subsequent 1-day CHO restoration (FAT-adapt trial) or an isoenergetic high-CHO diet (HCHO trial) while training on markers of skeletal muscle CHO and fat metabolism during exercise. Our results indicate that the pre-

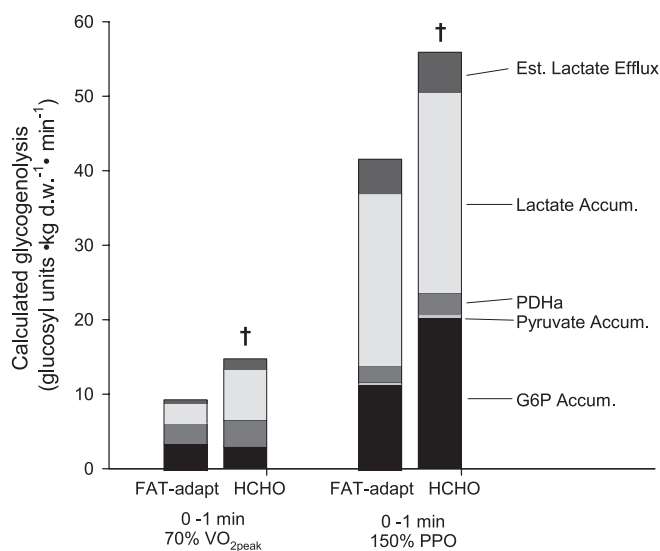


Fig. 3. Calculated glycogenolysis in the 1st min of steady-state cycling at  $\sim 70\%$   $\dot{V}O_{2\text{ peak}}$  and during the 1-min sprint at 150% of PPO on day 7, after either 5 days of FAT-adapt or HCHO. Values are means ± SE; *n* = 7. G6P, glucose 6-phosphate. †Significantly different from corresponding time point for FAT-adapt ( $P < 0.05$ ).

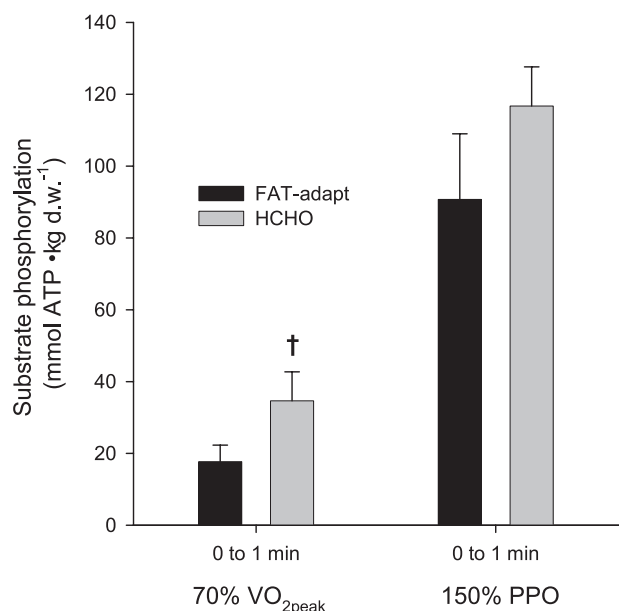


Fig. 4. Estimated substrate phosphorylation in the 1st min of steady-state cycling at  $\sim 70\%$   $\dot{V}O_{2\text{ peak}}$  and during the 1-min sprint at 150% of PPO on day 7, after either 5 days of FAT-adapt or HCHO. Values are means ± SE; *n* = 7. †Significantly different from corresponding time point for FAT-adapt ( $P < 0.05$ ).

viously reported decreases in whole body CHO oxidation and increases in fat oxidation following the FAT-adapt protocol are a function of changes within skeletal muscle. These include reduced estimated muscle glycogenolysis (Phos flux), decreased PDH activity (pyruvate oxidation), and a trend to increased HSL activity. The high-fat diet coupled with the maintenance of aerobic training altered the muscle's metabolic response during exercise to favor fat oxidation despite the restoration of muscle and blood CHO stores. It is not clear from the present results what metabolic signals account for the shift in muscle substrate use during cycling at 70%  $\dot{V}O_{2\text{ peak}}$ . However, lower accumulation of free ADP and AMP following 1 min at 150% PPO in the FAT-adapt trial may be responsible for the decreased glycogenolysis and PDH activation during sprinting.

*Chronic effects of diet and training on PDH activity at rest.* The resting PDH activity in the FAT-adapt trial remained 50% lower than in the HCHO trial despite 1 day of a high-CHO diet. Although previous work has also demonstrated decreases in PDH activity following a high-fat diet (29, 31, 36), we expected that CHO restoration would return resting PDH activity to levels seen with the carbohydrate diet. At rest, PDH kinase (PDK) is acutely (minute to minute) stimulated by high ratios of acetyl-CoA to CoA, ATP to ADP, and NADH to  $\text{NAD}^+$  and inhibited by pyruvate. Exposure to a high-fat diet has been shown to increase the acetyl-CoA/CoA ratio (31), but the decrease in resting PDH activity has been mainly attributed to longer-term (hourly, daily) increases in PDK activity (27, 29). Peters et al. (27) demonstrated that muscle PDK-4 protein content and total PDK activity increased after just 24 h on a high-fat diet. It has been proposed that increased PDK activity would decrease PDH activation by increased multisite phosphorylation of the entire PDH complex (33). The upregulation of PDK is thought to be due to the low insulin levels, which removes an inhibition on the production of PDK that is normally present and/or increased FFA, which may stimulate transcription factors within the cell (27).

A recent study in untrained subjects found that high-fat diet-induced increases in PDK activity can be rapidly reversed, such that PDH activity was restored within 3 h after CHO refeeding (2). The present study found no differences between dietary treatments in the acute regulators of PDH activity at rest (acetyl-CoA, ATP,  $\text{ADP}_f$ ,  $\text{AMP}_f$ , pyruvate contents). The fact that PDH activity was still attenuated after 24 h of CHO restoration in the FAT-adapt trial suggests that PDK activity also remained elevated. This may have been the result of the high-fat diet coupled with the intense training protocol that the endurance-trained subjects maintained during the FAT-adapt trial in the current study. Another explanation for the current study's results could be a training-induced increase in total PDH (PDHt). However, a recent training study did not show an increase in PDHt protein following 1 wk of training (23). Therefore, we would not expect an increase in either PDHt activity or protein following just 5 days of normal endurance training in already well-trained subjects while on the high-fat diet. Nevertheless, direct measurements of PDK isoform protein and total PDK and PDH activity are needed to test this hypothesis.

*Muscle glycogenolysis and PDH activity during onset of exercise at 70%  $\dot{V}O_{2\text{ peak}}$ .* A novel aspect of the current study was the quantification of glycogenolysis during the initial

minute of submaximal exercise. In support of one of our hypotheses, there was decreased muscle glycogenolysis after FAT-adapt compared with HCHO trials. The initially equivalent and augmented resting glycogen contents ( $\sim 800$  mmol/kg dry wt in both treatments) resulted in similar muscle G-6-P contents and pyruvate and lactate accumulations over the initial minute of exercise. However, there was substantially less pyruvate oxidation (via PDH flux) in the FAT-adapt trial, indicating decreased glycogenolysis. We hypothesized that decreases in the accumulation of the high-energy phosphates (free ADP and AMP) in the FAT-adapt condition would explain the decreased glycogenolysis (flux through Phos) and activation of PDH at the onset of exercise. We have previously argued that increased production of NADH from fat oxidation at the onset of exercise decreases the accumulation of free ADP and AMP and reduces the need to activate the CHO pathways (8, 24). Acute increases in the availability of fat increased muscle NADH content and decreased free ADP and AMP accumulations at rest and following the initial minute of exercise and corresponded with decreased PDH activity (8, 24). Although muscle NADH was not measured in the current study, a potential increase in the redox state may have occurred considering the increases in whole body fat oxidation. However, contrary to our hypothesis, this did not translate into reductions in the accumulation of  $\text{ADP}_f$  and  $\text{AMP}_f$ , and we cannot explain the reduced glycogenolysis and PDH activity by this mechanism. There was also no difference between dietary treatments in the response of epinephrine, so it is also unlikely that extracellular activation of glycogen Phos was responsible for the reported changes in glycogenolysis.

As previously noted, PDK activity may have been elevated in the FAT-adapt condition before exercise, as PDH activity was chronically lower at rest. It has been proposed that increased PDK activity would decrease PDH activation by a decreased sensitivity to the normal regulation by the substrate pyruvate at the onset of exercise (17). However, we expected that any effects that higher PDK activity would have on the activation of PDH during exercise would be overridden, as resting muscle glycogen levels were very high and pyruvate contents and accumulation were similar between the two dietary treatments. Again, there are few data regarding the potential for PDK activity changes following FAT-adapt to alter the response of PDH activity to exercise, and further studies are warranted in this area. The mechanism(s) underpinning the reduced glycogenolysis and PDH activity during exercise following FAT-adapt is unknown. The most likely candidate may be alterations in the interaction with  $\text{Ca}^{2+}$  during exercise. If the FAT-adapt condition decreased the  $\text{Ca}^{2+}$  transients in muscle during exercise or decreased the sensitivity of Phos and PDH to  $\text{Ca}^{2+}$ , it could explain the present results.

*Muscle glycogenolysis and PDH activity during sprint cycling.* Glycogenolysis during the 1-min sprint was reduced following the FAT-adapt treatment compared with HCHO. Despite high glycogen availability and no differences in plasma epinephrine levels, there were large decreases in G-6-P and pyruvate accumulations and PDH activity during the sprint, which accounted for the decrease in estimated glycogenolysis after FAT-adapt. Unlike the findings at 70%  $\dot{V}O_{2\text{ peak}}$  and consistent with our hypothesis, the findings of decreased  $\text{ADP}_f$  and  $\text{AMP}_f$  after the sprint in the FAT-adapt condition

could explain the decreased flux through both glycogen Phos and PDH.  $ADP_f$  and  $AMP_f$  are potent allosteric activators of the active form of Phos, and lower levels of  $ADP_f$  would favor maintained PDK activity and less PDH in the active form during exercise. It is important to remember that these findings do not preclude regulatory contributions from other factors that regulate Phos and PDH activities, such as  $Ca^{2+}$ .

**HSL activity measurement.** The present study, along with previous studies (3, 5, 6) demonstrated that whole body fat oxidation was approximately twofold higher during exercise at 70%  $\dot{V}O_{2\text{ peak}}$  following the FAT-adapt protocol despite there being no differences in plasma FFA and ample CHO availability in muscle and blood. We assumed that the increased fat oxidation following FAT-adapt was mainly due to increased skeletal muscle fat oxidation and examined HSL activity, as HSL plays a key regulatory role in the breakdown of intramuscular triacylglycerol (IMTG) (41). We report a 20–30% increase in HSL at rest and during cycling at 70%  $\dot{V}O_{2\text{ peak}}$  after the FAT-adapt compared with HCHO, although this did not reach statistical significance. To our knowledge, the current studies average submaximal exercise skeletal muscle HSL activity (FAT-adapt  $5.32 \pm 0.79$  vs. HCHO  $4.26 \pm 0.28$   $\text{mmol}\cdot\text{kg dry wt}^{-1}\cdot\text{min}^{-1}$  average for 1 and 20 min) are the highest ever reported in the literature. In contrast, previous studies using relatively untrained subjects ( $\dot{V}O_{2\text{ peak}} < 50$   $\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ) cycling at approximately the same exercise intensity as in the current study showed an average exercise HSL activation of only  $\sim 3$   $\text{mmol}\cdot\text{kg dry wt}^{-1}\cdot\text{min}^{-1}$  (39, 40, 42). The training status of the current subjects ( $\dot{V}O_{2\text{ peak}} 60.7 \pm 2.6$   $\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ) coupled with a high-fat diet while training played a role in elevating their HSL activity above what is normally seen in healthy recreationally active subjects.

The increased HSL activity at rest and during cycling in FAT-adapt cannot be explained by differences in the hormonal milieu, as epinephrine and insulin concentrations were similar in both trials. Therefore, the treatment difference of HSL activity originated from regulatory mechanisms within skeletal muscle. It is possible that HSL activity is influenced by substrate availability (i.e., IMTG content). Others have shown that increased dietary fat results in augmented resting IMTG contents (9, 19, 20, 37, 43), and IMTG contents have been correlated with increased fat oxidation during both rest and exercise (34). Although IMTG contents were not quantified in the current study, 5 days of a high-fat diet, coupled with training in well-trained subjects, who already have a heightened capacity for IMTG storage (13, 18), may have further increased the IMTG stores.

**Substrate phosphorylation at the onset of exercise and after 1-min sprint.** The decreased glycogenolysis and lactate accumulation in the high-FAT condition translated into decreased estimates of substrate phosphorylation during the initial minute of exercise despite a lack of difference in PCr utilization between trials. The same trend was found during the 1-min sprint, but here the difference was not statistically significant. The finding of decreased substrate phosphorylation after FAT-adapt suggests that oxidative phosphorylation was increased in the first minute of exercise. This may have resulted from increases in fat metabolism that ultimately provide more reducing equivalents (NADH) and partially relieves the metabolic inertia at exercise onset.

**Conclusions.** The FAT-adapt CHO restoration protocol utilized in the current investigation offers a unique model to study the interactive effects of alterations in fuel availability on subsequent muscle metabolism during both submaximal and maximal exercise. We found that PDH activity was lower during submaximal cycling, and estimated glycogenolysis and PDH activity were also lower during the transitions to exercise both during submaximal and sprint cycling in the FAT-adapt compared with the HCHO trial. Conversely, whole body fat oxidation was increased, and there was a trend toward significance ( $P = 0.09$ ) for increased HSL activity during submaximal cycling. Although there were decreased  $ADP_f$  and  $AMP_f$ , which helps explain the attenuated PDHa during the FAT-adapt treatment following the 1-min sprint, the mechanism(s) behind the decreased activation of PDHa or the increase in HSL after a high-fat diet at rest and during submaximal steady state cycling is unknown. These results indicate that the previously reported decreases in whole body CHO oxidation and increases in fat oxidation following the FAT-adapt protocol are a function of metabolic changes within the skeletal muscle. Perhaps a residual effect of the high-fat diet, when coupled with endurance training in these well-trained subjects, on increasing PDK or IMTG stores, despite the restoration of muscle glycogen stores, or an increase in the redox state of the cell after the FAT-adapt protocol, might explain the PDHa and HSL results, but these proposed mechanisms remain to be elucidated.

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