Effects of fat adaptation and carbohydrate restoration on prolonged endurance exercise

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CAREY, Andrew L., Heidi M. Staudacher, Nicola K. Cummings, Nigel K. Stepto, Vasilis Nikolopoulos, Louise M. Burke, and John A. Hawley. Effects of fat adaptation and carbohydrate restoration on prolonged endurance exercise. J Appl Physiol 91: 115–122, 2001.—We determined the effect of fat adaptation on metabolism and performance during 5 h of cycling in seven competitive athletes who consumed a standard carbohydrate (CHO) diet for 1 day and then either a high-CHO diet (11 g·kg⁻¹·day⁻¹ CHO, 1 g·kg⁻¹·day⁻¹ fat; HCHO) or an isoenergetic high-fat diet (2.6 g·kg⁻¹·day⁻¹ CHO, 4.6 g·kg⁻¹·day⁻¹ fat; fat-adapt) for 6 days. On day 8, subjects consumed a high-CHO diet and rested. On day 9, subjects consumed a preexercise meal and then cycled for 4 h at 65% peak O₂ uptake, followed by a 1-h time trial (TT). Compared with baseline, 6 days of fat-adapt reduced respiratory exchange ratio (RER) with cycling at 65% peak O₂ uptake [0.78 ± 0.01 (SE) vs. 0.85 ± 0.02; P < 0.05]. However, RER was restored by 1 day of high-CHO diet, preexercise meal, and CHO ingestion (0.88 ± 0.01; P < 0.05). RER was higher after HCHO than fat-adapt (0.85 ± 0.01, 0.89 ± 0.01, and 0.93 ± 0.01 for days 2, 8, and 9, respectively; P < 0.05). Fat oxidation during the 4-h ride was greater (171 ± 32 vs. 119 ± 38 g; P < 0.05) and CHO oxidation lower (597 ± 41 vs. 719 ± 46 g; P < 0.05) after fat-adapt. Power output was 11% higher during the TT after fat-adapt than after HCHO (312 ± 15 vs. 279 ± 20 W; P = 0.11). In conclusion, compared with a high-CHO diet, fat oxidation during exercise increased after fat-adapt and remained elevated above baseline even after 1 day of a high-CHO diet and increased CHO availability. However, this study failed to detect a significant benefit of fat adaptation to performance of a 1-h TT undertaken after 4 h of cycling.

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METHODS

Subjects. Seven well-trained competitive male cyclists or triathletes [age 23.9 ± 5.6 yr, body mass (BM) 74.9 ± 4.4 kg, $\dot{V}O_2$ peak 5.06 ± 0.26 l/min, peak sustained power output (PPO) 403 ± 176 W; values are mean ± SE] participated in this study, which was approved by the Human Research Ethics Committee of RMIT University and the Radiation Advisory Committee of Victoria. Tracer amounts of radioactivity were to be used and repeated blood samples taken, and all experimental procedures and possible risks were explained to each subject, who gave written, informed consent. Our subject pool included national and international athletes (three competed in the 2000 Hawaii Ironman Triathlon several months after this investigation) who did not consent to biopsy procedures.

Preliminary testing and familiarization. Two weeks before experimental testing (described below in Experimental trial), subjects performed an incremental cycling test to exhaustion on an electronically braked cycle ergometer (Lode, Groningen, The Netherlands). This test protocol has been described in detail previously (17). Volitional fatigue was defined as the point at which the cyclists’ cadence decreased >10 rpm and/or as a respiratory exchange ratio (RER) >1.14. Throughout the maximal test and during portions of the subsequently described experimental trials, subjects inspired air through a mouthpiece and low-resistance turbine attached to a Quark b2 gas-analysis system (Cosmed, Rome, Italy) interfaced to an IBM personal computer, which calculated the instantaneous rates of $O_2$ consumption ($\dot{V}O_2$), CO$_2$ production (VCO$_2$), minute ventilation, and the RER every 15 s from conventional equations. Before each maximal test and all experimental trials, the analyzers were calibrated with commercially available gases of known $O_2$ and CO$_2$ content. $V_{O_2\,\text{peak}}$ was defined as the highest $V_{O_2}$ a subject attained during any 60 s of the test, whereas 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. The results of the maximal test were used to determine the power output that corresponded to 65% of each subject’s $V_{O_2\,\text{peak}}$ (57.5% of PPO) to be used in the experimental trials.

In the week before an experiment, subjects reported to the laboratory for a familiarization session, during which they cycled for 2 h on the Lode cycle ergometer at the workload that they would be required to maintain for the first 4 h of the experimental rides. This was immediately followed by a 30-min TT on a Kingcycle ergometry system (Kingcycle, High Wycombe, Bucks, UK). During this familiarization TT and the subsequent 1-h TT performed during the experimental trials, subjects rode their own bicycles mounted on the Kingcycle ergometer. This system utilizes rolling resistance uniformly applied to the rear wheel of the bicycle via a computer-controlled air-braked flywheel to simulate outdoor conditions. The Kingcycle ergometry system has previously been reported to be reliable and valid, with a test-retest coefficient of 1.0 ± 0.5% for three 40-km TTs and performance times, on average, 8% faster than on-road performances (28). Before the beginning of each familiarization ride and the subsequent experimental TTs, the Kingcycle was calibrated as described by Palmer et al. (28).

Study design. Each subject undertook two 9-day periods of dietary intervention in a randomized, crossover design separated by an 18-day washout period. It was not possible to completely blind subjects to each treatment diet. On the day before the commencement of a dietary treatment (day 1), subjects were given a standardized diet containing 9 g/kg CHO, 1.8 g/kg fat, and 2.2 g/kg protein (58% CHO, 27% fat, and 15% protein; total energy, 0.25 MJ·kg$^{-1}$·day$^{-1}$) to consume on that day and were instructed to train lightly before 1200. The aim of this dietary control was to standardize muscle and liver glycogen stores before subsequent exercise testing and dietary intervention. Subjects then commenced 7 days of a supervised diet and training program. On the fat adaptation treatment (fat-adapt), they were prescribed a high-fat (4.6 g·kg$^{-1}$·day$^{-1}$ fat, 69% of energy), low-CHO (2.5 g·kg$^{-1}$·day$^{-1}$ CHO, 16% of energy) diet supplying 0.25 MJ·kg$^{-1}$·day$^{-1}$. The control treatment (HCHO) was an isocaloric diet providing 11 g·kg$^{-1}$·day$^{-1}$ and 70% of energy from CHO and 1.0 g·kg$^{-1}$·day$^{-1}$ and 15% of energy from fat. Diets were constructed to maximize, or at least match, absorbable energy. Fiber intake was kept to a mean daily intake of 50 g and was matched to within 5–10 g each day between treatments. Foods with a very-low-glycemic index or high content of resistant starch were generally avoided. All meals and snacks were supplied to subjects, with diets being individualized for food preferences as well as BM. A typical meal each day was eaten under supervision in the laboratory, with the remaining food for each 24-h period being provided in prepared packages. Subjects were required to keep a food diary and report all food and drink intake on a daily basis to maximize compliance to the designed diets.

On the morning of day 8, subjects on both dietary treatments were provided with a high-CHO diet that was identical in macronutrient composition to the HCHO. They then refrained from any training for the next 24 h. Such a diet-training regimen has previously been shown to supercompensate muscle glycogen stores independent of the previous dietary treatment (4). On the morning of day 9, subjects reported to the laboratory and ingested a standardized breakfast before commencing exercise testing (described below).

Training programs during the 9 days of the dietary intervention period were individualized for each subject according to his fitness level and present training load. In addition to the subjects’ normal training, two standardized, supervised laboratory interval-training sessions were included in each treatment. The first interval session was undertaken on day 2 immediately after a 20-min submaximal exercise test (described below). The intention of this session was to cause a rapid lowering of muscle glycogen concentrations on the first day and initiate a rapid differentiation among dietary treatments based on their ability to restore depleted muscle glycogen stores. The second laboratory session was undertaken on day 5 of each trial.

Submaximal rides. On the mornings of days 2, 5, and 8 of each dietary intervention, subjects reported to the laboratory between 0600 and 0800 after a 12- to 14-h overnight fast. They were weighed, then a 20-gauge Teflon catheter (Terumo, Tokyo, Japan) was inserted into an antecubital vein, and a resting blood sample was taken. The catheter was kept patent by periodic flushing with 1.0 ml of sterile saline. Subjects then warmed up on the Lode ergometer for 5 min before commencing 15 min of cycling at a work rate eliciting 65% $V_{O_2\,\text{peak}}$. During the final 7 min of this ride, pulmonary gas samples were measured via the previously described gas system. Blood samples were subsequently analyzed for plasma glucose, free fatty acid (FFA), insulin, lactate, and glycerol concentration. Heart rate was measured via a Polar Accurex Plus monitor (Polar Electro OY, Kempele, Finland).

Experimental trial. On the morning of day 9, subjects reported to the laboratory after a 12- to 14-h overnight fast and were weighed, and catheters were inserted into a vein in the antecubital space of one arm for blood sampling. Subjects
then ingested a breakfast similar in size and composition to what they might consume before an ultraendurance race (D. J. Angus, personal communication). This meal contained 3 g/kg BM of CHO. Sixty minutes after finishing breakfast, subjects were reweighed, and a further blood sample was taken. They then mounted the Lode cycle ergometer and began 4 h of constant-load cycling at a work rate eliciting 65% of V\textsubscript{O}\textsubscript{2peak} (232 ± 9 W). During the 4-h ride, subjects were provided with 7 ml/kg of a 10 g/100 ml glucose solution that they were required to ingest every 30 min. The solution was labeled with trace amounts of a [U-\textsuperscript{14}C]glucose tracer (Ambersham Biotech, Sydney, Australia) for determination of the rates of ingested CHO oxidation and plasma glucose oxidation (described below in Rates of whole body CHO and fat oxidation, ingested CHO oxidation, and plasma glucose oxidation). Subjects ingested a total of 56 ml/kg of the glucose solution, for a total mean CHO intake of ~100 g/h. Subjects were provided with water ad libitum. After 10 min of exercise, a blood sample and expired respiratory gas data were collected, and subjects provided a rating of their perceived exertion (1). At this time, a sample of expired air was passed through a solution containing 1 ml hyamine hydroxide in methanol, 1 ml 96% ethanol, and two drops of 1% phenolphthalein indicator. Expired air was bubbled through this mixture for 2–3 min until the phenolphthalein turned from pink to clear, at which point 1 mM of CO\textsubscript{2} had been absorbed (34). Liquid scintillation cocktail (10 ml; Ready Gel, Beckman) was then added to the solution, and \textsuperscript{14}CO\textsubscript{2} disintegrations per minute were counted in a liquid scintillation counter (Packard 1500 Tri-Carb, Downers Grove, IL). This sample collection protocol was repeated after each 15-min period during the first hour and every 30 min thereafter.

On completion of the 4-h ride, subjects voided before mounting their own bicycles on the Kingcycle ergometer. They then completed a TT during which they rode as fast as possible for 1 h. Subjects were provided with a sports drink containing 7 g/100 ml of CHO (Gatorade, Spring Valley Beverages, Cheltenham, Australia) and water to consume ad libitum during the TT. At the beginning of the TT and after 30 min, subjects were provided with a CHO gel (Gu, Sports Street Marketing, Berkeley, CA) containing 25 g CHO. The volume of drinks and the amount of gel consumed during the first TT were provided during the second TT. The information available to subjects during the TT was for 5-min elapsed time periods. Subjects were only given the results of their TT for the entire study was completed.

**Blood sampling and analyses.** Unless otherwise specified, 12 ml 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 in duplicate for plasma glucose and lactate concentrations using an automated method (EML-105, Radiometer, Copenhagen, Denmark). Five milliliters of blood were mixed in a tube containing lithium-heparin and were spun in a centrifuge; from this, 500 µl of plasma were placed in a tube containing 500 µl of ice-cold 3 M perchloric acid, mixed vigorously on a vortex mixer, and spun. Eight hundred microliters of this supernatant were added to a tube containing 200 µl of 6 M potassium hydroxide (KOH), mixed, and spun. The resultant supernatant was analyzed for plasma glycerol concentration using an enzymatic spectrophotometric analysis (30). The remaining plasma from the lithium-heparin tube was used for analysis of plasma insulin concentrations by radioimmunoassay (Incstar, Stillwater, MN). The remaining blood was added to an aliquot of preservative consisting of EGTA and reduced glutathione in normal saline, mixed gently, and spun in a centrifuge. The plasma was later analyzed for plasma FFA concentration using an enzymatic colorimetric method (NEFAC code 279–75409. Wako, Tokyo, Japan).

Rates of fatty acid oxidation (µmol·kg\textsuperscript{-1}·min\textsuperscript{-1}) were determined by converting the gram-per-minute rate of triglyceride oxidation to its molar equivalent, assuming the average weight of human triglyceride to be 855.26 g/mol, and multiplying the molar rate of triglyceride oxidation by 3, because each molecule contains 3 mol of fatty acid. Rates of CHO oxidation (µmol·kg\textsuperscript{-1}·min\textsuperscript{-1}) were determined by converting the gram-per-minute rate of CHO oxidation to its molar equivalent, assuming 6 mol of O\textsubscript{2} are consumed and 6 mol of CO\textsubscript{2} are produced for each mole (180 g) oxidized. Total CHO and fat oxidation during the 240 min of steady-state exercise were estimated from the area under the CHO oxidation vs. time curve for each subject.

The rates of total plasma glucose oxidation were calculated from the following equation

\[
\text{Glu}_{\text{ox}} = (\text{SA CO}_2/\text{SA}_\text{glu}) \cdot \text{V}_\text{CO}_2
\]

where \text{Glu}_{\text{ox}} is the rate of plasma glucose oxidation in mmol/min, later converted to g/min; \text{SA CO}_2 is the specific (radio)activity of expired \text{14}CO\textsubscript{2} in disintegrations·min\textsuperscript{-1}·mmol\textsuperscript{-1}; \text{SA}_\text{glu} is the corresponding specific (radio)activity of the plasma glucose in disintegrations·min\textsuperscript{-1}·mmol\textsuperscript{-1}; and V\textsubscript{CO}_2 is the volume of expired CO\textsubscript{2} in mmol/min, calculated from the liter per minute V\textsubscript{O}_2 value and the 22.4 lmol gas volume. Because the complete conversion of one molecule of [U-\textsuperscript{14}C]glucose to six molecules of \textsuperscript{14}CO\textsubscript{2} decreases the disintegrations per minute per millimole specific radioactivity by a factor of six, the V\textsubscript{CO}_2 values did not need to be divided by six to allow for six CO\textsubscript{2} molecules arising from oxidation of one glucose molecule (10).

The same equation was used to determine the rates of exogenous (ingested) CHO oxidation. However, in this case, \text{SA}_\text{glu} was the specific radioactivity of the drink in disintegrations per minute per millimole.

These formulas do not take into account the time taken to equilibrate \textsuperscript{14}CO\textsubscript{2} with the HCO\textsubscript{3} pool. Although this time has been reported to vary between 5 min (7) and 90 min (6), it can be predicted from the flux of CO\textsubscript{2} through the body HCO\textsubscript{3} stores that equilibration is essentially complete in 20–30 min (2). More to the point, any systematic lag in the time curve for each subject.

**Statistical analyses.** Data from the two trials were compared by using a two-factor (diet and time) ANOVA with repeated measures. Separate analyses were undertaken to compare data from 20-min rides on day 1, 4, and 6 and data collected at different time points during the experimental ride. Newman-Keuls post hoc tests were undertaken when ANOVA revealed a significant interaction. Differences in dietary intakes, estimated glycogen utilization, and TT performances between trials were compared by using Student's t-tests. Significance was accepted when \( P < 0.05 \). All data are reported as means ± SE. The statistical analyses were undertaken by using Statistica software for Windows (StatSoft, version 5.1, 1997, Statistica, Tulsa, OK).
RESULTS

Diet and training compliance. All subjects completed the prescribed dietary treatments and the training requirements of the study, except for one subject who failed to complete one of the laboratory interval-training sessions during the fat-adapt treatment. Dietary analysis after the treatment interventions revealed that subjects consumed 19,370 ± 415 kJ of energy (68% CHO, 16% fat, 16% protein) during the 6-day HCHO and 19,179 ± 439 kJ (16% CHO, 68% fat, 16% protein) during fat-adapt. On day 8 (CHO restoration), subjects consumed 19,593 ± 500 kJ of energy (70% CHO, 15% fat, 15% protein) during both treatments.

Training volume during the 8 days of intervention was 787 ± 96 min of cycling and 265 ± 77 min of other athletic activities (swimming, running, resistance training) for the HCHO and 692 ± 79 min of cycling and 329 ± 83 min of other athletic activities for fat-adapt. Time spent cycling was not different between diets, and overall total training time (cycling plus other athletic training activities) was not different between diets.

BM measured on days 2, 5, 8, and 9 did not change significantly during the HCHO intervention. However, compared with HCHO, during the fat-adapt treatment, BM was reduced on days 5 and 8 compared with day 2 (day 2: 75.6 ± 2.1 vs. 76.1 ± 2.3; day 8: 75.5 ± 2.1 vs. 74.2 ± 2.2; day 9: 75.2 ± 2.2 vs. 75.2 ± 2.1 kg for HCHO and fat-adapt, respectively; \( P < 0.05 \)). BM during fat-adapt returned to baseline values and was similar to that on day 9 of the HCHO treatment after 1 day of a high-CHO diet.

Plasma metabolites. Plasma glucose, lactate, and insulin concentrations during the experimental ride are summarized in Fig. 1. There was no main effect of either diet or time or any interaction effect for resting plasma glucose, insulin, lactate, or glycerol concentrations on days 2, 5, and 8. However, plasma FFA concentration was significantly greater on day 8 than on days 2 and 5 of fat-adapt and day 8 of the HCHO treatment (fat-adapt: day 2, 0.71 ± 0.06; day 5, 0.91 ± 0.07; day 8, 1.19 ± 0.14; HCHO day 8, 0.55 ± 0.03 mmol/l; \( P < 0.05 \)). Plasma FFA concentrations were also significantly elevated from day 2 to day 5 during the HCHO treatment, but they returned to baseline values on day 8 (day 2: 0.6 ± 0.3; day 5: 0.89 ± 0.09; day 8: 0.55 ± 0.03 mmol/l; \( P < 0.05 \)).

Plasma insulin concentrations immediately before the experimental ride and 1 h after the preexercise meal were significantly greater than resting values on day 8 (day 8: 26 ± 3 and 21 ± 7; day 9: 118 ± 20 and 107 ± 23 pmol/l for HCHO and fat-adapt, respectively; \( P < 0.05 \)). No main effect of diet and no interaction were observed for plasma glucose or lactate concentrations during the experimental ride, although there was a significant main effect of time (Fig. 1; \( P < 0.05 \)). A significant interaction of diet and time was observed in plasma insulin concentration during the experimental ride (Fig. 1; \( P < 0.05 \)). Plasma FFA and plasma glycerol concentrations measured during the experimental ride are summarized in Fig. 2. A significant main effect of time was observed for plasma FFA and glycerol concentrations (Fig. 2; \( P < 0.05 \)). Plasma FFA and plasma glycerol concentrations steadily increased over time.

CHO and fat oxidation during exercise. Figure 3 summarizes the RER data collected during the 20-min submaximal rides on days 2, 5, and 8 and throughout the 4-h experimental ride (Fig. 3, top), along with the rates of CHO (middle) and fat (bottom) oxidation for the same timeframe. At baseline (day 2), RER was similar between trials (0.85 ± 0.01 and 0.85 ± 0.02 for HCHO and fat-adapt, respectively). However, fat-adapt reduced RER values so that RER had declined to 0.79 ± 0.01 by day 5 and to 0.78 ± 0.01 by day 8 (\( P < 0.05 \)). One day of high-CHO diet and rest, together with a high-CHO preexercise meal and CHO ingestion during exercise, significantly elevated RER during the first 15 min of the experimental ride (0.88 ± 0.01; \( P <
such that it was higher than the fasting value on day 2. After 8 days of HCHO, on day 8, RER was significantly elevated from day 2 (0.89 ± 0.01 vs. 0.85 ± 0.01; \( P < 0.05 \)). During the experimental ride, RER during the fat-adapt trial was significantly lower (mean RER: 0.90 vs. 0.87 for HCHO and fat-adapt, respectively; \( P < 0.05 \)) than during the HCHO trial at all time points except 210 min (Fig. 3). RER was maintained during both diets during the experimental ride until 150 min, after which it was lower than at the beginning of exercise (HCHO: 0.93 ± 0.01 vs. 0.89 ± 0.01; fat-adapt: 0.88 ± 0.01 vs. 0.85 ± 0.01 for 15-min vs. 150-min time points; \( P < 0.05 \); Fig. 3).

Rates of fat and CHO oxidation were similar at baseline (day 2) during exercise (\( P < 0.05 \)). There was a significant main effect of diet (\( P < 0.05 \)), with rates of fat oxidation being significantly higher and CHO oxidation being correspondingly lower at all time points after baseline during fat-adapt, except for fat oxidation at 210 min during the experimental ride and CHO oxidation at 90 min during the experimental ride (Fig. 3). During both experimental rides, there was a gradual increase in fat oxidation and concomitant decrease in CHO oxidation over the 240 min of cycling, such that, between 90 and 120 min, rates of CHO oxidation were significantly lower and rates of fat oxidation were significantly higher than at the beginning of the experimental ride (Fig. 3; \( P < 0.05 \)).

Total substrate utilization during the 4-h experimental ride is presented in Fig. 4. In the fat-adapt trial, there was a significant sparing of endogenous CHO stores compared with the HCHO trial (594 ± 39 vs. 475 ± 33 g; 9,274 ± 509 vs. 7,349 ± 466 kJ for HCHO and fat-adapt, respectively; \( P < 0.05 \); Fig. 4). Accordingly, there was an increased utilization of fat (119 ± 14 vs. 171 ± 12 g; 4,407 ± 536 vs. 6,309 ± 447 kJ for HCHO and fat-adapt, respectively; \( P < 0.05 \); Fig. 4).

Fig. 2. Plasma free-fatty acid (FFA) and plasma glycerol concentrations measured during 4 h of cycling at 65% of maximal oxygen uptake after 6-day adaptation to a high-fat diet and 1-day CHO restoration. Values are means ± SE; \( n = 7 \) subjects. *Significantly different from time 0, \( P < 0.05 \).

Fig. 3. Respiratory exchange ratio and fat and CHO oxidation rates (\( \mu \text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \)) measured during cycling at 65% of maximal oxygen uptake on day 2 (d2; baseline), day 5 (d5; after 3 days of a high-fat diet), and day 8 (d8; after 6 days of a high-fat diet) for 4 h (15–240 min). Values are means ± SE; \( n = 7 \) subjects. Significantly different from *HCHO, *day 2, and *time = 15 min: \( P < 0.05 \).
DISCUSSION

This is the first study to determine the effect of short-term fat adaptation on metabolism and performance during 5 h of continuous exercise in highly trained athletes. We originally hypothesized that fat adaptation would result in a significant sparing of CHO during 4 h of submaximal cycling and in an enhancement of a subsequent 1-h TT. With regard to our first hypothesis, we did find substantial CHO sparing after fat adaptation. However, despite marked changes in the patterns of fuel utilization favoring a substantial increase in the rates of fat oxidation during the first 4 h of exercise, fat adaptation failed to enhance subsequent 1-h TT performance, compared with a high-CHO diet, when both trials were undertaken under conditions in which CHO was consumed before and during the exercise bout.

Previous investigations, in which highly trained subjects consumed a high-fat diet, have also found no change in performance during exercise lasting 2–3 h (4, 5, 14, 29). However, the studies by Burke et al. (4, 5) were the only other investigations in which subjects were fed a CHO restoration diet before exercise. Despite a similar order of magnitude of CHO sparing as in the present study, the exercise protocols of Burke et al. (4, 5) were likely to have been too short in duration to severely deplete muscle glycogen and, as such, affect the performance of a subsequent TT.

Large interindividual performance responses after high-fat diets have been reported previously (4, 29). This was most evident in the study by Phinney et al. (29), in which a huge performance enhancement by one subject after fat adaptation improved the group mean such that it was not different from the control diet. In the present study, we do not know the reliability of a 1-h TT undertaken at the end of such a prolonged preload. However, the possibility exists that the reliability of this measure may be sufficiently large that it fails to detect the small but worthwhile enhancements in performance with sample sizes of only 6–10 subjects. On average, subjects rode the TT at a power output that was 11% higher after fat adaptation, and, although this performance enhancement failed to reach statistical significance, it represented an ~4% improvement, which would certainly be worthwhile and meaningful for an ultraendurance athlete (19).
The large increases in fat oxidation during exercise in the present study are in agreement with previous investigations that have used a similar dietary intervention (4, 5, 14, 26, 29). Such increases are impressive in light of the already high capacity for fat oxidation in these highly trained athletes. Accompanying the enhanced rate of fat oxidation was a concomitant reduction in the rate of CHO oxidation, such that ~120 g of endogenous CHO was “spared” during the 4-h steady-state ride after fat adaptation. Why such a sparing did not result in an enhancement of subsequent TT performance is difficult to explain.

One possible scenario is that muscle glycogen is not the only factor influencing performance of prolonged, submaximal exercise. Alternatively, it could be that subjects commenced the TT with sufficient muscle glycogen stores after both dietary interventions, enabling them to complete 1 h of intense (280–312 W) cycling. Indeed, any shortfall in CHO availability would have been entirely compensated for by the aggressive CHO ingestion regimen during the TT, a practice consistent with the real-life dietary strategies used by athletes who compete in single ultraendurance events (e.g., Ironman Triathlon). In this regard, it should be noted that, of the ~400 g of CHO ingested, only one-third (~125 g) was oxidized during the first 4 h of submaximal cycling. Such a finding is consistent with previous studies that have measured both the rates of gastric emptying and oxidation of ingested CHO during submaximal cycling and have reported that the amount oxidized is always far less than the amount ingested (for review see Ref. 21). It has been suggested that, if the glucose supply from the intestine is too high (i.e., greater than the maximal rate of exogenous CHO oxidation by muscle, ~1 g/min), glycogenesis may be activated in the liver (21). However, such a hypothesis seems unlikely as hepatic tissue retains very little glucose directly, even in the presence of supraphysiological concentrations of glucose and insulin (9). Instead, we propose that the modest hyperinsulinemia that accompanied CHO ingestion would have been likely to accelerate glucose transport and hexokinase activity, whereas the prevailing (~5.5 mmol/l) concentrations of glucose would accelerate intracellular glucose-6-phosphate concentration and promote nonworking muscle glycogen synthesis. Direct evidence for this hypothesis comes from Kuipers et al. (25), who reported that, when well-trained cyclists ingested large amounts of CHO (~500 g) during 3 h of submaximal cycling, there was net glycogen synthesis in nonactive muscle fibers.

Romijn et al. (33) demonstrated previously that, in well-trained fasted subjects riding at 65% of maximum VO2, the contribution from muscle glycogen to total CHO oxidation was ~80%. In the present study, total CHO oxidation after the high-fat diet during the 4-h steady-state ride approached 600 g, of which ~20% came directly from the exogenous CHO drink ingested during exercise. As consuming CHO during constant-load cycling suppresses endogenous glucose production (3, 11, 23) but does not reduce muscle glycogenolysis (3, 8, 13, 15), and because the contribution of plasma glucose to total CHO and plasma-derived glucose oxidation was very small, it can be estimated that, after fat adaptation, ~460 g of muscle glycogen were oxidized during the 4-h ride. On the other hand, total CHO oxidation during the steady-state ride after the high-CHO diet was >700 g, of which muscle glycogen oxidation contributed an estimated 580 g. This latter figure approaches the upper limit of the glycogen content of the active muscle mass involved in cycling (20) and running (27).

Despite maximizing CHO availability before and during exercise in the present study, the rate of fat oxidation after fat adaptation remained significantly elevated above that of the high-CHO diet treatment. The mechanisms underlying this observation are difficult to explain. Changes in selected muscle enzymes, such as increased carnitine acyl transferase (12, 14), carnitine palmitoyl transferase-1 (14), 3-hydroxyacyl-CoA dehydrogenase (18), and reduced muscle hexokinase activity (14), have previously been reported in human subjects. Other investigations have found that exposure of trained subjects to high-fat diets for varying time periods increases intramuscular triglyceride stores (20, 24, 36) or oxidation of plasma-derived triglycerides (35), which might provide an extra substrate pool to account for additional fat utilized during exercise. The precise mechanisms underlying the increased oxidation of fat after only 6 day of fat adaptation in the face of high-CHO availability remains to be determined.

In conclusion, this is the first investigation to determine the effects of a high-fat diet and CHO restoration on metabolism and performance during ultraendurance exercise. We found that 6 days of exposure to a high-fat, low-CHO diet, followed by 1 day of CHO restoration, increased fat oxidation during prolonged, submaximal exercise, yet, despite this sparing of CHO, this study failed to detect a statistically significant benefit to performance of a 1-h TT undertaken after 4 h of continuous cycling.

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