Adaptations to short-term high-fat diet persist during exercise despite high carbohydrate availability

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ABSTRACT

BURKE, L. M., J. A. HAWLEY, D. J. ANGUS, G. R. COX, S. CLARK, N. K. CUMMINGS, B. DESBROW, and M. HARGREAVES. Adaptations to short-term high-fat diet persist during exercise despite high carbohydrate availability. *Med. Sci. Sports Exerc.*, Vol. 34, No. 1, 2002, pp. 83–91. **Purpose:** Five days of a high-fat diet produce metabolic adaptations that increase the rate of fat oxidation during prolonged exercise. We investigated whether enhanced rates of fat oxidation during submaximal exercise after 5 d of a high-fat diet would persist in the face of increased carbohydrate (CHO) availability before and during exercise. **Methods:** Eight well-trained subjects consumed either a high-CHO (9.3 $g \cdot kg^{-1} \cdot d^{-1}$ CHO, 1.1 $g \cdot kg^{-1} \cdot d^{-1}$ fat; HCHO) or an isoenergetic high-fat diet (2.5 $g \cdot kg^{-1} \cdot d^{-1}$ CHO, 4.3 $g \cdot kg^{-1} \cdot d^{-1}$ fat; FAT-adapt) for 5 d followed by a high-CHO diet and rest on day 6. On day 7, performance testing (2 h steady-state (SS) cycling at 70% peak O_2 uptake [$\dot{V}O_{2peak}$] + time trial [TT]) of 7 $kJ \cdot kg^{-1}$) was undertaken after a CHO breakfast (CHO 2 $g \cdot kg^{-1}$) and intake of CHO during cycling (0.8 $g \cdot kg^{-1} \cdot h^{-1}$). **Results:** FAT-adapt reduced respiratory exchange ratio (RER) values before and during cycling at 70% $\dot{V}O_{2peak}$; RER was restored by 1 d CHO and CHO intake during cycling (0.90 ± 0.01, 0.80 ± 0.01, 0.91 ± 0.01, for days 1, 6, and 7, respectively). RER values were higher with HCHO (0.90 ± 0.01, 0.88 ± 0.01 (HCHO > FAT-adapt, $P < 0.05$), 0.95 ± 0.01 (HCHO > FAT-adapt, $P < 0.05$)). On day 7, fat oxidation remained elevated (73 ± 4 g vs 45 ± 3 g, $P < 0.05$), whereas CHO oxidation was reduced (354 ± 11 g vs 419 ± 13 g, $P < 0.05$) throughout SS in FAT-adapt versus HCHO. TT performance was similar for both trials (25.53 ± 0.67 min vs 25.45 ± 0.96 min, NS). **Conclusion:** Adaptations to a short-term high-fat diet persisted in the face of high CHO availability before and during exercise, but failed to confer a performance advantage during a TT lasting ~ 25 min undertaken after 2 h of submaximal cycling. **Key Words:** FAT ADAPTATION, METABOLISM, CYCLING TIME TRIAL

In contrast to the detrimental effects of very short-term exposure (≤ 3 d) to high-fat diets, which reduce resting muscle glycogen content without compensation for the reduced carbohydrate (CHO) availability (2,5,8,27), longer periods of adaptation to high-fat, low-CHO diets increase the capacity of the muscle to oxidize fat (9,20,24). However, the reports of muscle glycogen sparing as a consequence of fat adaptation in these studies are uncertain, as this finding could be an artifact arising from a striking reduction in starting muscle glycogen content (20,24). Also equivocal is the effect of fat adaptation *per se* on exercise performance. When undertaken by trained subjects, fat adaptation has been reported to either enhance (20,22) or fail to alter (9,24) exercise capacity. Alternatively, when sedentary subjects are exposed to high-fat diets while commencing a training program, gains in endurance are either impaired (15) or unchanged (16) compared with a control group consuming a high-CHO diet. The combination of an enhanced capacity for fat utilization and increased CHO availability might provide a potential benefit for endurance performance compared with the reliance on one or other of these fuel sources alone.

In a previous study, we reported that 5 d of a high-fat, low-CHO diet in well-trained subjects caused marked changes in substrate utilization during prolonged, submaximal exercise (4). Five days of this dietary intervention combined with high-volume, intense training elicited an almost twofold increase in the rate of fat oxidation during steady-state submaximal exercise compared with baseline values (4). At least some of the changes in substrate oxidation observed in that study were independent of CHO availability, since a greater rate of fat oxidation persisted despite subsequent restoration of muscle glycogen concentration after 1 d of high CHO intake (4). Both direct and indirect estimation of muscle glycogen disappearance showed that the decreased rate of CHO oxidation could almost entirely be accounted for by the decreased rate of muscle glycogen utilization (4).

Despite promoting muscle glycogen sparing during 2 h of submaximal exercise, fat adaptation strategies in that study did not provide a clear benefit to the subsequent

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performance of a time trial lasting ~ 30 min (4). Indeed, the performance results were highly variable and appeared, in part, to be influenced by the better preservation of blood glucose concentration following the fat adaptation treatment in some subjects, which otherwise declined throughout the steady-state exercise. Such a decline in blood glucose concentrations is expected in prolonged exercise undertaken in the baseline metabolic conditions chosen for this study; subjects were overnight fasted and consumed only water throughout the exercise bout. However, such conditions are not representative of the nutrition practices recommended by sports nutritionists (1).

The present study was undertaken to determine whether the enhanced rates of fat oxidation during submaximal exercise reported after 5 d of a high-fat diet in the fasting condition persist in the face of high CHO availability before and during exercise. Such nutritional strategies are typically practiced during endurance sports. We hypothesized that a high-CHO preexercise meal and CHO ingestion throughout exercise, in addition to glycogen restoration with 1 d of high CHO intake, would abolish the enhanced rates of fat oxidation induced by a short-term high-fat diet. In addition to investigating the hierarchy of metabolic regulation during exercise with fat-adaptation and high-CHO availability, we wished to determine if such strategies would confer a performance benefit to an endurance task under sport-specific conditions.

SUBJECTS AND METHODS

Eight well-trained male cyclists or triathletes (age, 27.9 ± 1.7 yr; weight, 73.7 ± 2.3 kg; $\dot{V}O_{2max}$, 68.6 ± 1.9 $mL \cdot kg^{-1} \cdot min^{-1}$; peak sustained power output (PPO), 399 ± 10 W, mean ± SEM) 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 subsequently described experimental trials, each subject performed an incremental cycling test to exhaustion on an electronically braked cycle ergometer (Lode, Groningen, The Netherlands). The test protocol has been described in detail previously (13). During the maximal test, which typically lasted between 10 and 12 min, subjects inspired air through a two-way Hans Rudolf valve attached to a custom built automated Douglas bag gas analysis system (Australian Institute of Sport, ACT, Australia) for which calibration and operation details have been previously described (10). The incremental test to exhaustion was used to measure each subject's $\dot{V}O_{2max}$ and PPO. These values were used to determine the work rate corresponding to 70% of each subject's $\dot{V}O_{2max}$ (~ 63% of PPO) to be used in the experimental trials.

Study design. Each subject undertook two trials in a randomized, double-blind crossover design separated by a 2-wk washout period. Blinding of the dietary treatments was achieved by developing recipes to allow certain foods or dishes to be made in indistinguishable high-fat or low-fat forms; adding CHO (Polyjoule™) or fats (oils or cream) to other foods or dishes to modify the fat or CHO content; and using "decoy" foods on each diet, i.e., placing *perceived* CHO-rich foods in conspicuous ways in high-fat menus (e.g., bread, fruit, salads), and conversely, making high-fat foods (e.g., butter) obvious in the high-CHO trial. A sample day's menu incorporating these strategies is presented in Table 1. At the end of each dietary treatment, subjects were asked to nominate the diet they thought they had received, how they made this decision, and when they first became sure of the decision. To further remove any opportunity for observer bias, we ensured that the investigator responsible for the collection of performance data was kept blind to the order of treatments by minimizing her interaction with subjects or the metabolic data collection.

On day 1 of each trial, subjects reported to the laboratory after a 12- to 14-h overnight fast. Subjects were then weighed, and a resting blood sample was collected by venipuncture from an antecubital vein. After sitting quietly for 10 min, subjects mounted the ergometer and cycled for 20 min at 70% of $\dot{V}O_{2max}$ (251 ± 6 W). Pulmonary gas data were collected for the last 5 min of the ride, as previously described.

Subjects then commenced 5 d of a supervised diet/training program. On the fat-adaptation trial (FAT-adapt), they were prescribed a high-fat (4.3 $g \cdot kg^{-1} \cdot d^{-1}$ fat, 70% of energy), low CHO (2.5 $g \cdot kg^{-1} \cdot d^{-1}$ CHO, 18% of energy) diet supplying 0.22 $MJ \cdot kg^{-1}$ body mass (BM). The control treatment (HCHO) was an isoenergetic diet providing 9.3 $g \cdot kg^{-1} \cdot d^{-1}$ and 70% of energy from CHO and 1.1 $g \cdot kg^{-1}$ and 18% 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 < 50 g and matched to within 5 to 10 g each day between dietary 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 body mass. At least one meal each day was eaten under supervision in the laboratory, with the remaining food for each 24-h period being provided in preprepared 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.

Training programs were individualized for each subject according to their fitness level and current training load. It was intended that the program would correspond to the habitual training volume undertaken by each subject, translated into road cycling hours. In addition to their normal training, we included two prescribed laboratory-supervised interval-training sessions in each trial. The first interval session was undertaken at the commencement of each trial, immediately following the steady-state exercise test. The intention of this session was to cause a rapid lowering of muscle glycogen concentrations on the first day, and initiate a rapid differentiation between dietary treatments on the basis of their ability to restore depleted muscle glycogen stores. The second interval session was undertaken on day 4 of each trial, to allow comparison of the capacity to perform high-intensity exercise with each dietary treatment. Each training session was completed under supervision; being undertaken either in the laboratory or on the road accom-

TABLE 1. Example of diets in HCHO and FAT-adapt treatments for 76-kg athlete.

Meal	HCHO	FAT-Adapt
Breakfast	135 g museli (rolled oats, all-bran, sugar, dried apricots, pumpkin kernels, sultanas, cashew nuts) 300 g skim milk 2 slices mixed grain bread (10 g margarine, 15 g honey, 15 g jam) 280 g orange juice (+ 30 g of Polyjoule™)	100 g museli (linseed and almond mix, macadamia nuts, pumpkin kernels, sesame seeds, rolled oats, oil) 280 g milk 580 g milk shake (milk, cream and low-joule topping)
Lunch	300 g cream of chicken and vegetable soup (+ 22 g skim milk powder) 90 g bread roll 10 g margarine	230 g chicken and mushroom soup (+ cream) 50 g bread roll 12 g margarine
Dinner	665 g lasagne (onion, garlic, lean mince, mushrooms, pasta sauce, tomato soup, pasta sheets, natural yogurt, reduced fat cheese) 200 g salad (lettuce, tomato, carrot, cucumber) 20 g reduced-fat mayonnaise 84 g garlic bread 355 g trifle (sponge cake, jelly with Polyjoule™, custard, sugar, reduced-fat cream and grated chocolate)	650 g lasagne (onion, oil, garlic, regular mince meat, pasta sauce, tomato soup, crushed macadamia nuts, pasta sheets, sour cream, cheese) 140 g salad (lettuce, tomato, cucumber) 30 g Italian dressing 290 g trifle (low-joule jelly, custard, cream, sponge cake, macadamia nuts)
Snacks	42 g chocolate chip biscuits	80 g carrot sticks 50 g celery sticks 45 g French onion dip 65 g avocado and natural yogurt dip
Training fluid	160 g 25% cordial made up to 1 L (+ 60 g Polyjoule™)	160 g low-joule cordial (made up to 1 L)
Nutritional analysis		
Energy (kJ)	16,890	16,942
Carbohydrate (g)	698	203
Fat (g)	87	309
Protein (g)	127	121

panied by one of the researchers. Each subject completed an identical training program during both of his trials.

On the morning of day 6, subjects returned to the laboratory and repeated the testing protocol undertaken on day 1. After completing the 20-min cycle, subjects were provided with a high-CHO diet providing CHO 10 $g \cdot kg^{-1}$ BM and rested for the next 24 h. This phase was an attempt to normalize muscle glycogen stores independent of the previous dietary treatment. On the morning of day 7, subjects reported to the laboratory after an overnight fast to undertake a performance ride that consisted of 2 h cycling at 70% of $\dot{V}O_{2max}$ (SS) followed by a 7-$kJ \cdot kg^{-1}$ BM time trial (TT) anticipated to last ~ 30 min.

Performance ride. On arrival in the laboratory, subjects were weighed, and catheters (Terumo 20G, Tokyo, Japan) were inserted into a vein in the antecubital space of each arm for blood sampling. A basal blood sample was collected from the sampling catheter, which was kept patent by flushing with 0.5 mL of saline. Subjects were then fed a standard breakfast (fruit juice, toasted bread and jam, and a Power Bar™), providing a CHO intake of 2 $g \cdot kg^{-1}$. This meal was consumed within 15 min and subjects then rested in the laboratory while postprandial blood samples were taken at 30, 60, 90, and 120 min. Subjects then mounted the cycle ergometer and began SS (120 min of steady-state exercise at 70% of $\dot{V}O_{2max}$). From 15 to 20 min, respiratory gas data and a blood sample were collected, and subjects provided a rating of their perceived exertion (RPE) according to the Borg scale (3). This protocol was repeated during each subsequent 20-min period throughout the ride. Fluid intake was standardized during the ride: subjects were provided with 3.3 $mL \cdot kg^{-1}$ of a commercial CHO-electrolyte drink providing 6% (weight:volume) CHO to be consumed each 20 min. At 120 min, after the final blood sample was taken, subjects stopped cycling for a standardized rest period of 2 min while the Lode bike was changed to linear mode. A further intake of 4 $mL \cdot kg^{-1}$ of sports drink was provided for intake during the TT.

Subjects were instructed to complete the 7-$kJ \cdot kg^{-1}$ TT "as fast as possible." The same researcher supervised each time trial and provided standardized feedback to each subject. The only information available to subjects during the TT was elapsed work as a percentage of the final work; furthermore, subjects were given the results of their TT performances only after the entire study was completed. No respiratory or blood data were collected during the TT. On completion of the TT, subjects were towel-dried and weighed.

Blood sampling and analyses. Twelve milliliters of blood were collected at each sampling time, of which 5 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 by radioimmunoassay (Incstar, Stillwater, MN). A further aliquot of blood was mixed in a tube containing lithium heparin and spun in a centrifuge. 500 μL of plasma was placed in a tube containing 500 μL of ice-cold 3 mol perchloric acid, mixed vigorously on a vortex mixer, and spun; 800 μL of this supernatant was added to a tube containing 200 μL of 6 mol potassium hydroxide (KOH), mixed, and spun. The resultant supernatant was analyzed for glycerol using an enzymatic spectrophotometric analysis (25). 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 free fatty acid (FFA) concentration using an enzymatic colorimetric method (Wako, NEFAC code 279–75409, Tokyo, Japan).

Rates of fat and CHO oxidation. Whole body rates of CHO and fat oxidation ($g \cdot min^{-1}$) were calculated from the respiratory data collected during the 20-min cycle bouts, and from the data collected every 20 min during the steady-state phase of the performance ride. The calculations were made from $\dot{V}CO_2$ and $\dot{V}O_2$ measurements assuming a non-protein RER value, according to the following equations (23): CHO oxidation = 4.585 $\dot{V}CO_2$ − 3.226 $\dot{V}O_2$; and fat oxidation = 1.695 $\dot{V}O2$ − 1.701 $\dot{V}CO_2$.

Total fat and CHO oxidation over the 120 min of steady state exercise were estimated by calculating the area under the oxidation ($g \cdot min^{-1}$) versus time curves for each subject. Rates of fatty acid oxidation ($\mu mol \cdot kg^{-1} \cdot min^{-1}$) were determined by converting the rate of triglyceride oxidation ($g \cdot kg^{-1} \cdot min^{-1}$) to its molar equivalent assuming the average molecular weight of human triglyceride to be 855.3 $g \cdot mol^{-1}$ and multiplying the molar rate of triglyceride oxidation by 3, because each molecule contains three molecules of fatty acids. Rates of CHO oxidation ($\mu mol \cdot kg^{-1} \cdot min^{-1}$) were determined by converting the rate of CHO oxidation ($g \cdot min^{-1}$) to its molar equivalent assuming 6 mol of O_2 are consumed and 6 mol of CO_2 produced for each mole (180 g) oxidized. Fat and CHO oxidation rates ($\mu mol \cdot kg^{-1} \cdot mL^{-1}$) for this study were plotted against the values calculated for subjects in our previous study (4).

Statistical analyses. Data from the two trials were compared using a two-factor (diet and time) analysis of variance (ANOVA) with repeated measures. Separate analyses were undertaken to compare data from day 1, day 6, the first 20 min of SS on day 7, and data collected at different time points during SS. Newman-Keuls *post hoc* tests were undertaken when ANOVA revealed a significant difference or interaction between factors. Differences in TT performances between trials were compared using Student's *t*-tests. Significance was accepted at $P < 0.05$. All data are reported as mean ± SEM. The statistical analyses were undertaken using Statistica software for Windows (version 5.1, 1997, StatSoft, Inc., Tulsa, OK).

RESULTS

Training and dietary control. All subjects completed the dietary and training requirements of both treatments. Mean training volume was 495 ± 26 km and 16.7 ± 0.8 h for the HCHO trial and 491 ± 27 km, 16.7 ± 0.8 h for the FAT-adapt trial. All subjects reported experiencing symptoms of mild headaches, lethargy, and increased fatigue during the high-fat dietary treatment compared with the HCHO treatment. Although the full training program was completed on the FAT-adapt diet, all subjects experienced difficulties in at least one training session, particularly the interval session on day 4, either complaining of increased perception of effort or difficulty in maintaining the training pace. The generalized symptoms appeared to decrease as the week progressed. Changes in BM from day 1 to day 7 revealed a mean loss of 1.6 ± 0.2 kg with FAT-adapt and 1.4 ± 0.2 kg with HCHO. Although we observed rapid fluctuations in BM, which could be partially explained by changes in gastrointestinal contents because of a lower residue diet and changes in muscle concentrations of glycogen and water, subjects experienced a mild energy deficit during their dietary treatments.

Seven of the eight subjects correctly identified the order of their dietary treatments. The time point they nominated as being sure of their dietary intervention ranged between the evenings of day 1 and day 4. The main clue used to identify diets was the sense of well-being or exercise tolerance. The one subject, who was unable to identify the diets correctly, received the HCHO treatment first and judged this to be the FAT-adapt treatment on the basis of feeling fatigued. Food-related factors were of secondary importance in decoding the dietary treatments, although subjects noted the sustained absence or presence of key foods over the 5 d of eating, as well as the absence or presence of oily residue on crockery and cutlery. Four subjects were able to correctly identify the treatment on which they achieved their fastest TT performance.

Blood metabolites. Fasting plasma glucose and insulin concentrations did not differ between trials or as a result of the dietary treatments; values were the same on day 1, day 6, and day 7 (data not shown). Fasting plasma FFA concentrations were higher after 5 d of the high-fat diet (day 1, 0.43 ± 0.04 vs 0.38 ± 0.07 $mmol \cdot L^{-1}$; day 6, 0.43 ± 0.05 vs 0.93 ± 0.1 $mmol \cdot L^{-1}$, $P < 0.05$) for HCHO and FAT-adapt, respectively). However, after 1 day of CHO restoration, fasting FFA concentrations declined to similar levels as for day 1 (0.69 ± 0.10 $mmol \cdot L^{-1}$) and were not different from the corresponding time point for the HCHO trial (0.47 ± 0.10 $mmol \cdot L^{-1}$). Similarly, fasting plasma glycerol concentrations increased after 5 d of the high-fat diet and were elevated at day 6 (0.04 ± 0.01 vs 0.09 ± 0.02 for HCHO and FAT-adapt, $P < 0.05$). However, they were not different from those of the HCHO trial on day 7 (0.06 ± 0.01 vs 0.04 ± 0.01).

Plasma glucose, insulin, and lactate concentrations during testing on day 7 are summarized in Figure 1. This figure displays fasting values, responses to the preride CHO meal, and responses during 120 min of SS cycling for these metabolites. There was a time effect for plasma glucose and lactate concentrations ($P < 0.05$), but no effect of dietary treatments. In both trials, plasma glucose concentrations were increased 30 min after intake of the CHO meal ($P < 0.05$), and declined thereafter so that concentrations at 90 and 120 min after the meal were below fasting values ($P < 0.05$) (Fig. 1A). The combination of cycling and intake of the CHO-containing drink increased glucose concentrations above values at the onset of exercise ($P < 0.05$). Plasma glucose concentration remained elevated above preexercise values throughout the 120 min of SS cycling. Plasma lactate concentrations increased above fasting values in response to the CHO meal and were maintained throughout the remain-

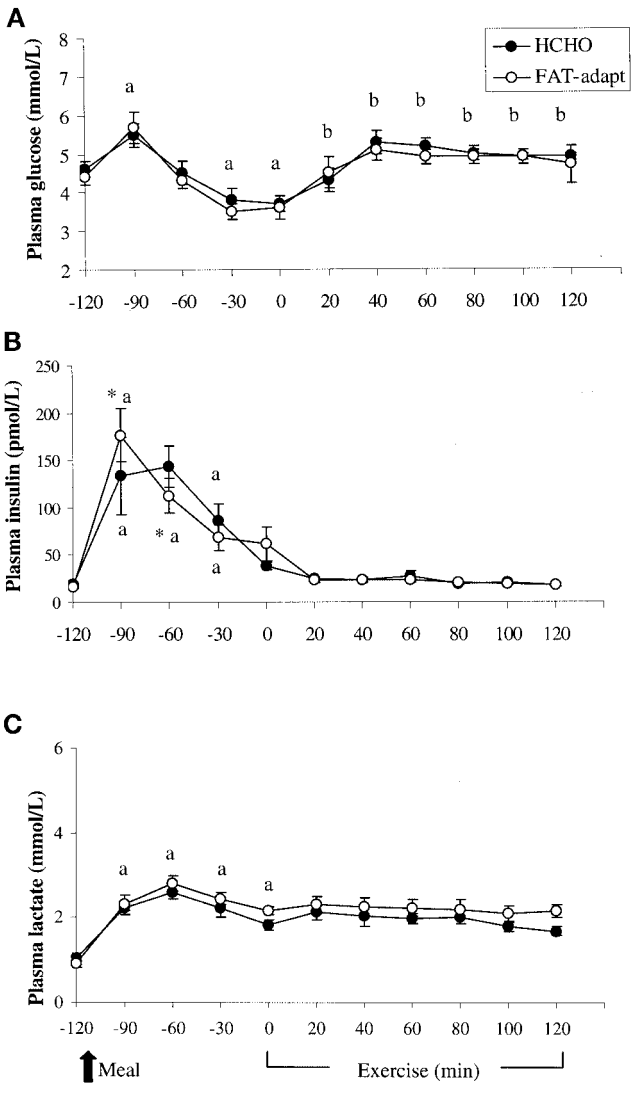

FIGURE 1—Plasma concentrations of glucose, insulin, and lactate on day 7 after 5 d adaptation to high-fat diet and 1 d CHO restoration. Values are measured for fasting, for 120 min after a CHO-rich meal, and throughout 120 min of steady-state cycling at 70% $\dot{V}O_{2max}$. Values are mean ± SEM for eight subjects. *Different from HCHO trial; [a]different from fasting values; [b]different from values at onset of exercise.

der of the preride and SS time points ($P < 0.05$) (Fig. 1C). There was a significant interaction of diet and time for plasma insulin concentration (Fig. 1B). Plasma insulin concentrations were increased above fasting values for 90 min after the ingestion of the preride CHO meal in both trials ($P < 0.05$). The insulin response differed for dietary treatments, with the FAT-adapt trial appearing to produce a more rapid and shorter-lasting peak insulin value compared with the HCHO trial. In both trials, insulin concentrations fell to fasting values by the onset of exercise and remained at similarly low concentrations throughout the SS cycling.

Plasma FFA and glycerol concentrations during testing on day 7 are shown in Figure 2. There was a significant interaction of time and diet for plasma glycerol concentration. In both trials, plasma glycerol concentrations remained at fasting values throughout the preexercise period (Fig. 2A). However, in the FAT-adapt trial plasma glycerol concentrations gradually increased during exercise so that from 100 min onwards, glycerol concentrations were above fasting values and above values at the corresponding time points in the HCHO trial ($P < 0.05$). There was also a significant interaction of time and diet for plasma FFA concentrations, with fasting values being greater in the FAT-adapt trial than the HCHO trial ($P < 0.05$) (Fig. 2B). Plasma FFA concentrations declined equally in both trials in response to the CHO meal until the onset of exercise ($P < 0.05$). During exercise, FFA concentrations increased gradually and equally in both trials so that values were greater from 80 min onwards than the immediate preexercise concentration ($P < 0.05$).

CHO and fat oxidation during exercise. Figure 3 summarizes RER data collected during 20 min of cycling at 70% of $\dot{V}O_{2max}$ on day 1, day 6, and day 7 (first 20 min of SS), as well as throughout SS. Five days of training and the FAT-adapt dietary intervention reduced RER values from day 1 (0.90 ± 0.01) to day 6 (0.80 ± 0.01, $P < 0.05$). RER values were maintained from day 1 to day 6 with the high CHO dietary treatment. One day of high-CHO diet and rest together with the high CHO preevent meal increased RER values in the FAT-adapt treatment so that RER at the start of the SS ride was returned to day 1 values (see Fig. 1). In the HCHO trial, 1 d of rest and high-CHO diet plus the preride CHO intake increased RER during the first 20 min

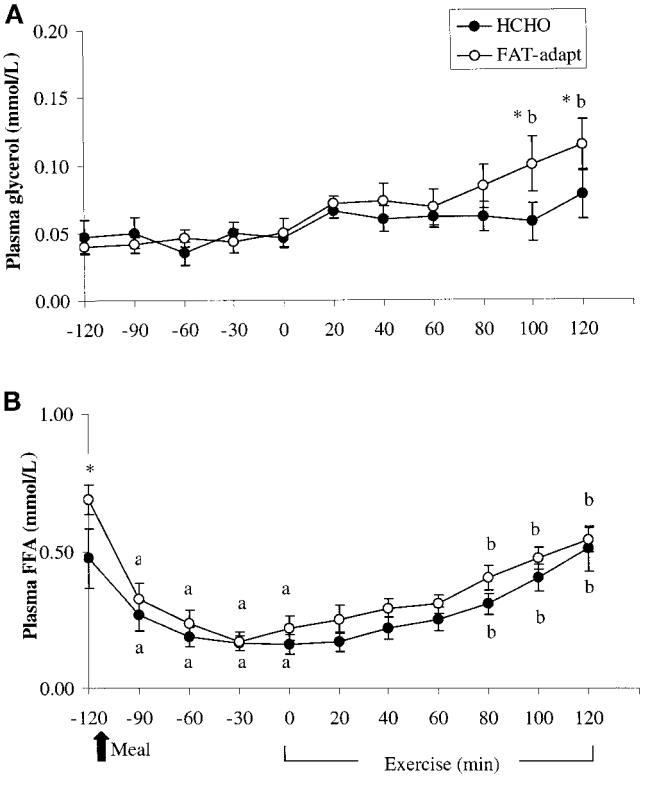

FIGURE 2—Plasma concentrations of glycerol and free fatty acids (FFA) on day 7 after 5 d adaptation to high-fat diet and 1 d CHO restoration. Values are measured for fasting, for 120 min after a CHO-rich meal, and throughout 120 min of steady-state cycling at 70% $\dot{V}O_{2max}$. Values are mean ± SEM for eight subjects. *Different from HCHO trial; [a]different from fasting values; [b]different from values at onset of exercise.

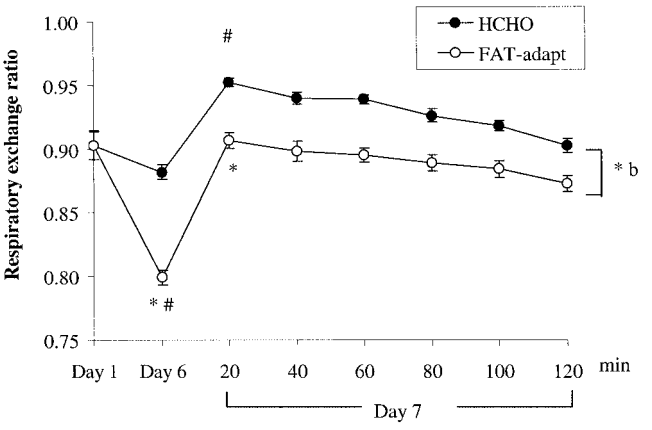

FIGURE 3—Effect of 5 d adaptation to high-fat diet and 1 d CHO restoration (FAT-adapt) on respiratory exchange ratio during cycling at 70% $\dot{V}O_{2max}$ compared with control trial (HCHO). Values are mean ± SEM for eight subjects at day 1 (baseline), day 6 (adaptation), and during 120 min of steady-state cycling on day 7. Comparison of data for 20 min cycling (day 1 vs day 6 vs day 7): #Different from day 1; *different from HCHO trial. Comparison of data from 120-min steady-state cycling (SS) during Performance ride: *different from HCHO trial, bdifferent to onset of exercise.

of SS to values higher than seen during exercise on day 1 (0.90 ± 0.01 vs 0.95 ± 0.0 for day 1 and day 6, $P < 0.05$) and for day 6 in the FAT-adapt trial (0.91 ± 0.01, $P < 0.05$). There was a progressive decline in RER values during the 120 min of SS in the performance ride with both treatments ($P < 0.05$). However, there was a significant treatment effect of diet, with RER values in HCHO trial being higher than with the FAT-adapt treatment ($P < 0.05$).

Rates of CHO and fat oxidation during submaximal cycling on days 1, 6, and 7 are summarized in Figure 4, and compared with the results collected in our previous study (4). Data from the present study are labeled as FAT-adapt + CHO and HCHO + CHO to denote the intake of CHO before and during the trial on day 7. Data from the first study (4) are labeled FAT-adapt − CHO and HCHO − CHO to denote that the two investigations were conducted with an identical protocol apart from the absence of CHO intake on performance testing (day 7) testing in the first study (4). The data from the present study follow the RER data previously outlined. The 5-d adaptation to a high fat intake increased rates of fat oxidation ($P < 0.05$) and decreased rates of CHO oxidation ($P < 0.05$) during exercise compared with baseline values. One day of high CHO intake, together with a CHO-rich breakfast and CHO intake during exercise, increased rates of CHO oxidation ($P < 0.05$) and reduced rates of fat oxidation ($P < 0.05$) back to baseline values. However, there was a main effect of diet ($P < 0.05$), with the rates of fat oxidation being significantly higher, and CHO oxidation being correspondingly lower during SS cycling on day 7 in the FAT-adapt trial than with the HCHO trial ($P < 0.05$). In both trials, there was a gradual but significant increase in rates of fat oxidation and a concomitant decrease in rates of CHO oxidation over the 120 min of SS cycling ($P < 0.05$).

The superimposition of data from the first study shows that identical changes in rates of fat and CHO oxidation were achieved as a result of 5 d of high fat intake in both studies. Furthermore, the ingestion of CHO on day 7 (before and during exercise) resulted in an upward shift in the rate of CHO oxidation and a downward shift in rates of fat oxidation compared with the first study. However, the pattern of change in rates of both fat and CHO oxidation throughout the 120 min of SS cycling paralleled the changes reported in the first study, with the differences between the HCHO and FAT-adapt trials maintained throughout exercise.

Estimates of total substrate utilization during 120 min of SS cycling are presented in Figure 5. In the FAT-adapt trial, we observed a sparing of CHO utilization compared with the HCHO trial (354 ± 11 g vs 419 ± 13 g, $P < 0.05$), and an increased utilization of fat (73 ± 4 g vs 45 ± 3 g, $P < 0.05$).

Performance. Changes in body mass over the performance ride (SS and TT) did not differ between trials (0.6 ± 0.1 kg vs 0.7 ± 0.1 kg for FAT-adapt and CHO trials, respectively), suggesting that a similar fluid deficit was incurred in each trial. There was no difference in the time to complete 7 $kJ \cdot kg^{-1}$ of work between trials (25.53 ± 0.96

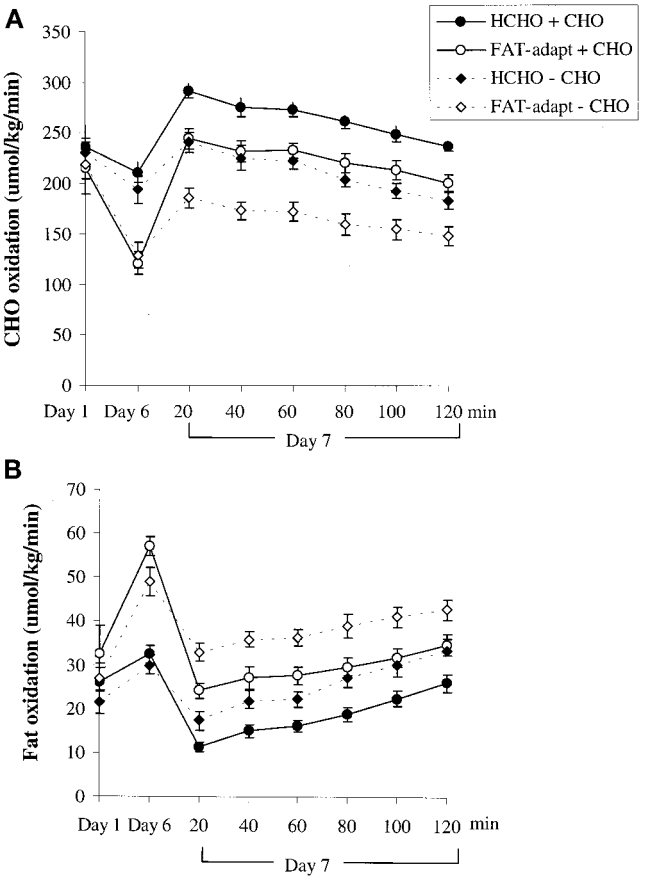

FIGURE 4—Effect of 5 d adaptation to high-fat diet and 1 d CHO restoration (FAT-adapt) on rate of CHO oxidation and rate of fat oxidation during cycling at 70% $\dot{V}O_{2max}$ compared with control trial (HCHO). Values are mean ± SEM for eight subjects at day 1 (baseline), day 6 (adaptation), and during 120 min of steady-state cycling on day 7. Data from present trial = HCHO + CHO and FAT-adapt + CHO to denote intake of CHO before and during cycling on DAY 7. Data from Burke et al. (4) = HCHO − CHO and FAT-adapt − CHO to denote no further intake of CHO on day 7.

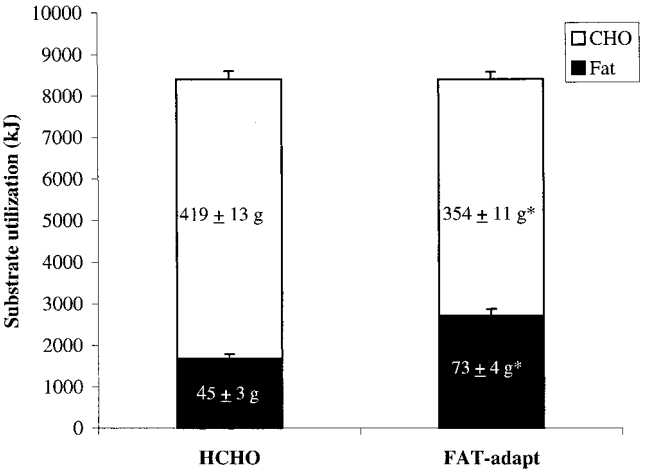

FIGURE 5—Estimated contribution of CHO and fat to substrate oxidation during 120 min of steady-state cycling at 70% $\dot{V}O_{2max}$ on day 7 following 5 d adaptation to high-fat diet and 1 d CHO restoration. Values are mean ± SEM for eight subjects. *Different from HCHO (control) trial.

min vs 25.45 ± 0.67 min for FAT-adapt and HCHO, respectively, $P = 0.86$). The mean difference in TT performance was a 0.7% reduction in performance with the FAT-adapt treatment (95% CI, −1.7% to 0.4%).

DISCUSSION

The main finding from the present study was that 5 d of a high-fat diet enhanced rates of fat oxidation during submaximal exercise despite increased CHO availability before and during exercise. However, the most novel finding from the current investigation was that, in contrast to our hypothesis, the CHO sparing observed after short-term adaptation to a high-fat diet was not abolished by this increased CHO availability. Indeed, the CHO sparing observed during 2 h of submaximal exercise in the present study (~ 65 g) was similar to that reported in our previous investigation undertaken in the fasted condition with no CHO supplementation (4). Despite such changes in fuel utilization during exercise, fat adaptation in association with increased CHO availability still had no beneficial effect on the performance of a TT undertaken at the end of 2 h of submaximal cycling compared with the high-CHO diet.

Dietary periodization to optimize the contributions of both fat and CHO oxidation to fuel metabolism during submaximal exercise is an appealing concept (14). Strategies to increase CHO availability are known to enhance endurance and performance of prolonged exercise tasks, although the general effect is to increase rates of CHO oxidation, and/or to supply an additional CHO source rather than spare limited muscle glycogen stores (11,14,28). Alternatively, strategies that promote the availability of fat as an exercise fuel can reduce reliance on endogenous CHO stores (12).

The dietary periodization protocol used in this study combined a 5-d phase of training on a high-fat, low-CHO intake with 1 d of high CHO intake and rest. This is in contrast to the design of previous studies of fat adaptation in which more prolonged periods (2–7 wk) of high fat intake have been undertaken (15,16,20,24). However, our recent work (4) and a study by Goedecke et al. (9) have shown that significant increases in fat oxidation occur after as little as 5 d of exposure to a high-fat diet. More to the point, short-term exposure to a high-fat diet offers a more manageable period for radical dietary change and an advantage in minimizing the potential health and training disadvantages arising from longer periods on high fat intakes. We chose a 1-d period of CHO recovery to restore muscle glycogen levels without allowing a "washout" of adaptations incurred by the high-fat phase (4).

In agreement with our previous findings (4), the dietary fat treatment utilized in this study resulted in large shifts in fat oxidation during exercise. Five days of high-fat intake combined with training produced an almost twofold increase in the rate of fat oxidation during cycling at 70% $\dot{V}O_{2max}$ compared with baseline values. This increase is particularly impressive in light of the already high capacity for fat oxidation in highly trained subjects. Although muscle glycogen content was not monitored in the present study, the 1 d of rest and a high-CHO diet that followed the adaptation phase has previously been shown to increase muscle glycogen stores above normal resting, regardless of previous dietary treatment (4). When this glycogen restoration strategy was combined with a high-CHO breakfast, rates of fat oxidation at the onset of exercise in both trials were reduced compared with the corresponding day 6 (fasting) values. Concomitantly, rates of CHO oxidation were increased.

In the case of the HCHO trial, rates of CHO oxidation at the onset of exercise on day 7 (glycogen restored, CHO fed) were elevated above the baseline values taken during exercise on day 1 in both trials (overnight fasted). These findings are consistent with other studies that have reported that intake of CHO in the hours before exercise causes a long-lasting suppression of FFA mobilization in response to the rise in insulin concentration, leading to increased rates of CHO oxidation and decreased fat oxidation during subsequent exercise (6,17,21). In contrast to our previous study in which plasma glucose concentration fell over the course of the 2-h steady-state cycling on day 7 when subjects ingested water (4), the intake of a 6% CHO-electrolyte drink maintained plasma glucose concentrations and rates of CHO oxidation throughout exercise. Previous studies have reported that the intracellular availability of glucose determines subsequent substrate oxidation during moderate intensity exercise (7,26). However, these observations fail to explain why, in the current study, despite the restoration of endogenous CHO stores and increased CHO availability, rates of fat oxidation were persistently elevated throughout steady-state exercise when subjects were fat-adapted compared with fed CHO. Indeed, as previously noted, the amount of CHO "spared" during the 2 h of steady-state cycling after fat-adaptation was similar in magnitude to the glycogen sparing observed in our previous observations (4).

The source(s) of the additional fat oxidized during prolonged cycling after fat-adaptation and the mechanism underlying this change are more speculative. It is possible that there is an

up-regulation of enzymes involved in fat transport and oxidation, although these changes are likely to be minimal in such highly trained athletes. It is possible that intramuscular triglyceride (IMTG) might provide an additional substrate pool to account for some or all of the additional fat utilized after fat-adaptation, since other studies have found that trained subjects exposed to a high-fat diet for up to 28 d increase IMTG stores (18,19). However, recent observations from a study of previously sedentary males who undertook a 7-wk program of training on a high-fat or high-CHO diet cast doubt on the importance of IMTG as a fuel substrate during exercise. In that study, the high-fat group showed greater fat utilization during submaximal exercise compared with the high-CHO group, but there was only minimal utilization of IMTG, as determined from muscle biopsy samples. Instead, very-low-density lipoprotein (LDL) triglyceride and an unaccounted source of fat (hypothesized to be intrafascicular lipid) accounted for the additional fat utilization during exercise by subjects adapted to the high fat diet (J. W. Helge, personal communication).

Despite marked changes in fuel utilization during the steady-state ride preceding the TT, we did not find evidence of improved cycling performance. Differences in TT performance between treatments were neither statistically significant nor likely to be meaningful to the outcome of real-life sports events, where even small improvements in performance seem useful to top competitors. It appears that CHO sparing, even in the form of muscle glycogen, is not beneficial to the performance of our specific exercise task. High work rates, and presumably concomitantly high rates of CHO oxidation, were sustained throughout the TT. Thus, it appears that CHO substrate is not limiting for this specific performance in highly trained athletes who undertake dietary strategies to consume CHO immediately before and during exercise. Alternatively, subjects may have started the TT with adequate muscle glycogen stores after *both* dietary conditions.

Although we attempted to present dietary treatments in a double-blind manner, seven out of eight subjects correctly identified the order of their treatments. However, the most important clue reported by subjects was their sense of well-being and exercise tolerance rather than any food-related observations. This suggests that even if formula diets are utilized in such extreme dietary manipulations, subjects are likely to be capable of guessing their treatment order. Nevertheless, we are confident that placebo effects did not have a significant bearing on the outcome of our performance measures, since only half of our subjects were able to correctly identify the week on which they recorded their best TT outcome.

In conclusion, 5 d of a high-fat diet caused marked changes in substrate utilization during submaximal exercise. At least some of these changes were independent of CHO availability, since the enhanced capacity for fat oxidation persisted despite the restoration of muscle glycogen before, and provision of exogenous CHO during exercise. Such strategies are typical of those recommended by sports nutritionists to competitive athletes. Despite promoting CHO sparing during exercise, fat adaptation provided no benefit to the subsequent performance of a TT lasting ~ 30 min. Accordingly, the results of this study do not support the practice of fat-adaptation strategies by endurance athletes competing in events of 2 to 3 h duration. Whether such strategies would be of benefit to ultraendurance events lasting $>$ 4 h where fat oxidation has the potential to meet most of the fuel requirements of work remains to be determined.

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