Effect of fat adaptation and carbohydrate restoration on metabolism and performance during prolonged cycling

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Burke, Louise M., Damien J. Angus, Gregory R. Cox, Nicola K. Cummings, Mark A. Febbraio, Kathryn Gawthorn, John A. Hawley, Michelle Minehan, David T. Martin, and Mark Hargreaves. Effect of fat adaptation and carbohydrate restoration on metabolism and performance during prolonged cycling. *J Appl Physiol* 89: 2413–2421, 2000.— For 5 days, eight well-trained cyclists consumed a random order of a high-carbohydrate (CHO) diet (9.6 g·kg^{-1}·day^{-1} CHO, 0.7 g·kg^{-1}·day^{-1} fat; HCHO) or an isoenergetic high-fat diet (2.4 g·kg^{-1}·day^{-1} CHO, 4 g·kg^{-1}·day^{-1} fat; Fat-adapt) while undertaking supervised training. On *day 6,* subjects ingested high CHO and rested before performance testing on *day 7* [2 h cycling at 70% maximal O$_2$ consumption (SS) + 7 kJ/kg time trial (TT)]. With Fat-adapt, 5 days of high-fat diet reduced respiratory exchange ratio (RER) during cycling at 70% maximal O$_2$ consumption; this was partially restored by 1 day of high CHO [0.90 ± 0.01 vs. 0.82 ± 0.01 ($P <$ 0.05) vs. 0.87 ± 0.01 ($P <$ 0.05), for *day 1, day 6,* and *day 7,* respectively]. Corresponding RER values on HCHO trial were [0.91 ± 0.01 vs. 0.88 ± 0.01 ($P <$ 0.05) vs. 0.93 ± 0.01 ($P <$ 0.05)]. During SS, estimated fat oxidation increased [94 ± 6 vs. 61 ± 5 g ($P <$ 0.05)], whereas CHO oxidation decreased [271 ± 16 vs. 342 ± 14 g ($P <$ 0.05)] for Fat-adapt compared with HCHO. Tracer-derived estimates of plasma glucose uptake revealed no differences between treatments, suggesting muscle glycogen sparing accounted for reduced CHO oxidation. Direct assessment of muscle glycogen utilization showed a similar order of sparing (260 ± 26 vs. 360 ± 43 mmol/kg dry wt; *P* = 0.06). TT performance was 30.73 ± 1.12 vs. 34.17 ± 2.48 min for Fat-adapt and HCHO (*P* = 0.21). These data show significant metabolic adaptations with a brief period of high-fat intake, which persist even after restoration of CHO availability. However, there was no evidence of a clear benefit of fat adaptation to cycling performance.

[6,6-^{2}H]glucose; glycogen sparing; cycling time trial; substrate oxidation

WHEREAS SHORT-TERM (1–3 days) adherence to a high-fat, low-carbohydrate (CHO) diet reduces resting muscle glycogen stores and impairs capacity for prolonged (>90 min) submaximal [∼70% maximal oxygen uptake ($\dot{V}O_{2\ max}$)] exercise (1, 3, 7, 28), longer periods of adherence (>7 days) to such diets are associated with metabolic adaptations that enhance fat oxidation during exercise and compensate for the reduced CHO availability (19, 24). Although reduced rates of muscle glycogen oxidation during exercise have been reported after "fat adaptation" (19, 24), such observations might be explained by the low initial concentrations of muscle glycogen after high-fat diets as much as by "glycogen sparing" per se. Nevertheless, there is evidence of muscle adaptation after chronic exposure to high-fat diets even in well-trained subjects, with some studies reporting an increase in some of the enzymes involved in fat transport and oxidation (6, 14).

The effect of these metabolic adaptations on exercise capacity or performance during prolonged exercise is equivocal. Although some studies have reported performance benefits (19, 21), the results of these investigations are confounded by unorthodox factors in the methodological design, such as ordered allocation of treatments or carryover effects from various measurements of performance conducted in succession. Other studies have reported that long-term high-fat diets produce no change in performance (16, 24) or an impaired ability to adapt to a training program (15). An alternative model involving fat adaptation involves a period of exposure to high-fat, low-CHO intake, followed by the restoration of muscle glycogen stores with a high-CHO diet. Such "dietary periodization" (12) aims to enhance the capacity of both glycolytic and lipolytic systems to oxidative metabolism during prolonged exercise by increasing the contribution from fat to substrate metabolism while potentially sparing intact muscle glycogen stores.

Although previous studies of fat adaptation have used 2- to 7-wk periods of adherence to high-fat intakes (15, 16, 19, 24), such diets are impractical for human

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subjects to maintain and, furthermore, may impair the high-intensity training programs of athletes. A recent study indicated that significant increases in fat oxidation occurred after 10 days of adherence to a high-fat, low-CHO diet and were possibly present after 5 days (8). We decided to investigate this more fully because a 5-day timeframe represents a more manageable period for radical dietary change and should minimize the potential health and training disadvantages arising from longer periods on high-fat intakes. A one-day period of CHO recovery was chosen in an attempt to restore muscle glycogen levels without allowing a "washout" of adaptations incurred by the high-fat phase.

Accordingly, the aims of this study were to investigate the effects of 5-day adaptation to high-fat diet, followed by 1 day of CHO restoration, on metabolism and performance of prolonged cycling. To allow us to investigate baseline metabolic conditions and to provide an exercise situation that might benefit most from glycogen sparing, cyclists undertook the cycling bout without further CHO intake.

METHODS

Subjects and preliminary testing. Eight well-trained male cyclists and triathletes [age 29.3 ± 3.0 (SE) yr; weight 74.4 ± 2.1 kg; $\dot{V}O_{2\,max}$ 64.4 ± 1.8 ml·kg^{-1}·min^{-1}; peak sustained power output (PPO) 373 ± 14 W] were recruited for 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, informed consent.

Before the experimental trials, each subject undertook an incremental cycling test to exhaustion (13) on an electronically braked cycle ergometer (Lode, Groningen, The Netherlands). During this test, which typically lasted between 10 and 12 min, subjects inspired air through a two-way Hans Rudolph valve attached to a custom-built automated Douglas bag gas analysis system (Australian Institute of Sport, Australian Capital Territory, Australia) for which calibration and operation details have been previously described (9). The incremental test to exhaustion was used to determine $\dot{V}O_{2\,max}$ and PPO for each subject (13). These data were used to determine the work rate corresponding to 70% of each

subject's $\dot{V}O_{2\,max}$ (~63% of PPO) to be used in the subsequently described experimental trials.

Study design. Each subject undertook two trials in a randomized, crossover design with a 2-wk washout period separating each trial. Because it was not possible to completely blind the diets, subjects were aware of the treatment being received. However, the investigator responsible for the collection of performance data was kept blind to the order of treatments.

An overview of the study protocol is summarized in Fig. 1. On *day 1* of each trial, subjects reported to the laboratory after an overnight fast. They were then weighed, and a resting blood sample was collected by venipuncture from an antecubital vein. A muscle sample was then taken from the vastus lateralis using the percutaneous biopsy technique. After 10-min rest, subjects cycled for 20 min at 70% $\dot{V}O_{2\,max}$ (232 ± 8 W) on the Lode cycle ergometer with pulmonary gas data being collected for the last 5 min of the ride, as previously described (9). On completion of the ride, a second blood sample was collected.

Subjects then commenced 5 days of a supervised diet and training program. On the fat-adaptation treatment (Fat-adapt), they were prescribed a high-fat (>65% of energy), low-CHO (<20% of energy) diet supplying 0.22 MJ/kg body mass. The control treatment (HCHO) was an isoenergetic diet providing 70–75% of energy from CHO 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 50 g and matched to within 5–10 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. Actual dietary intakes reported by subjects are summarized in Table 1.

Training programs were individualized for each subject according to fitness level and current training load, and a summary of the general program is provided in Fig. 1. We intended that the program would correspond to the habitual training volume undertaken by each subject, translated into road cycling hours. Two interval training sessions were in-
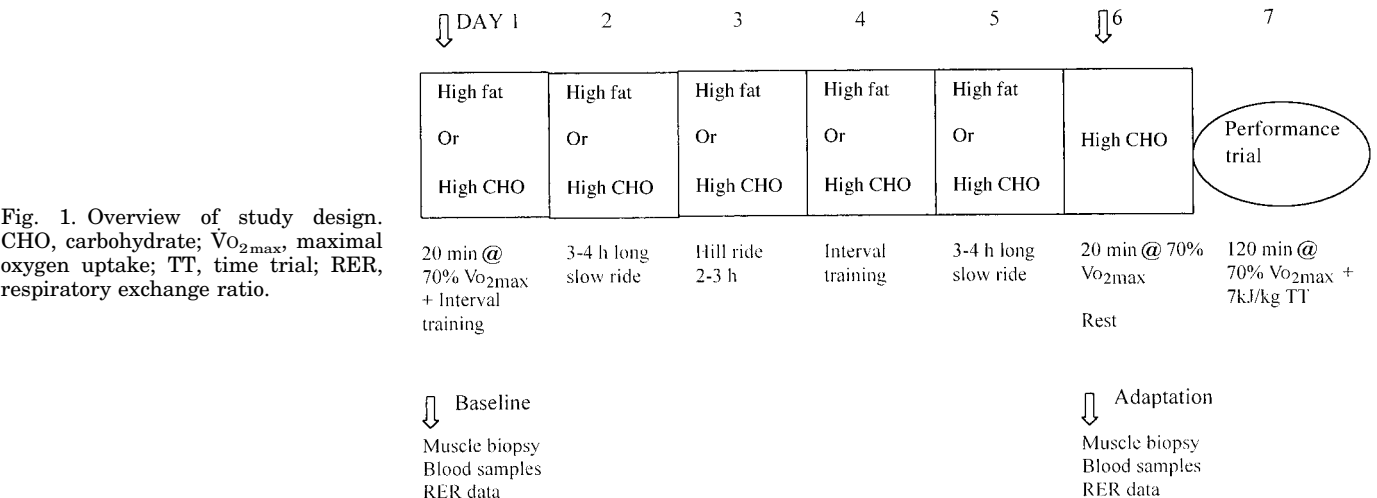

Fig. 1. Overview of study design. CHO, carbohydrate; $\dot{V}O_{2\,max}$, maximal oxygen uptake; TT, time trial; RER, respiratory exchange ratio.

Table 1. *Reported dietary intake during treatments*

Energy		CHO			Fat			Protein			Energy		CHO			Fat		Protein	
MJ	MJ/kg	g	g/kg	%E	g	g/kg	%E	g	g/kg	%E	MJ	MJ/kg	g	g/kg	%E	g	%E	g	%E
HCHO trial (mean daily intake days 1–5)											*HCHO trial (day 6)*								
16.18	0.22	709	9.6	74	56	0.7	13	129	1.7	13	16.68	0.23	733	9.9	75	60	13	130	12
±0.39	±0.00	±17	±0.1	±0.2	±2	±0.0	±0.2	±2	±0.0	±0.1	±0.48	±0.01	±20	±0.2	±0.0	±3	±0.3	±4	±0.2
Fat-adapt trial (mean daily intake days 1–5)											*Fat-adapt trial (day 6)*								
16.11	0.22	177	2.4	19	297	4.0	68	130	1.7	13	16.7	0.23	737	9.9	75	62	14	133	13
±0.39	±0.00	±7*	±0.1*	±0.4*	±7*	±0.0*	±0.2*	±4	±0	±0.2	±0.4	±0.0	±21	±0.3	±0.5	±3	±0.3	±5	±0.3

Values are means ± SE for 8 subjects. CHO, carbohydrate; E, energy. Fat-adapt, high-fat and low-CHO diet. *Different from high-CHO (HCHO) trial, $P < 0.05$.

cluded in each trial. The first interval session was undertaken at the commencement of each trial, immediately after 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 to initiate a rapid differentiation between dietary treatments based on 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; the session was undertaken either in the laboratory or on the road accompanied by one of the researchers. Each subject completed an identical training program during both of his trials, averaging 13.7 h and 414 km over the week.

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 10 g CHO/kg body mass 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 of cycling at 70% $\dot{V}O_{2 \max}$ (SS) followed by a 7 kJ/kg body mass time trial (TT).

Performance ride. On arrival in the laboratory subjects were weighed, and catheters (20 gauge; Terumo, Tokyo, Japan) were inserted into a vein in the antecubital space of each arm for blood sampling or infusion of the tracer (described subsequently). A basal blood sample was collected from the sampling catheter, which was kept patent by flushing with 0.5 ml of saline containing heparin (10 IU/ml). A primed (3.3 mmol) continuous (~44 µmol/min) infusion of [6,6-²H]glucose (Cambridge Isotope Laboratories, Cambridge, MA) was then commenced and maintained until completion of SS.

After the commencement of the infusion, subjects rested for 120 min. During this period, a muscle sample was obtained as previously described, and an incision was made in the same leg, 2–3 cm from the first incision site for a further biopsy (4). After 60 min, subjects were fed a placebo meal consisting of 500 ml of low-energy jelly. This was provided so subjects thought they were receiving a preexercise breakfast while effectively maintaining fasting conditions. At 105, 110, and 115 min, further blood samples (2 ml) were taken for measurement of [6,6-²H]glucose enrichment. Subjects then voided and were weighed again.

Exactly 2 h after the commencement of the infusion, subjects mounted the cycle ergometer and began SS (120 min of steady-state exercise at 70% of $\dot{V}O_{2 \max}$). 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 (2). 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/kg of water to be consumed each 20 min. At 120 min, after the final blood sample was taken, subjects stopped cycling and a muscle sample was taken quickly from the second biopsy site.

On completion of SS, a 3-min rest period was allowed before subjects commenced the TT. Subjects were instructed to complete the TT "as fast as possible." The same researcher supervised each TT 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.

Because one subject was unable to undertake one of the TTs because of discomfort from the muscle biopsy, performance data were collected from seven subjects. In addition, one subject was unable to finish the TT after the CHO treatment because of extreme fatigue and stopped cycling after 26 min, having completed 321 kJ or 62% of the TT. Because this outcome was an important finding of the study, it was considered critical to include these data in the final TT results. Thus a predicted time for the TT was estimated for this cyclist by plotting his decline in power output over the duration of the TT and extrapolating the time it would have taken him to complete the full amount of work.

Blood sampling and analyses. Unless otherwise specified, 12 ml 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. Five hundred microliters 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 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 acids (FFAs) using an enzymatic colorimetric method (NEFAC code 279-75409, Wako, Tokyo, Japan).

In the case of plasma samples collected during SS, and for the 2 ml samples taken during the 15 min before this trial, an aliquot was stored for measurement of [6,6-^{2}H]glucose enrichment as previously described (20). Briefly, 500 μl of plasma were mixed with 500 μl of 0.3 Ba(OH)$_2$ and 500 μl of ZnSO$_4$ and spun. The supernatant was passed down an ion-exchange column, washed with distilled water, and dried. The samples were then resuspended with distilled water, placed in glass vials, dehydrated overnight, and derivatized with the addition of pyridine and acetic anhydride. The derivatized samples were then measured using a gas chromatograph-mass spectrometer (5890 series 2 gas chromatograph 5971 mass spectrometer detector, Hewlett-Packard, Avondale, PA).

Muscle analyses. Muscle samples were dissected free of blood, connective tissue, and fat and frozen within 15 s in liquid N$_2$. Samples were subsequently freeze-dried and weighed. Muscle glycogen content was determined after acid hydrolysis using an enzymatic fluorometric technique (22).

Glucose kinetics. Glucose kinetics during exercise were calculated with a modified one-pool non-steady-state model (29) by assuming a pool fraction of 0.65 and estimating the apparent glucose space as 25% of body mass. Rates of appearance (R$_a$) and disappearance (R$_d$) of plasma glucose were determined from the changes in percent enrichment of [6,6-^{2}H]glucose. The metabolic clearance rate (MCR) of glucose was calculated by dividing the glucose R$_d$ by the plasma glucose concentration.

Rates of fat and CHO oxidation. Whole body rates of carbohydrate and fat oxidation (g/min) were calculated from the respiratory data collected during the 20-min cycle bouts, and from the data collected every 20 min during the SS phase of the performance ride. The calculations were made from carbon dioxide production ($\dot{V}CO_2$) and oxygen uptake ($\dot{V}O_2$) measurements assuming a nonprotein respiratory exchange ratio (RER) value, according to the following equations (23).

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

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

Total fat and CHO oxidation over the 120 min of SS exercise were estimated by calculating the area under the oxidation vs. time curves for each subject. Rates of plasma glucose oxidation, assuming 100% oxidation of tracer-determined glucose R$_d$, enabled an estimation of total plasma glucose oxidation over the 120 min. Differences between total CHO oxidation and plasma glucose oxidation provided an indirect estimate of muscle glycogen utilization during this time.

Statistical analyses. Data from the two trials were compared using a two-factor (diet and time) ANOVA with repeated measures. Separate analyses were undertaken to compare data from *day 1*, *day 6,* and 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 interaction. Differences in dietary intakes, glycogen utilization and TT performances between trials were compared using Student's *t*-tests. Significance was accepted when *P* was <0.05. All data are reported as means ± SE. The statistical analyses were undertaken using Statistica software for Windows (StatSoft, version 5.1, 1997, Tulsa, OK).

RESULTS

All subjects completed the dietary and training requirements of both treatments in this study. According to questionnaires completed each day, all subjects experienced 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, either complaining of increased perception of effort or having difficulty in maintaining the training pace. Three of the subjects experienced such symptoms in the second interval session on *day 4*. The generalized symptoms appeared to decrease as the week progressed. Changes in body mass from *day 1* to *day 7* revealed a mean loss of 0.9 ± 0.3 kg with Fat-adapt and 1.0 ± 0.2 kg with HCHO. Although we observed rapid fluctuations in body mass, which could be partially explained by changes in gastrointestinal contents and muscle concentrations of glycogen and water, we acknowledge that our subjects experienced a mild energy deficit during their dietary treatments.

Muscle glycogen concentrations measured during each experimental trial are shown in Table 2. Muscle glycogen declined with 5 days of training and a high-fat, low-CHO intake such that *day 6* concentrations after Fat-adapt treatment were lower than in the HCHO trial and were below levels determined on *day 1*. Muscle glycogen concentrations were maintained throughout the training program by the CHO treatment. Regardless of previous dietary treatment, 24 h of high CHO intake and rest rapidly restored muscle glycogen concentrations above *day 1* values. Accordingly, subjects commenced the performance ride on *day 7* with similarly elevated muscle glycogen stores on both treatments.

Table 2. *Muscle glycogen concentrations after 5 days of fat adaptation, 1 day of CHO restoration, and 2 h of steady-state cycling at 70% $\dot{V}O_{2max}$*

Trial	Day 1	Day 6	Day 7 Preexercise (SS)	Day 7 Postexercise (SS)	Day 7 Utilization during SS
Fat-adapt	451 ± 32	255 ± 24*†	554 ± 45*	294 ± 23‡	260 ± 26
HCHO	470 ± 24	464 ± 42	608 ± 51*	248 ± 20‡	360 ± 43

Values are means ± SE for 8 subjects given in mmol/kg dry wt. $\dot{V}O_{2max}$, maximal oxygen consumption; SS, steady-state cycling. *Different from *day 1*, $P < 0.05$. †Different from HCHO trial, $P < 0.05$. ‡Different from preexercise, $P < 0.05$.

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 days of the high-fat diet (*day 1*: 0.37 ± 0.06 vs. 0.43 ± 0.07 mmol/l; *day 6*: 0.43 ± 0.06 vs. 0.86 ± 0.09 mmol/l for HCHO and Fat-adapt, respectively; $P < 0.05$). However, after 1 day of CHO restoration, fasting FFA concentrations declined to similar levels as for *day 1* (0.59 ± 0.06 mmol/l) and were not different from the corresponding time point for the HCHO trial (0.38 ± 0.06 mmol/l). Similarly, fasting plasma glycerol concentrations increased after a 5-day high-fat diet and were elevated at *day 6* (0.03 ± 0.01 vs. 0.10 ± 0.01 mmol/l for HCHO and Fat-adapt, respectively; $P < 0.05$). However, they remained higher than those of the CHO trial on *day 7* (0.01 ± 0.01 vs. 0.05 ± 0.01 mmol/l; $P < 0.05$).

Figure 2 summarizes RER data, rates of CHO, and fat oxidation estimated during 20 min of cycling at 70% $\dot{V}O_{2\,max}$ on *day 1*, *day 6,* and *day 7* (first 20 min of SS), as well as throughout SS. Five days of training and dietary intervention reduced RER, such that *day 6* values were below *day 1* values with both treatments (Fig. 2). However, at *day 6* the decrease in RER with Fat-adapt treatment was greater than with the HCHO trial (0.82 ± 0.01 vs. 0.88 ± 0.01; $P < 0.05$). In the HCHO trial, 1 day of rest and high-CHO diet restored RER during the first 20 min of SS to values higher than seen during exercise on *day 1*. One day of high-CHO diet and rest also increased RER values in the Fat-adapt treatment. However, RER values during the first 20 min of SS in the Fat-adapt trial (0.87 ± 0.01) was below values on *day 1* (0.90 ± 0.01; $P < 0.05$) and below the corresponding SS values in the HCHO trial (0.93 ± 0.01, $P < 0.05$) (Fig. 2). There was a progressive decline in RER values during the 120 min of SS in the Performance ride with both treatments. However, RER values in Fat-adapt remained significantly lower than in the CHO trial at all corresponding time points.

Rates of CHO oxidation during exercise were reduced after 5 days of training with both treatments ($P < 0.05$). However, on *day 6*, rates of CHO oxidation were lower with Fat-adapt treatment compared with the CHO trial (1.73 ± 0.18 vs. 2.59 ± 0.19 g/min; $P < 0.05$; Fig. 2). One day of CHO restoration increased rates of CHO oxidation during the first 20 min of SS cycling in both trials. However, at *day 6* with the Fat-adapt treatment, CHO oxidation rates remained below *day 1* values (2.48 ± 0.14 vs. 2.92 ± 0.19 g/min; $P < 0.05$) and were lower than values in the CHO trial (3.21 ± 0.14 g/min; $P < 0.05$). Rates of CHO oxidation declined progressively throughout the 120 min of SS with both treatments. There was a main effect ($P < 0.05$) of diet, with rates of CHO oxidation remaining lower in the Fat-adapt trial than with the HCHO treatment (Fig. 2).

Five days of high-fat intake significantly increased rates of fat oxidation during 20 min of cycling at 70% $\dot{V}O_{2\,max}$. On *day 6* with Fat-adapt, fat oxidation rates were elevated above the values observed on *day 1*
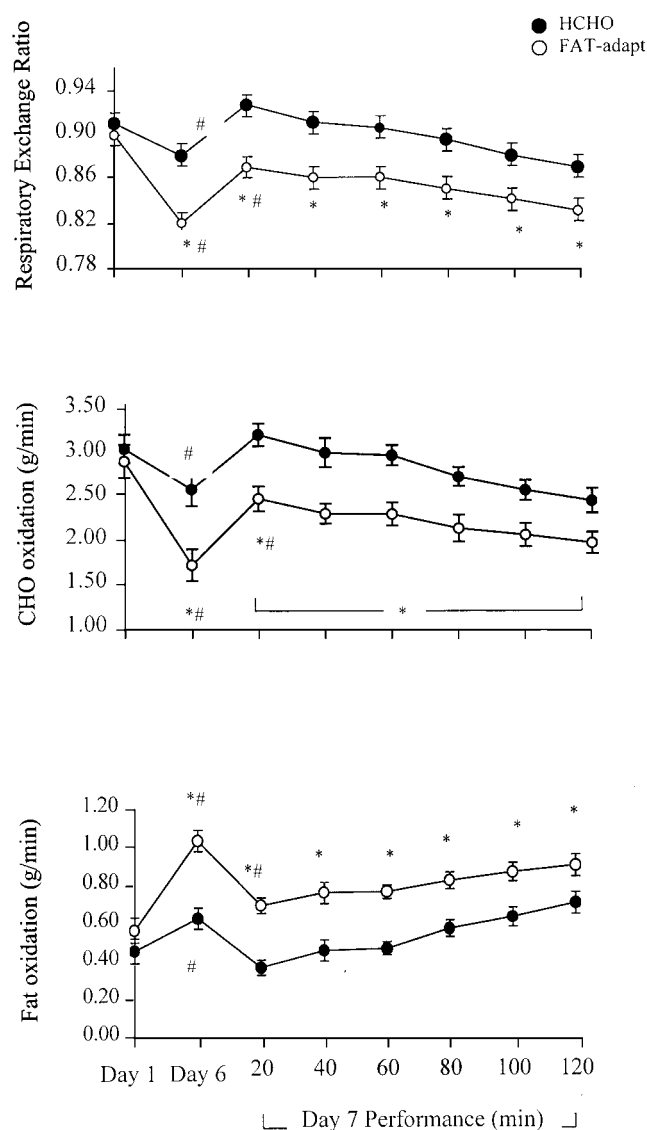

Fig. 2. Effect of 5 days of adaptation to high-fat diet and 1 day of CHO restoration (Fat-adapt) on respiratory exchange ratio, rate of CHO oxidation, and rate of fat oxidation during cycling at 70% $\dot{V}O_{2\,max}$ compared with control trial (HCHO). Values are means $\pm$ SE for 8 subjects on *day 1* (baseline), on *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*, $P < 0.05$; *different from HCHO trial, $P < 0.05$. Comparison of data from 120 min of steady-state cycling during performance ride: *different from HCHO trial, $P < 0.05$.

(1.04 ± 0.07 vs. 0.57 ± 0.07 g/min; $P < 0.05$) and were greater than the corresponding values for the HCHO trial (0.63 ± 0.06 g/min; $P < 0.05$; Fig. 2). One day of high-CHO intake attenuated these rates of fat oxidation during the first 20 min of SS on *day 7*. However, with the Fat-adapt trial, fat oxidation remained elevated above *day 1* values ($P < 0.05$) and above the rates measured in the HCHO trial (0.70 ± 0.05 vs. 0.37 ± 0.04 g/min; $P < 0.05$). Fat oxidation increased over the 120 min of SS with both treatments but remained significantly higher in the Fat-adapt trial at all time points.

Fig. 3. Plasma concentrations of glucose, insulin, free fatty acids (FFA), and glycerol during 120 min of steady-state cycling at 70% $\dot{V}o_{2\,max}$ on *day 7* after 5 days of adaptation to high-fat diet and 1 day of CHO restoration. Values are means $\pm$ SE for 8 subjects. Plasma insulin decreased over time in both trials ($P < 0.05$). Plasma FFA and glycerol concentrations rose during steady-state cycling in both trials ($P < 0.05$). *Different from HCHO trial, $P < 0.05$. #Different from time = 0 min, $P < 0.05$.
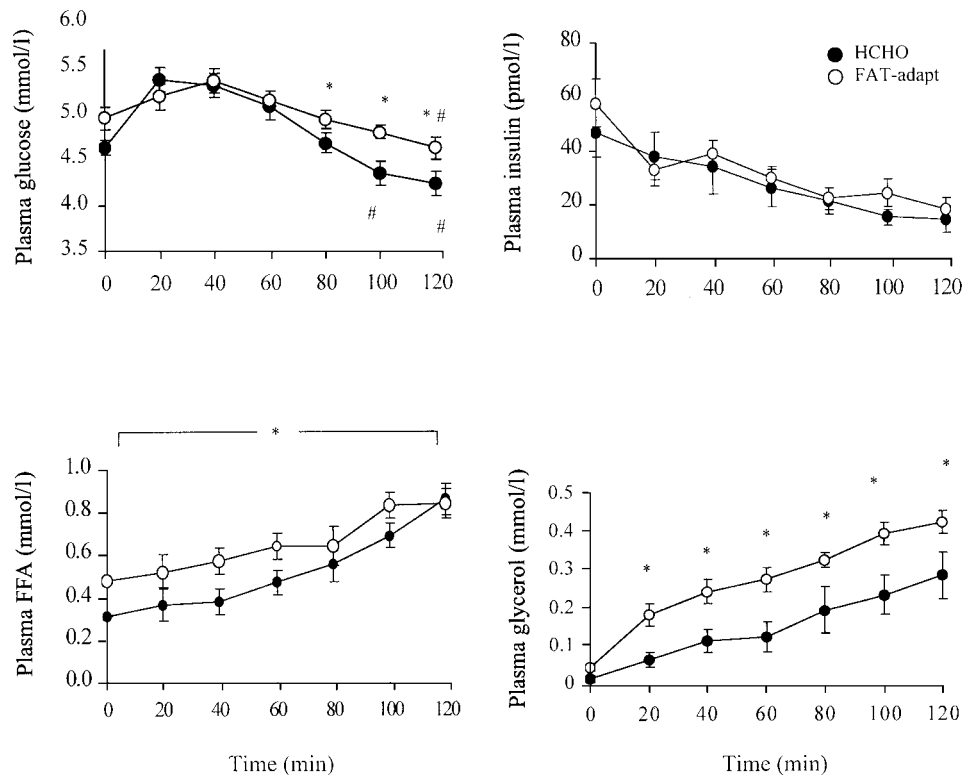
Figure 3 summarizes the concentrations of plasma metabolites during 120 min of SS on *day 7*. Although plasma glucose concentrations gradually fell over the 120 min of cycling in both trials, there was an interaction of time and diet ($P < 0.05$) such that plasma glucose was better maintained in the Fat-adapt treatment over the last 40 min of the steady-state ride (Fig. 3). In the HCHO trial, two subjects showed symptoms of severe fatigue toward the end of SS and during the TT in association with lower plasma glucose concentrations at the end of SS. Plasma insulin decreased over time in both trials ($P < 0.05$); however, there were no differences between treatments (Fig. 3). Plasma FFA concentrations rose during SS in both trials ($P < 0.05$). There was a significant main effect of diet, with higher plasma FFA concentrations occurring with the Fat-adapt treatment (Fig. 3). Plasma glycerol concentrations increased above values at time = 0 min in both trials and were higher at all time points with the Fat-adapt treatment than during the HCHO trial ($P < 0.05$; Fig. 3).

Plasma glucose R_a, plasma glucose R_d, and MCR increased during the 120 min of SS on *day 7*; however, no differences were observed between treatments with regard to glucose kinetics. Plasma glucose R_a increased from 29.8 ± 3.0 μmol·kg^{-1}·min^{-1} to a peak value of 47.6 ± 4.6 μmol·kg^{-1}·min^{-1} in the Fat-adapt trial and from 28.8 ± 2.5 to 41.3 ± 2.7 μmol·kg^{-1}·min^{-1} in HCHO. Increases in plasma glucose R_d were from 26.7 ± 2.6 to 48.9 ± 4.8 and from 23.3 ± 2.9 to 42.9 ± 2.8 μmol·kg^{-1}·min^{-1} for Fat-adapt and HCHO trials, respectively. MCR increased from 5.3 ± 0.4 to $10.5 \pm$

1.0 ml·kg^{-1}·min^{-1} with Fat-adapt and from 4.7 ± 0.4 to 10.2 ± 0.8 ml·kg^{-1}·min^{-1} in the HCHO trial.

Total substrate oxidation from plasma glucose, muscle glycogen, and fat derived from glucose kinetic and RER data is summarized in Fig. 4. Total fat oxidation was increased ($P < 0.05$), with a concomitant decrease ($P < 0.05$) in CHO oxidation in the Fat-adapt trial. Because whole body glucose oxidation did not differ between trials, a significant reduction in estimated muscle glycogen utilization appeared to account for the reduced CHO oxidation with the Fat-adapt treatment. Differences in muscle glycogen utilization measured from muscle biopsy samples were 260 ± 26 and $360 \pm$
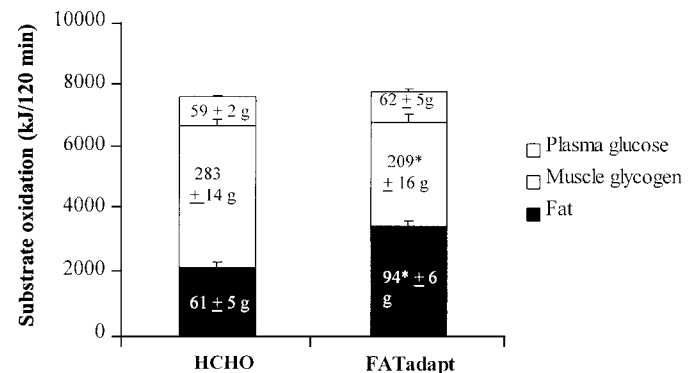

Fig. 4. Estimated contribution of plasma glucose, muscle glycogen, and fat to substrate oxidation during 120 min of steady-state cycling at 70% $\dot{V}o_{2\,max}$ on *day 7* after 5 days of adaptation to high-fat diet and 1 day of CHO restoration. Values are means $\pm$ SE for 8 subjects. *Different from HCHO trial, $P < 0.05$.
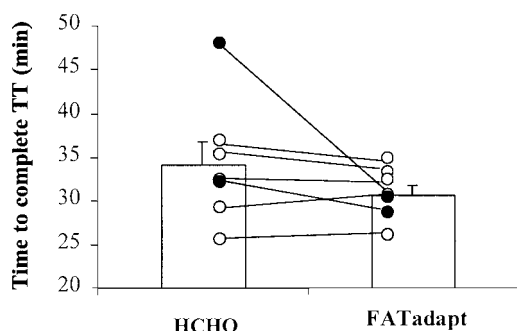
Fig. 5. Time to complete cycling TT (7 kJ/kg) at the end of 120 min of steady-state exercise on *day* 7 after 5 days of adaptation to high-fat diet and 1 day of CHO restoration. Values are means $\pm$ SE for 7 subjects. $\circ$, Individual subjects; $\bullet$, subjects reporting severe fatigue during HCHO trial. $P = 0.21$.

43 mmol/kg dry wt for Fat-adapt and HCHO treatments, respectively ($P = 0.06$; Table 2). The extent of muscle glycogen sparing assessed via these two methods were in close agreement; glycogen utilization after Fat-adapt was 74 and 72% of that observed in the control (HCHO) trial according to indirect and direct estimation methods, respectively.

Changes in body mass over the performance ride (SS and TT) did not differ between trials (1.1 ± 0.4 vs. 0.8 ± 0.2 kg for Fat-adapt and HCHO trials, respectively), suggesting that a similar fluid deficit was incurred in each trial. The results of the TT are summarized in Fig. 5. There was no difference in time to complete 7 kJ/kg of work (30.73 ± 1.12 vs. 34.17 ± 2.62 min for Fat-adapt and HCHO, respectively; $P = 0.21$; $n = 7$). Mean power outputs during the TT were 281 ± 14 and 260 ± 24 W for Fat-adapt and HCHO ($P = 0.24$; $n = 7$). Although these differences are not significant, mean TT time was 8% faster with Fat-adapt treatment compared with the HCHO trial [95% confidence interval (CI) = -6 to 21%], whereas the difference in mean power output during the TT was 21 W [95% CI = -18–60 W]. However, most of these differences are explained by superior performance on the Fat-adapt trial by two subjects; these were the subjects who exhibited symptoms of severe fatigue in the latter stages of SS with HCHO treatment. Mean TT performance of the remaining five subjects was 31.53 ± 1.42 and 31.98 ± 2.12 min for Fat-adapt and HCHO ($P = 0.59$), a mean difference of 0.8% (95% CI = -5.6–7.4%)

DISCUSSION

This study was undertaken to investigate the effects of a practical "dietary periodization" strategy on metabolism and performance of endurance cycling. We observed that 5 days of adherence to a high-fat, low-CHO diet enhanced fat oxidation during exercise, with these adaptations being independent of CHO availability. Indeed, adaptations increasing fat oxidation during exercise persisted despite the restoration of muscle glycogen levels and were associated with muscle glycogen sparing. However, despite striking changes in fuel utilization during exercise, fat-adaptation and glyco-

gen-restoration strategies did not produce a clear benefit to the performance of a TT undertaken at the end of 2 h of cycling.

Previous studies have reported that periods of adaptation to high-fat, low-CHO diets increase the capacity of the muscle for fat oxidation, in contrast to the detrimental effects of short-term exposure to high-fat diets, which reduce resting muscle glycogen content without compensation for the reduced CHO availability (1, 3, 7, 28). Phinney and colleagues (24) were the first to compare the effects of a long-term (28 days) ketogenic (<20 g CHO/days) high-fat diet with an isoenergetic diet (66% energy as CHO) in five well-trained cyclists. They found that the high-fat diet increased rates of fat oxidation during moderate intensity (63% $\dot{V}_{O_2 \max}$) exercise and reduced the rates of both muscle glycogen utilization and plasma glucose oxidation (24). Lambert et al. (19) employed a crossover design to study the effects of 14 days of either a high-fat (67% of energy) or a high-CHO (74% of energy) diet in five trained cyclists. They also reported marked decreases in the rate of CHO oxidation (2.2 vs. 1.4 g/min) and a concomitant increase in fat oxidation (0.3 vs. 0.6 g/min) during submaximal cycling (60% $\dot{V}_{O_2 \max}$) that was preceded by several bouts of high-intensity exercise (19). In both of these studies, muscle glycogen sparing was reported as a consequence of fat adaptation. However, this sparing could be an artifact, arising from the striking reduction in starting muscle glycogen content ($\sim$50% of control concentrations) (11). A true sparing of muscle glycogen stores can only be proven when lower rates of utilization are measured in subjects commencing exercise with similar starting concentrations.

Despite the brevity of the adaptation period, the dietary fat treatment utilized in this study achieved 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% $\dot{V}_{O_2 \max}$ compared with baseline values. This increase is particularly impressive in light of the already enhanced capacity for fat oxidation in our highly trained subjects. One day of rest and a high-CHO diet was sufficient to increase muscle glycogen stores above normal resting levels, regardless of previous dietary treatment. However, despite the restoration of CHO availability, elevated rates of fat oxidation persisted throughout SS on the Fat-adapt trial and total fat oxidation over 2 h of cycling was elevated by $\sim$50% over control trial estimates. Higher plasma glycerol concentrations with the Fat-adapt treatment confirmed increased rates of whole body lipolysis, but we were unable to distinguish between potential sources of fat.

Concomitant with increased fat utilization during submaximal cycling after fat adaptation was a reduced reliance on CHO. Despite equally elevated muscle glycogen stores at the onset of the performance ride, total CHO oxidation over 2 h of cycling after Fat-adapt treatment was decreased by $\sim$70 g compared with the control HCHO treatment. In contrast to observations of reduced plasma glucose oxidation and very low CHO

oxidation rates during exercise after 28 days of adaptation to a high-fat diet (24), we did not observe any differences in whole body plasma glucose uptake to account for this reduced CHO oxidation. The indirect estimate of muscle glycogen utilization, derived from the difference between estimates of total and plasma CHO utilization, showed a significantly reduced contribution of glycogen to exercise fuel metabolism after fat adaptation. Direct assessment of muscle glycogen utilization from biopsy samples showed a similar order of magnitude of glycogen sparing. Thus this is the first study to report evidence of true muscle glycogen sparing in response to fat adaptation strategies. Furthermore, we observed a tight agreement between direct and indirect measurements of muscle glycogen sparing.

Plasma glucose concentrations declined during the 2 h of submaximal cycling in both trials, as is typically observed during prolonged bouts of exercise undertaken after an overnight fast and without CHO intake (5). However, the decline in plasma glucose was attenuated during the final 20 min of the SS bout after the Fat-adapt treatment, with modest differences in glucose concentrations being observed immediately before the commencement of the TT (4.2 vs. 4.6 mmol/l). Importantly, two subjects suffered severe symptoms of fatigue in concert with lower plasma glucose concentrations at the end of the SS ride on the HCHO trial. Similar problems were not observed with the Fat-adapt trial. No differences in hepatic glucose production (glucose R_a) or whole body glucose disposal (glucose R_d) could be detected, either for these two subjects or for the group mean values. This apparent discrepancy must be explained by the lack of sensitivity of the measurement of plasma glucose kinetics; larger differences in plasma glucose concentrations are needed before clear differences in glucose R_a or R_d can be detected.

It is not clear whether the metabolic changes observed after fat-adaptation strategies involve upregulation of fat oxidation during exercise, a downregulation of CHO oxidation, or a combination of both. Other human studies have reported changes in the activity of β-hydroxyacyl-CoA dehydrogenase (14), carnitine palmitoyltransferase-1 (6), and pyruvate dehydrogenase (26) after several days or several weeks of high-fat, low-CHO intake. Whether these or other changes occur over the period of fat exposure used in our study or in highly trained athletes who continue to undertake extensive training program needs to be investigated. Other studies have found that exposure of trained subjects to 1–28 days of a high-fat intake increases muscle triglyceride stores (17, 28). It is possible that this might provide an additional substrate pool to account for some or all of the additional fat utilized after the Fat-adapt treatment. Nevertheless, the precise mechanism to explain the preferential use of this or other fat substrates in the face of plentiful glycogen stores remains to be elucidated.

It should be noted that the results of this study appear to contradict the theory presented by Sidossis and Wolfe (27), that intracellular availability of glucose determines the relative contribution of substrate oxidation during steady-state exercise. Data from their clamp study suggested that a reversal of the traditional "glucose-fatty acid cycle" exists, whereby intracellular CHO availability promotes CHO oxidation during exercise, despite constant FFA concentrations (27). According to their theory, the restoration of muscle glycogen concentrations on *day 7* in our subjects should have caused an inhibition of fat oxidation during subsequent exercise. Further work is needed to fully understand the complex regulation of substrate utilization during exercise.

We were interested in the effect of these metabolic adaptations on the performance of endurance cyclists because fat adaptation has recently been promoted in some sporting circles as an ergogenic strategy for competition preparation. Previous studies of long-term fat adaptation have reported conflicting results with respect to performance benefits. When undertaken by trained subjects, fat adaptation has been reported both to enhance (19, 21) or to fail to alter (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 literature is difficult to review because of the unorthodox design of some studies (19, 21), small subject numbers (19, 24), and lack of relevance of performance measurements to outcomes in competitive sport (19, 24). We chose a fixed-work TT as the outcome measure of this study because of its application to sport and its documented reliability even when undertaken after a steady-state exercise preload (coefficient of variation = ~3.5%) (18). In this initial study, cyclists performed after an overnight fast and while consuming water during their prolonged exercise bout. Although this is not representative of the real-life nutritional practices recommended for endurance athletes (10), the withholding of CHO intake immediately before and during the performance ride provided baseline metabolic conditions.

In our study, we did not find evidence of improved cycling performance despite observing marked changes in fuel utilization during the steady-state ride preceding the TT. Two individuals performed significantly better after Fat-adaptation treatment. More correctly, these subjects performed badly on the control (HCHO) trial due to the onset of severe symptoms of fatigue at the end of the SS and during the TT. It appears that fat adaptation may be of benefit to individuals who are at risk of developing symptomatic hypoglycemia during prolonged exercise when deprived of CHO, in so far as that it may allow better maintenance of blood glucose concentration. However, these subjects will also benefit from strategies to consume CHO during prolonged exercise (5), practices that are commonly recommended and easier to achieve than 5 days of extreme dietary change. Overall, despite higher muscle glycogen stores and an enhanced capacity for fat utilization at the onset of the TT, subjects did not show a clear perfor-

mance benefit after the Fat-adapt treatment. The 95% CIs delineated the likely range of true effects from this strategy range from a moderate performance decrement to a moderate performance increase. A larger sample size would be needed to determine whether either of these outcomes is real. More importantly, metabolic and performance outcomes need to be assessed when fat-adapted subjects undertake an endurance cycling bout under the preferred conditions of CHO intake in a preevent meal and during the exercise session.

In summary, we found that 5 days of exposure to a high-fat, low-CHO diet caused clear changes in fuel substrate utilization during submaximal exercise. At least some of these changes were independent of CHO availability because enhanced capacity for fat oxidation persisted despite restoration of muscle glycogen stores. Despite promoting glycogen sparing during prolonged exercise, fat-adaptation strategies did not provide a clear benefit to the subsequent performance of a TT lasting ~30 min. 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.

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