Metabolic Adaptations to a High-Fat Diet in Endurance Cyclists

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We examined the time course of metabolic adaptations to 15 days of a high-fat diet (HFD). Sixteen endurance-trained cyclists were assigned randomly to a control (CON) group, who consumed their habitual diet (30% ± 8% mJ fat), or a HFD group, who consumed a high-fat isocaloric diet (69% ± 1% mJ fat). At 5-day intervals, the subjects underwent an oral glucose tolerance test (OGTT); on the next day, they performed a 2.5-hour constant-load ride at 70% peak oxygen consumption (VO_{2peak}), followed by a simulated 40-km cycling time-trial while ingesting a 10% ^{14}C-glucose + 3.44% medium-chain triglyceride (MCT) emulsion at a rate of 600 mL/h. In the OGTT, plasma glucose concentrations at 30 minutes increased significantly after 5 days of the HFD and remained elevated at days 10 and 15 versus the levels measured prior to the HFD ($P < .05$). The activity of carnitine acyltransferase (CAT) in biopsies of the vastus lateralis muscle also increased from 0.45 to 0.54 μmol/g/min over days 0 to 10 of the HFD ($P < .01$) without any change in citrate synthase (CS) or 3-hydroxyacyl-coenzyme A dehydrogenase (3-HAD) activities. Changes in glucose tolerance and CAT activity were associated with a shift from carbohydrate (CHO) to fat oxidation during exercise ($P < .001$), which occurred within 5 to 10 days of the HFD. During the constant-load ride, the calculated oxidation of muscle glycogen was reduced from 1.5 to 1.0 g/min ($P < .001$) after 15 days of the HFD. Ingestion of a HFD for as little as 5 to 10 days significantly altered substrate utilization during submaximal exercise but did not attenuate the 40-km time-trial performance.

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FATIGUE during endurance exercise appears to be associated with a depletion of muscle glycogen[1] and can be delayed by maximizing these stores prior to an event.[1] Hence, many endurance athletes increase their carbohydrate (CHO) intake for several days before competition, using a regimen first described by Ahlborg et al.[2] However, CHO "loading" does not appear to "spare" muscle glycogen.[3] Instead, CHO loading increases muscle glycogen utilization[4] and attenuates the increase in fat oxidation[5] during endurance exercise. In contrast, studies in rats[6-9] and humans[10-12] have shown that a high-fat diet (HFD) for more than 7 days increases fat oxidation and decreases muscle glycogen utilization during exercise. Prolonged (>7 days) exposure to a HFD did not adversely affect exercise endurance[10] and, in some studies, improved the endurance performance.[9,12]

The mechanisms involved in the shift in fuel oxidation from CHO to fat with a prolonged HFD are not completely understood. Rat studies have shown that a prolonged (1 to 12 weeks) HFD increases skeletal muscle carnitine acyltransferase (CAT) and 3-hydroxyacyl-coenzyme A dehydrogenase (3-HAD) activities relative to the activity of citrate synthase (CS).[7,8,13] Fisher et al[14] found that 4 weeks of a ketogenic HFD in well-trained cyclists resulted in a 46% reduction in muscle hexokinase activity and a 35% increase in muscle CAT activity. More recently, Helge and Kiens[15] demonstrated a 25% increase in muscle 3-HAD activity in cyclists after 7 weeks of a HFD.

A HFD also causes insulin resistance, which can increase fat oxidation by suppressing CHO metabolism. In rats, exposure to a HFD for 3 days resulted in liver insulin resistance,[16] while 3 weeks of exposure to a HFD decreased insulin-stimulated peripheral glucose utilization[16] and reduced the intrinsic activity of glucose transporters.[17] More prolonged (>10 weeks) exposure to a HFD caused a decrease in GLUT-4 glucose transporter mRNA.[18]

Therefore, the aim of this study was to examine the effects of a HFD on glucose tolerance, certain muscle enzyme activities, and substrate metabolism during constant-load exercise.

SUBJECTS AND METHODS

Subjects and Preliminary Testing

Sixteen endurance-trained male cyclists participated in the investigation, which was approved by the Research and Ethics Committee of the Faculty of Medicine of the University of Cape Town. Because trace amounts of [U-^{14}C]glucose were ingested by the subjects on a HFD and blood samples and muscle biopsies were taken, the risks were carefully explained to the subjects before their written informed consent was obtained. The total radiation dose received by each subject was about 20 mrem. The radiation dose accepted as safe in South Africa is 500 mrem/yr or 130 mrem/13 wk.[19]

Table 1 shows the characteristics of the subjects assigned randomly to either the HFD group or the control (CON) group. The percent body fat was determined using the sum of seven skinfolds with the equations of Jackson and Pollock.[20] Peak oxygen consumption (VO_{2peak}) and peak sustained power output (W_{peak}) were measured on an electronically braked cycle ergometer (Lode, Groningen, Holland) modified with toe clips and racing handlebars as described by Hawley and Noakes.[21] Work rates were started at 3.33 W/kg body mass and increased first by 50 W and then by 25 W every 150 seconds until the subject was exhausted. Exhaustion was defined as a greater than 10% reduction in pedaling frequency, a respiratory exchange ratio greater than 1.10, or both. W_{peak} was defined as the highest exercise intensity the subject completed for

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150 seconds (in watts), plus the fraction of time spent in the final work rate times 25 W. W_{peak} values were used to set the work rates in the experimental trials to correspond to 63% of each subject's W_{peak} (approximately 70% VO_{2peak}).

During the progressive exercise test, ventilation volume (V_E), oxygen uptake (VO_2), and CO_2 production (VCO_2) were measured over 15-second intervals using a breath-by-breath Oxycon Alpha analyzer (Jaeger, Wuerzburg, The Netherlands). Before each test, the gas meter was calibrated with a Hans Rudolph 3-L syringe (Vacumed, Ventura, CA) and the analyzers were set with room air and a 4% CO_2/96% N_2 gas mixture.

Following VO_{2peak} and W_{peak} measurements, each subject performed a 2-hour familiarization ride at the predetermined exercise intensity on the electronically braked cycle ergometer. This familiarization ride was designed to minimize any learning effect and to ensure that the subjects were sufficiently well-trained to complete the experimental trial.

Dietary Manipulations

The subjects were then instructed to complete a 3-day dietary record consisting of 2 weekdays and 1 weekend day. These dietary records were analyzed with the Food Finder program (Medtech 1991, Tygerberg, South Africa) to determine the subjects' energy intake and macronutrient consumption. From this dietary information, a 15-day isocaloric HFD was devised for the HFD group (Table 2). To aid adherence to the HFD, subjects indicated their food preferences and meals were provided together with a diary to record any deviations from the diet. Subjects in the CON group were asked to adhere to their habitual diet throughout the 15-day trial.

Oral Glucose Tolerance Test

During the trials, an oral glucose tolerance test (OGTT) was administered to the HFD group on the days before each experimental ride (described later) and to the CON group on the days before the first and last experimental rides. All tests were conducted in the morning after an overnight fast. Upon arrival to the laboratory, a flexible 20-gauge cannula was inserted into a forearm antecubital vein and attached to a three-way stopcock (Industrias Palex, Barcelona, Spain). Venous blood samples (~5 mL) were drawn 15 minutes before and 30, 60, 90, and 120 minutes after the subjects ingested 75 g glucose in 400 mL water. After each blood sample, the cannula was kept patent by flushing with 1 mL sterile saline.

One aliquot of each blood sample (2 mL) was placed into a tube containing potassium oxalate and sodium fluoride for the subsequent analysis of plasma glucose concentrations. The remaining aliquot (3 mL) was placed into a tube containing lithium heparin for the subsequent analysis of plasma insulin concentrations. All samples were kept on ice until centrifugation at 3,000 rpm at 4°C for 10 minutes upon completion of the trial. Samples of the supernatant were then stored at −20°C for subsequent analyses of plasma glucose and insulin concentrations. Plasma glucose was determined using the glucose oxidase method (Glucose Analyzer 2; Beckman Instruments, Fullerton, CA).

Table 1. Subject Characteristics

Characteristic	CON (n = 8)	HFD (n = 8)	*P*
Age (yr)	30 ± 9	24 ± 3	NS
Weight (kg)	75 ± 5	73 ± 11	NS
Height (m)	179 ± 8	178 ± 10	NS
Body fat (%)	12.8 ± 5	7.5 ± 2	<.05
W_{peak} (W)	356 ± 41	364 ± 38	NS
VO_{2peak} (mL/kg/min)	61.0 ± 8.0	66.0 ± 4.0	NS

NOTE. Values are the mean ± SD.
Abbreviation: NS, nonsignificant.

Table 2. Dietary Intake

Parameter	Habitual Diet		Experimental Diet
	CON Group	HFD Group	HFD Group
kJ	13,210 ± 5,974	14,334 ± 3,382	16,442 ± 4,686
% CHO	53 ± 10	48 ± 10	19 ± 1*
% fat	30 ± 8	32 ± 8	69 ± 1*
% protein	13 ± 3	14 ± 2	10 ± 1
% alcohol	0.98 ± 2	3.2 ± 3	0
PS ratio	0.58 ± 0.2	0.72 ± 0.2	0.82 ± 0.2

NOTE. Values are the mean ± SD.
Abbreviation: PS ratio, polyunsaturated to saturated fat ratio.
*$P < .001$, HFD *v* habitual diet of CON and HFD groups.

Plasma insulin was determined by radioimmunoassay (Count-A-Coat Insulin; Diagnostic Products, Los Angeles, CA).

Experimental Rides

The day after the OGTTs, each subject completed one of four experimental trials at 5-day intervals (days 0, 5, 10, and 15). Between trials, subjects were asked to maintain regular training, but to refrain from training on the days preceding the experimental trials. Each trial consisted of a 2.5-hour ride at 63% W_{peak} on an electronically braked cycle ergometer, followed immediately by a simulated 40-km time-trial. The 40-km time-trial was performed on the subject's own bicycle mounted on a Kingcycle Trainer (EDS Portapromt, High Wycombe, Bucks, UK). The calibration and reliability of the Kingcycle has been described previously.[22] The coefficient of variation for eight subjects who undertook three 40-km time-trials was 1.0% ± 0.5%.[22] During the rides, the subjects were cooled with an electronic fan and the laboratory was maintained at an ambient temperature of about 22°C. In each ride, the only feedback to the subjects was their elapsed distance. They were not informed of their times or speeds until the completion of the experiments.

The experimental trials were performed in the morning after an overnight fast. Ninety minutes before the start of a trial, the subjects consumed 400 mL 4.3% (wt/vol) medium-chain triacylglycerol (MCT) emulsion (Liprocil; Adcock Ingram Critical Care Limited, Johannesburg, South Africa) suspended in artificially sweetened, orange-flavored water. The MCT emulsion consisted of 80% MCTs (C6-10) and 20% long-chain triacylglycerol (C > 12). The test solution therefore contained 3.4% MCT. At the start of the trial, the subjects ingested 400 mL of the same emulsion containing 10% glucose, and thereafter, they drank 200 mL of the MCT + glucose drink every 20 minutes until 40 minutes into the time-trial. MCT was given to the subjects to provide a readily oxidizable source of fat.[23]

On days 0, 10, and 15, the drinks consumed by the HFD group during the constant-load rides were labeled with [U-^{14}C]glucose (Amersham International, Buckinghamshire, UK) at a final specific (radio)activity of 6.3 kBq/g (0.17 μCi/g). A [U-^{14}C]glucose label was added to the drinks so that the rates of plasma and ingested glucose oxidation could be calculated from drink and plasma ^{14}C-glucose specific activities and expired $^{14}CO_2$ specific activity. A total of 36 μCi [U-^{14}C]glucose was consumed during each of the three trials.

VO_2, VCO_2, and $^{14}CO_2$ Measurements

Steady-state VO_2 and VCO_2 values were measured over 5-minute intervals every 30 minutes during the constant-load exercise. On days 0, 10, and 15, $^{14}CO_2$ expired by the HFD group was trapped by passing a sample of expired air through a solution containing 1 mL 1N hyamine hydroxide in methanol (United Technologies, Packard, Meriden, CT), 1 mL 96% ethanol (Saarchem, Krugersdorp, South Africa), and 1 to 2 drops of phenolphthalein (Saarchem). The expired air was bubbled

through the trapping mixture until the solution became clear, at which point 1 mmol CO_2 had been absorbed.[24] Liquid scintillation cocktail (Ready Gel; Beckman) was then added and $^{14}CO_2$ radioactivity was counted in a liquid scintillation counter (Packard Tri-Carb 4640). Gas exchange was not measured during the 40-km time-trials in case it interfered with performance.

Blood Sampling and Analyses

For the same reason, venous blood samples (~12 mL) were only drawn at rest and at 30-minute intervals during the constant-load exercise. One aliquot (5 mL) was placed into a tube containing potassium oxalate and sodium fluoride for subsequent analyses of plasma glucose, lactate, and ^{14}C-glucose concentrations. Another aliquot (3 mL) was placed into a tube containing lithium heparin for subsequent analysis of plasma insulin concentrations. The remaining aliquot (3 mL) was placed into a tube containing gel and clot activator for determination of serum free (nonesterified) fatty acids (FFAs), glycerol, and β-hydroxybutyrate concentrations. All samples were kept on ice and then centrifuged at 3,000 rpm at 4°C for 10 minutes at the end of the trial, and supernatants were then stored at −20°C for later analyses.

Plasma glucose and insulin were determined as described previously. Plasma lactate and serum FFAs were determined by spectrophotometric measurements using commercial kits (Lactate Pap; Bio Merieux, Marcy-L'Etiole, France; and FFA Half-micro test; Boehringer, Mannheim, Germany). Circulating glycerol and β-hydroxybutyrate concentrations were determined in neutralized $HClO_4$ extracts of serum using enzymatic spectrophotometric assays.[25]

Plasma Glucose and Lactate Specific Activity

Plasma (1 mL) for measurement of glucose and lactate specific radioactivities was added to tubes containing 3.5 mol/L $HClO_4$ (70 μL). Acidified samples were then mixed and centrifuged at 5,000 rpm for 10 minutes at 4°C, and the protein-free and HCO_3^--free supernatants were removed and stored on ice. The precipitate was then twice resuspended in 0.13 mol/L $HClO_4$ (0.78 mL) and recentrifuged, and the supernatant was added to that previously saved. Combined supernatants were then adjusted to pH 7 to 8 with K_2CO_3 (3 mol/L) in 0.01 mol/L Tris buffer (pH 8.0), recentrifuged, and passed through 500-mg anion-exchange columns (SAX; Bakerbond, Cape Town, South Africa) that were preconditioned with 20 mL ethanol followed by 20 mL distilled water, adjusted to about pH 8 with trace amounts of NaOH. Glucose was fully eluted into a scintillation vial with 3 mL distilled water (~8 pH). Lactate was subsequently eluted into a second scintillation vial with 2 mL $CaCl_2$ (1 mol/L) adjusted to pH 2.0 with HCl. Eluates were evaporated to near dryness at 70°C for about 20 hours before liquid scintillation cocktail (Ready Gel; Beckman) was added for ^{14}C radioactivity determinations (dpm) using a liquid scintillation counter (Packard Tri-Carb 4640). For each time glucose and lactate were separated, a nonlabeled plasma sample was spiked with a known quantity of [U-^{14}C]glucose and analyzed simultaneously to correct the measured dpm values for the percent recovery. Such recoveries exceeded 85% for all samples. Since there were negligible counts in the lactate fraction, no corrections were made for the contribution to $V^{14}CO_2$ from ^{14}C-lactate oxidation.

Plasma Glucose and Ingested Glucose Oxidation

The rate of plasma glucose oxidation (R_{OX}) in grams per minute was calculated from the equation,

$$R_{ox} = [(^{14}CO_2 \times 6)/SAGIc] \times VCO_2 \times 1.35,$$

where $^{14}CO_2 \times 6$ is the expired CO_2 specific radioactivity (dpm per millimole) multiplied by 6, because there are six carbon atoms per molecule of glucose; SA Glc is the plasma [^{14}C]glucose specific activity (dpm per millimole); VCO_2 is the volume of expired CO_2 (liters per minute); and 1.35 is the grams of glucose oxidized to produce 1 L of CO_2.

The rate of ingested CHO oxidation (grams per minute) was calculated from the same equation except that plasma glucose specific radioactivity was replaced with drink glucose specific radioactivity. These formulae do not account for the $^{14}CO_2$ retained in the bicarbonate pool. The time required to equilibrate $^{14}CO_2$ with the plasma HCO_3^- pool during moderate-intensity exercise (60% to 70% VO_2max) has been reported to vary between 5[26] and 90[27] minutes and, in our opinion, is probably 20 to 30 minutes.[19]

Total CHO and Fat Oxidation

The overall rates of CHO and fat oxidation (grams per minute) were calculated from the formulae of Frayn,[28] assuming a nonprotein respiratory exchange ratio:

$$\text{total CHO oxidation} = 4.55VCO_2 - 3.21VO_2 \text{ and}$$

$$\text{total fat oxidation} = 1.67(VO_2 - VCO_2).$$

In these formulae, VCO_2 is the volume of CO_2 in the expired air (liters per minute) and VO_2 is the corresponding oxygen uptake (liters per minute). Differences between total CHO oxidation and plasma glucose oxidation provided an estimate of the rate of direct and indirect (via lactate) oxidation of muscle glycogen.

Muscle Biopsies

On days 0, 10, and 15, a biopsy was obtained from the vastus lateralis muscles of the HFD group using the method of Bergstrom[29] as modified by Evans et al.[30] The muscle samples were frozen rapidly in liquid N_2 and stored at −80°C for subsequent analyses of CAT, CS, and 3-HAD activities. These enzyme activities were measured in muscle samples (15 to 50 mg) homogenized on ice in a phosphate buffer (1:19 wt/vol). Homogenates were sonicated on ice three times for 10 seconds (Virsonic 60; Vitrus, New York, NY) and divided into two aliquots. One aliquot was used to determine CAT activity by the method of Crabtree and Newsholme.[31] The other aliquot was used to determine HAD and CS activities using techniques described by Bass et al[32] and Srere,[33] respectively.

Statistical Analyses

All data are expressed as the mean ± SD. One-way ANOVA for repeated measures was used to determine changes in muscle enzyme activities, plasma glucose and ingested glucose oxidation rates, and circulating metabolite concentrations in the HFD group. Least-significant difference post hoc analysis was used. Two-way ANOVA for repeated measures was used to determine differences in total fat and CHO oxidation rates and time-trial performance times between the HFD and CON groups. Differences between groups for the OGTT and circulating blood metabolites were determined using two-way ANOVA for repeated measures at days 0 and 15.

RESULTS

OGTT

The HFD did not cause any significant differences in the area under the plasma glucose and insulin concentration curves during the OGTT (Table 3). However, plasma glucose concentrations measured at 30 minutes during the OGTT increased significantly after day 5 and remained elevated at days 10 and 15 of the HFD (Fig 1; $P < .05$). Conversely, in the CON group, plasma glucose at 30 minutes following glucose ingestion was

Table 3. Area Under the Curve for Plasma Glucose and Insulin Concentrations During the OGTT

	Days on Trial			
Parameter	0	5	10	15
Glucose (mmol/L · 120 min)				
HFD	657 ± 85	710 ± 88	706 ± 130	700 ± 56
CON	751 ± 136			701 ± 102
Insulin (mU/L · 120 min)				
HFD	2,473 ± 1,171	2,392 ± 502	2,965 ± 1,679	2,410 ± 502
CON	2,835 ± 834			2,974 ± 847

NOTE. Values are the mean ± SD.

similar at days 0 and 15. Furthermore, plasma glucose concentrations at 120 minutes during the OGTT increased significantly after 5 days of the HFD (Fig 1; $P < .05$). There were no differences in plasma insulin concentrations between the 2 groups (Fig 2).

Circulating Metabolite Concentrations During Constant-Load Exercise

The HFD had no effect on plasma glucose, lactate, and insulin concentrations during the 150 minutes of constant-load exercise on the day following OGTT (Table 4). Decreases in mean serum FFA concentrations from 0.41 ± 0.12 to 0.31 ± 0.13 mmol/L after 15 days of the HFD were not significant (Table 5). Serum β-hydroxybutyrate concentrations also were not different between the HFD and CON groups (Table 5).

In contrast, exposure to the HFD for as little as 5 days significantly increased serum glycerol concentrations throughout the constant-load exercise (Fig 3; $P < .001$). After 5 days of the HFD, final serum glycerol concentrations were significantly increased from 0.43 ± 0.44 to 0.69 ± 0.65 mmol/L. Thereafter, final serum glycerol concentrations at 10 and 15 days declined to 0.63 ± 0.50 and 0.52 ± 0.50 mmol/L, respectively. After 15 days of the HFD, serum glycerol concentrations were only different from baseline values at day 0 at 30 and 60 minutes of the constant-load exercise ($P < .01$). When serum glycerol concentrations in the HFD and CON groups were compared on days 0 and 15, serum glycerol in the HFD group was significantly higher at 30, 60, and 90 minutes of the constant-load exercise ($P < .05$).

Total CHO and Fat Oxidation

Increases in serum glycerol concentrations with the HFD corresponded with increases in the rate of fat oxidation during the constant-load exercise (Fig 4; $P < .01$). The mean rate of fat oxidation increased from 0.67 ± 0.16 to 0.91 ± 0.20 g/min in the first 5 days of the HFD and then tended to plateau over the remainder of the trial. The mean rate of fat oxidation in the CON group remained unchanged at about 0.60 g/min and was significantly lower than the value in the HFD group during the constant-load exercise (Fig 4; $P < .05$).

Increases in the rate of fat oxidation with the HFD resulted in corresponding decreases in the rate of total CHO oxidation from about 2.5 to 2.0 g/min during the constant-load exercise (Fig 5; $P < .05$). The latter rate was significantly lower versus the CON group over the 15-day trial ($P < .05$).

Although there was a relatively large decrease in total CHO oxidation after 5 days of the HFD, there was only a modest reduction in mean plasma glucose oxidation from 0.60 ± 0.08 to 0.50 ± 0.12 g/min over the 15-day HFD (Table 6). Differences between the mean rate of total CHO oxidation and the mean rate of plasma glucose oxidation suggested that the

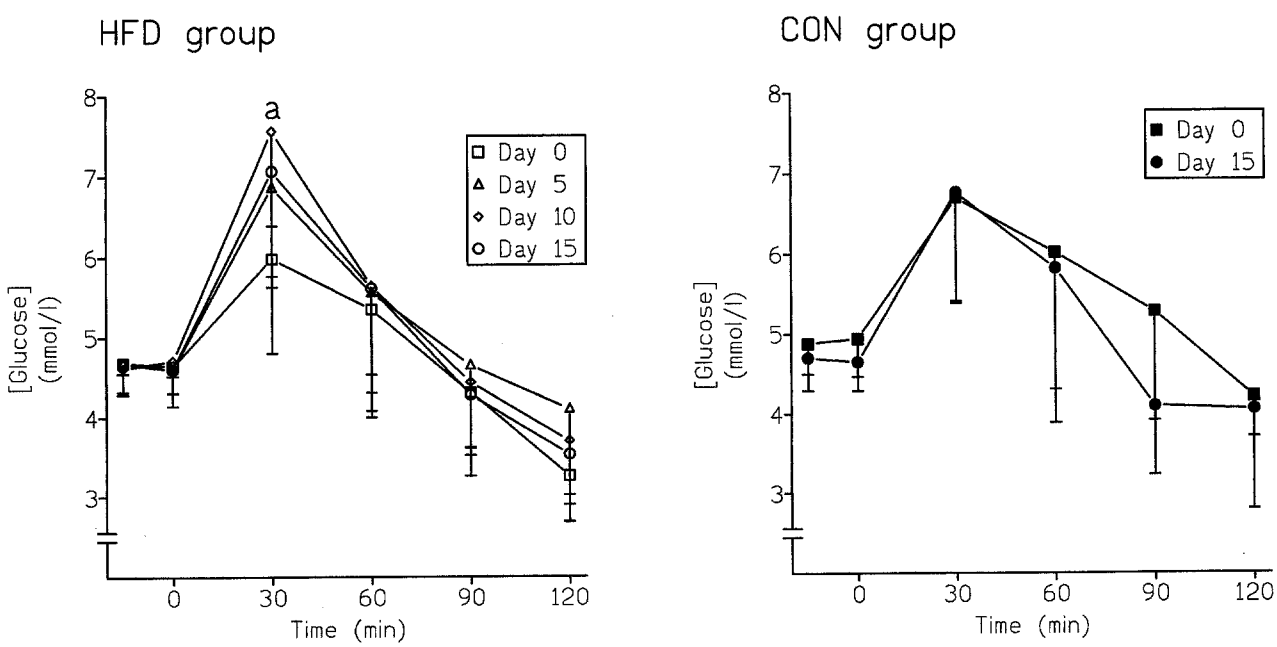

Fig 1. Plasma glucose concentrations in the OGTT during the 15-day trial in HFD and CON groups. Data are the mean ± SD. [a]Significant ($P < .05$) increase in plasma glucose concentrations from day 0 to days 5, 10, and 15.

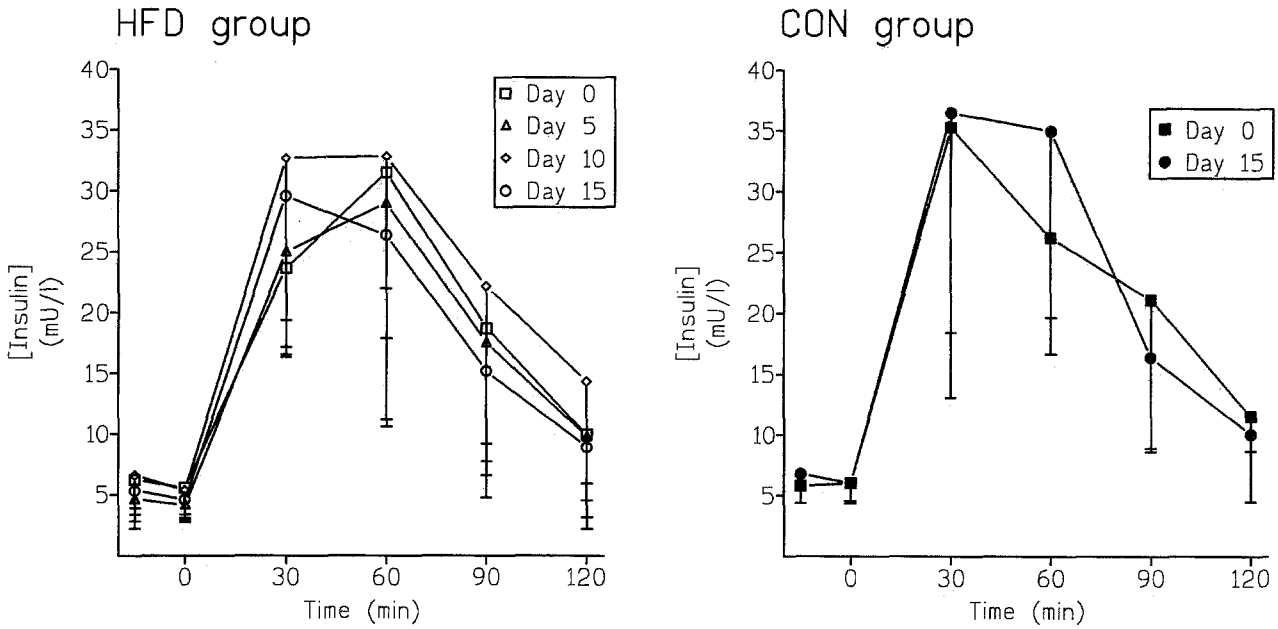

Fig 2. Plasma insulin concentrations in the OGTT during the 15-day trial in HFD and CON groups. Data are the mean ± SD.

HFD largely reduced the direct and/or indirect (via lactate) oxidation of muscle glycogen (Table 6). The estimated rates of direct and/or indirect (via lactate) muscle glycogen oxidation decreased from 1.9 ± 0.2 to 1.1 ± 0.3 and to 1.0 ± 0.3 g/min after 10 and 15 days of the HFD ($P < .005$ and $P < .001$, respectively).

Muscle Enzyme Activities

Changes in CAT, CS, and 3-HAD activities in the HFD group are shown in Table 7. CS and 3-HAD activities did not change significantly over the 15-day HFD, but CAT activity increased progressively from 0.45 ± 0.09 to 0.54 ± 0.08 μmol/g/min from days 0 to 10 ($P < .005$) and to 0.62 ± 0.11 μmol/g/min after 15 days of the HFD ($P < .005$).

Exercise Performance

Although exercise performance tended to improve to a greater extent in the HFD group versus the CON group, we were unable to detect a significant effect of diet on repeated 40-km time-trial performances (Table 8). The difference in exercise performance between the HFD and CON groups was 0.29 min (95% confidence interval, −2.4 to 3.0 minutes) over the 15-day trial.

Table 4. Mean Plasma Glucose, Lactate, and Insulin Concentrations During Steady-State Exercise Over the 15-Day Trial

Parameter	Days on Trial 0	5	10	15
Glucose (mmol/L)				
HFD	4.9 ± 0.7	4.8 ± 0.4	4.9 ± 0.4	4.9 ± 0.3
CON	4.7 ± 0.4			4.8 ± 0.4
Lactate (mmol/L)				
HFD	2.7 ± 0.7	2.5 ± 0.9	3.1 ± 1.4	2.4 ± 0.8
CON	2.7 ± 1.2			2.3 ± 0.9
Insulin (mU/L)				
HFD	6.1 ± 2.2	5.7 ± 0.9	6.5 ± 1.9	7.3 ± 2.1
CON	5.1 ± 0.6			6.5 ± 1.5

NOTE. Values are the mean ± SD.

DISCUSSION

In this study, we examined the metabolic adaptations that occur in well-trained cyclists in response to a HFD. The first finding was a marked increase in fat oxidation after 5 days of the HFD, with no further changes over the remaining 10 days of high-fat feeding. Previous researchers have attributed this shift in substrate metabolism with a HFD, in part, to changes in skeletal muscle enzyme activities[7,8,13-15] and altered whole-body glucose disposal.[16,34]

Although it is limited by a lack of CON data on days 5 and 10, there is evidence to suggest that the HFD reduced glucose tolerance in this study. Altered glucose tolerance was demonstrated by a significant increase in plasma glucose concentrations at 30 minutes during OGTTs on days 5, 10, and 15 of the HFD (Fig 1). However, these results should be interpreted with caution, as there were no differences in the areas under the plasma glucose and insulin concentration curves. On the other

Table 5. Mean Serum FFA and β-Hydroxybutyrate Concentrations During Steady-State Exercise Over the 15-Day Trial

Parameter	Days on Trial 0	5	10	15
FFA (mmol/L)				
HFD	0.41 ± 0.20	0.39 ± 0.19	0.29 ± 0.17	0.30 ± 0.13
CON	0.31 ± 0.10			0.27 ± 0.06
β-Hydroxybutyrate (mmol/L)				
HFD	0.18 ± 0.10	0.28 ± 0.13	0.27 ± 0.16	0.20 ± 0.06
CON	0.26 ± 0.15			0.19 ± 0.15

NOTE. Values are the mean ± SD.

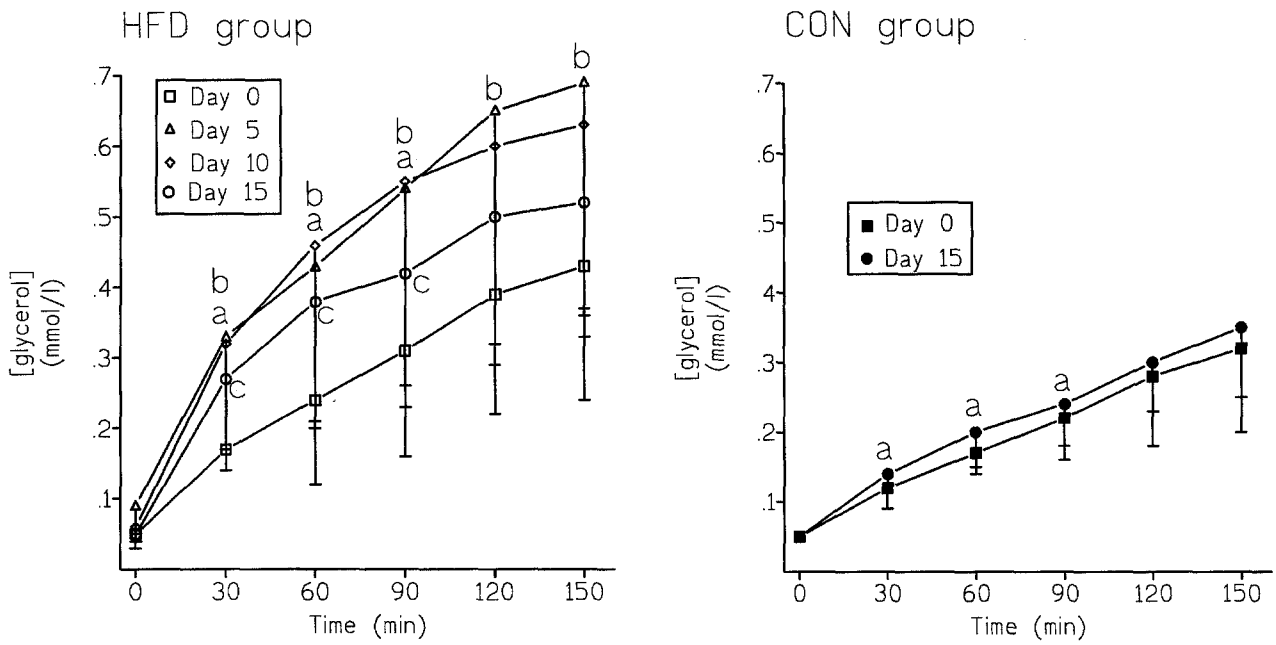

Fig 3. Serum glycerol concentrations during constant-load exercise in HFD and CON groups during the 15-day trial. Data are the mean ± SD. [a]Significant ($P < .05$) difference in serum glycerol between HFD and CON groups on days 0 and 15. [b]Significant ($P < .01$) increase in serum glycerol from day 0 to days 5 and 10. [c]Significant ($P < .01$) increase in serum glycerol from days 0 to 15.

hand, the OGTT is not as sensitive a measure of glucose tolerance and/or insulin sensitivity as the euglycemic clamp technique, and that technique has shown that 2 weeks of a HFD reduces insulin-stimulated glucose uptake in men.[34] Further, rat studies have demonstrated that exposure to a HFD for 3 days results in liver insulin resistance, manifested as a failure of glucose ingestion to suppress hepatic glucose output and promote liver glycogen synthesis.[16] A further 2 weeks of the HFD caused a decrease in insulin-stimulated rates of glucose oxidation.[16] The decrease in glucose tolerance that might have been expected with the HFD may have been attenuated by the training state of the subjects (Table 1). Secondly, the repeated bouts of high-intensity exercise in the experimental trials may have compensated for any insulin resistance induced by the HFD.[35] Furthermore, the HFD in this trial was rich in polyunsaturated fats (Table 2), which may have upregulated insulin action.[36]

During the exercise bout, there was no change in plasma

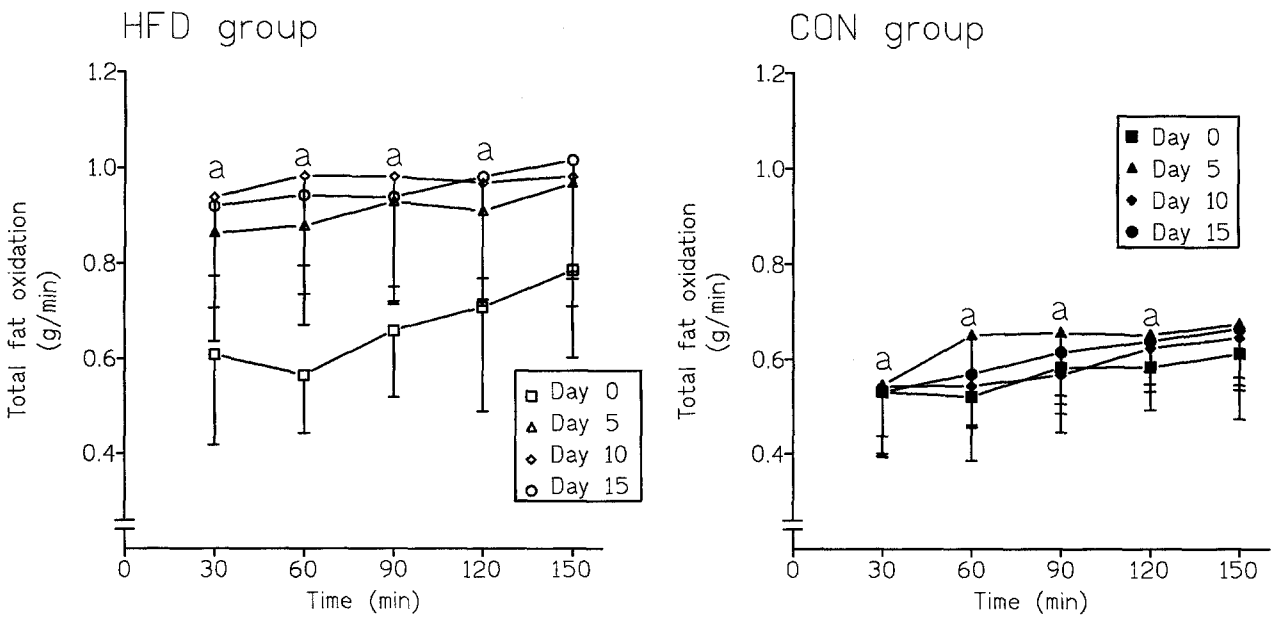

Fig 4. Total fat oxidation during constant-load exercise over 15 days in HFD and CON groups. Data are the mean ± SD. [a]Significant ($P < .01$) interaction effect between HFD and CON groups over the 15-day trial.

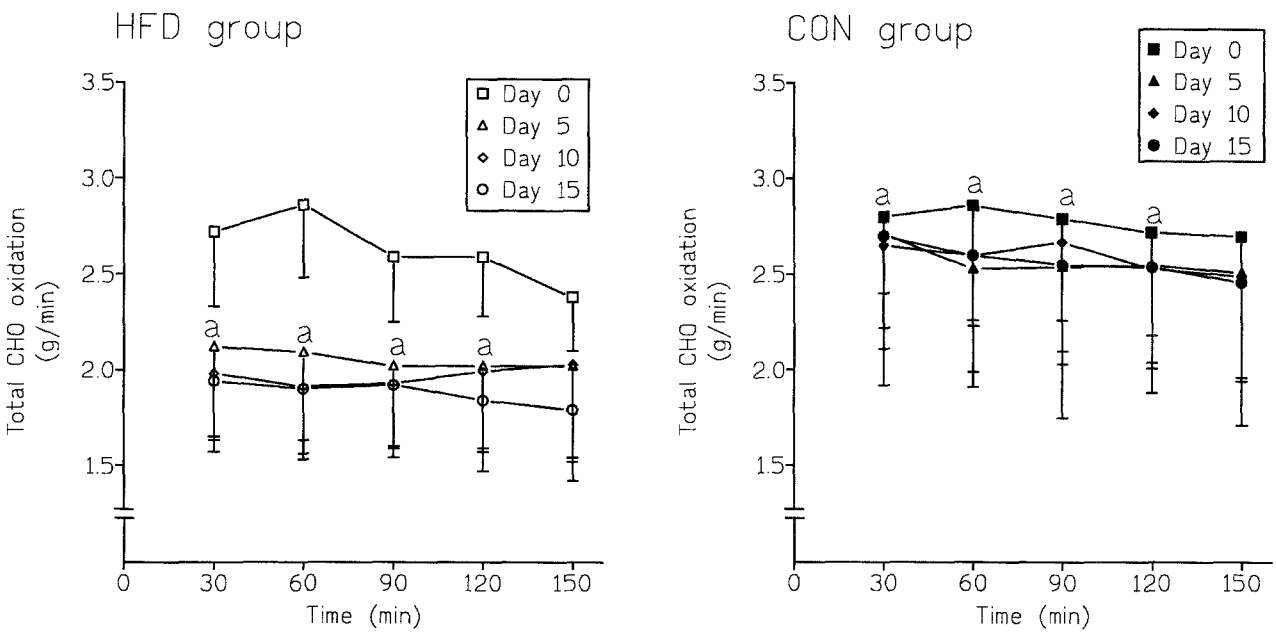

Fig 5. Total CHO oxidation during constant-load exercise over 15 days in HFD and CON groups. Data are the mean ± SD. [a]Significant ($P < .01$) interaction effect between HFD and CON groups over the 15-day trial.

glucose or insulin concentrations (Table 3). However, there was a significant increase in serum glycerol with the HFD (Fig 3). The changes in serum glycerol concentrations were not paralleled by changes in serum FFA concentrations (Table 5). Similar findings were reported by Jones et al,[37] who attributed the dissociation between serum FFA and glycerol concentrations during exercise, in part, to an increase in the rate of intramuscular triglyceride oxidation rather than an increase in adipose tissue lipolysis. They suggested that FFAs liberated from muscle triglyceride stores during high-intensity exercise are immediately oxidized.[37] Previous studies in humans have shown an increase in intramuscular triglyceride stores following both short-term (5 days)[11] and long-term (>2 weeks)[38] consumption of a HFD. Furthermore, Conlee et al[6] have shown both increased storage and utilization of intramuscular triglycerides during exercise in rats following 3 to 4 weeks of a HFD. Alternatively, it is possible that serum FFA concentrations were low due to rapid FFA oxidation rates.

Whatever the source, there was a significant increase in total fat oxidation during exercise with the HFD (Fig 4). Previous studies have also shown higher rates of fat oxidation during exercise in subjects who ingested a HFD for 2 or more weeks.[10,39] However, the adaptations that result in a shift in substrate utilization from CHO to fat oxidation with a HFD may not take more than 7 days, as previously thought.[40] We found that the major increase in fat oxidation (and serum glycerol concentrations) occurred in the first 5 days of the HFD. Mean total fat oxidation increased from 0.67 to 0.91 g/min in the first 5 days of the HFD and then plateaued at a maximum rate of 0.97 g/min at day 10 of the HFD. However, it is possible that this effect was magnified by the subjects ingesting MCTs before and during exercise. MCTs are a readily oxidizable source of fat, which were used in this study to maximize the rates of fat oxidation. With the ingestion of MCTs prior to exercise, the rates of fat oxidation in the CON group in this study were double those in our previous study wherein a high-CHO meal was ingested prior to exercise.[41]

Others have also shown that ingestion of a HFD for 3 to 5 days caused an increase in the rate of fat oxidation and a concomitant sparing of muscle glycogen.[1,11] Although we calculated muscle glycogen utilization only after 10 days of the HFD, we found that the estimated rates of direct and/or indirect

Table 6. Changes in the Rate of Plasma Glucose Oxidation, Ingested Glucose Oxidation, and Muscle Glycogen Oxidation With Exposure to a HFD

Parameter	Days on Trial 0	10	15
Plasma glucose oxidation (g/min)	0.60 ± 0.08	0.50 ± 0.14	0.50 ± 0.12
Ingested glucose oxidation (g/min)	0.42 ± 0.07	0.33 ± 0.10	0.40 ± 0.12
Muscle glycogen oxidation (g/min)	1.52 ± 0.24	1.08 ± 0.32*	1.00 ± 0.25†

NOTE. Values are the mean ± SD.
*$P < .005$, day 0 *v* day 10.
†$P < .001$, day 0 *v* day 15.

Table 7. Effects of HFD on Muscle Enzyme Activities

Enzyme	Days on Trial 0	10	15
CAT (μmol/g/min)	0.45 ± 0.09	0.54 ± 0.08*	0.62 ± 0.11†
3-HAD (μmol/g/min)	15.4 ± 3.3	16.3 ± 4.7	13.6 ± 4.4
CS (μmol/g/min)	28.9 ± 3.3	32.8 ± 7.8	31.7 ± 7.5

NOTE. Values are the mean ± SD.
*$P < .05$, day 0 *v* day 10.
†$P < .005$, day 0 *v* day 15.

Table 8. 40-km Time-Trial Performance Times of the HFD and CON Groups During the 15-Day Trial

Time (min)	Days on Trial			
	0	5	10	15
HFD	69.3 ± 7.6	67.1 ± 6.5	64.7 ± 5.8*	63.4 ± 5.3†
CON	69.9 ± 8.0	68.2 ± 7.3	64.9 ± 7.5‡‖	65.6 ± 6.6§

NOTE. Values are the mean ± SD.
*$P < .05$, day 0 *v* day 10.
†$P < .05$, day 0 *v* day 15.
‡$P < .005$, day 0 *v* day 10.
§$P < .001$, day 0 *v* day 15.
‖$P < .05$, day 10 *v* day 15.

(via lactate) muscle glycogen oxidation had decreased significantly by this time (Table 6). It may be argued that the decrease in muscle glycogen utilization measured at 10 days of the HFD may be due to some labeling of the body glycogen stores by the ^{14}C-glucose administered on day 0. However, 70% of the ^{14}C-glucose ingested during the experimental trial on day 0 was oxidized. Furthermore, in the 10 days between ^{14}C-glucose trials, the subjects continued training for an average of 500 minutes at intensities that would be expected to result in CHO oxidation rates of at least 2 g/min. The resultant utilization of about 1,000 g CHO is approximately equivalent to two times the whole-body CHO stores and would therefore be expected to eliminate any previously ^{14}C-labeled glycogen.

The shift from CHO to fat oxidation with a HFD or low-CHO diet may be related to a low muscle glycogen content. In a recent study from our laboratory, Weltan et al[42] demonstrated that low muscle glycogen content significantly increased fat oxidation and reduced muscle glycogen utilization during exercise. In their study, muscle glycogen content was manipulated by a glycogen-depleting exercise regimen followed by 2 days of either a low-CHO diet or an unrestricted diet. The muscle glycogen content in the low-CHO group was 40% of that in the normal-CHO group and resulted in a mean rate of fat oxidation of 0.87 versus 0.52 g/min, respectively. Interestingly, the 0.87-g/min rate of fat oxidation in the low–muscle glycogen group was similar to the 0.91-g/min rate of fat oxidation measured in our study after 5 days of the HFD.

Although the prevailing muscle glycogen content may influence substrate utilization during exercise, other researchers have attributed the increase in fat oxidation with a HFD to an increase in the mitochondrial enzymes involved in fat oxidation.[7-9,13,14] Increases in CS, 3-HAD, and CAT activities have been demonstrated in rats exposed to a HFD for 1 to 12 weeks.[7-9,13] In the present study, the HFD was associated with an increase in CAT activity from 0.45 to 0.54 μmol/g/min after 10 days and a further increase to 0.62 μmol/g/min after 15 days of the HFD (Table 7). However, there were no concomitant changes in 3-HAD or CS activities.

Our interpretation of the changes in CAT activity in response to the HFD may be limited by the lack of control data. However, the significant increases in CAT activity from day 0 to days 10 and 15 were not paralleled by changes in the activity of CS. If the increases in CAT activity were simply a result of an increase in mitochondrial proliferation, one may expect similar increases in CS activity, as shown by training studies performed on previously sedentary individuals.[43] However, we cannot disregard the possibility that the increases in CAT activity may be due to the repeated bouts of high-intensity exercise. Unfortunately, we are not aware of any studies that have examined the effects of high-intensity training or repeated bouts of moderate- to high-intensity exercise on CAT and CS activities in already-trained individuals.

Fisher et al[14] also showed that a HFD increased CAT activity from 0.43 to 0.62 μmol/g/min without parallel changes in mitochondrial enzymes in trained cyclists exposed to a ketogenic diet for 4 weeks. They suggested that the enzymatic adaptations after 4 weeks of a HFD may be part of the reason that exercise performance was not adversely affected, as found in the early short-term (3-day) high-fat feeding studies.[1,11] Other researchers have also reported that 2 to 4 weeks' adaptation to a HFD does not adversely affect exercise performance in the postabsorptive state[10,12,38] and may improve endurance exercise performance even when muscle glycogen stores are depleted.[12] In the present study, the exercise performance of both the HFD and CON groups improved to a similar extent over the 15-day trial (Table 8). This improvement in performance in both groups was most likely due to the exposure to repeated high-intensity exercise bouts.

The similarity in time-trial performances between the two dietary treatments may be confounded by the ingestion of CHO and MCT during exercise. CHO and MCT ingestion maintained euglycemia and may have delayed the earlier onset of fatigue that might otherwise be expected with the HFD. Although it was not measured, exposure to the HFD (low-CHO diet) and repeated bouts of high-intensity exercise would be expected to reduce the body glycogen stores and to compromise performance.[1,3] On the other hand, the ability of the subjects in the HFD group to improve their exercise performance despite presumably low glycogen stores could be due to their enhanced capacity to oxidize fat early in exercise.

In conclusion, exposure to a HFD for as little as 5 to 10 days resulted in an increase in fat oxidation and a concomitant sparing of muscle glycogen during exercise. This shift in substrate metabolism may be due to increases in muscle CAT activity, low muscle glycogen contents, and possibly altered glucose tolerance.

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