High-Fat Diet Versus Habitual Diet Prior to Carbohydrate Loading: Effects on Exercise Metabolism and Cycling Performance

Estelle V. Lambert, Julia H. Goedecke, Charl van Zyl, Kim Murphy, John A. Hawley, Steven C. Dennis, and Timothy D. Noakes

We examined the effects of a high-fat diet (HFD-CHO) versus a habitual diet, prior to carbohydrate (CHO)-loading on fuel metabolism and cycling time-trial (TT) performance. Five endurance-trained cyclists participated in two 14-day randomized cross-over trials during which subjects consumed either a HFD (>65% MJ from fat) or their habitual diet (CTL) (30 ± 5% MJ from fat) for 10 day, before ingesting a high-CHO diet (CHO-loading, CHO > 70% MJ) for 3 days. Trials consisted of a 150-min cycle at 70% of peak oxygen uptake (\(\dot{V}O_2\)peak), followed immediately by a 20-km TT. One hour before each trial, cyclists ingested 400 ml of a 3.44% medium-chain triacylglycerol (MCT) solution, and during the trial, ingested 600 ml/hour of a 10% 14C-glucose + 3.44% MCT solution. The dietary treatments did not alter the subjects’ weight, body fat, or lipid profile. There were also no changes in circulating glucose, lactate, free fatty acid (FFA), and \(\beta\)-hydroxybutyrate concentrations during exercise. However, mean serum glycerol concentrations were significantly higher (\(p<.01\)) in the HFD-CHO trial. The HFD-CHO diet increased total fat oxidation and reduced total CHO oxidation but did not alter plasma glucose oxidation during exercise. By contrast, the estimated rates of muscle glycogen and lactate oxidation were lower after the HFD-CHO diet. The HFD-CHO treatment was also associated with improved TT times (29.5 ± 2.9 min vs. 30.9 ± 3.4 min for HFD-CHO and CTL-CHO, \(p<.05\)). High-fat feeding for 10 days prior to CHO-loading was associated with an increased reliance on fat, a decreased reliance on muscle glycogen, and improved time trial performance after prolonged exercise.

Key Words: high-fat diet, medium-chain triacylglycerols, muscle glycogen, glucose metabolism, fat metabolism

The authors are with the Research Unit for Exercise Science and Sports Medicine in the Department of Human Biology, Faculty of Health Sciences, University of Cape Town, Sports Science Institute of South Africa, Newlands 7725, Cape Town, South Africa.
Introduction

There is an ongoing debate regarding the impact of a fat-rich, low-carbohydrate (CHO) diet on the regulation of substrate oxidation and exercise performance during prolonged exercise at moderate to high intensities (11;15;18;19;25;28;30;31). The debate is exacerbated by methodological differences, including the nature of the exercise bout (duration and intensity), the measurement of exercise performance (endurance capacity, the amount of work completed in a specified time, or a time trial) and the definition of a fat-rich diet (% of energy and duration of intake). The effects of a high-fat diet may be further confounded by the nutritional state of the subjects prior to the trials (fed or fasted), the pre-exercise meal (the nutrient and energy composition) and the resultant hormonal milieu during the exercise bout.

Compared to a high-CHO diet, the consumption of a high-fat diet for 3–7 days has resulted in a decrease in submaximal exercise time to exhaustion (2, 5, 13, 21, 23, 27, 35). On the other hand, there is evidence that the ingestion of a fat-rich diet for longer periods (≥7 days) induces adaptive responses that are fundamentally different to the acute lowering of body CHO reserves or fasting (4, 11, 15, 18, 19, 30, 32). Studies examining the effects of more prolonged high-fat feeding (≥2 weeks) have shown an increase in carnitine-acyl transferase activity (11, 15), a decrease in hexokinase activity (11), a decrease in insulin-stimulated muscle glucose uptake (24, 32), a decreased oxidative glucose disposal by the muscle, and a reduction in skeletal muscle pyruvate dehydrogenase activity in exercise-trained humans (9).

Possibly due to these adaptations, exposure to a fat-rich, low-CHO diet for 1–5 weeks in well-trained individuals has been shown to maintain (15, 19, 30, 31) and even enhance (25) endurance capacity during exercise of low to moderate intensities. Furthermore, fat-rich diets are associated with decreased CHO oxidation during exercise, decreased reliance on muscle and liver glycogen stores, and increased glycogen synthase activity (9).

Conlee et al. (7) have shown that rats adapted to a high-fat diet for 4–5 weeks and subsequently fed a high-CHO diet for 3 days, rapidly repleted muscle glycogen stores to pre-diet levels and ran longer during constant-load treadmill exercise than the CHO-adapted rats. Conversely, Helge et al. (18) fed untrained young male subjects a high–fat diet or a high-CHO diet for a period of 7 weeks, followed by 1 week of CHO-loading, showed similar rates of fat oxidation and muscle glycogen depletion during exercise under the two conditions. Moreover, exposure to the high-fat diet impaired endurance performance during a steady-state cycle to exhaustion. Helge et al. (18) suggested that prolonged (7 week) high-fat feeding might alter the membrane phospholipid composition and change calcium transport in the cytoplasm, compromising endurance performance.

It was, therefore, hypothesized that adaptation to a high-fat diet for a period sufficient to elicit metabolic adaptations in favor of fat oxidation, followed by short-term exposure to a high-CHO diet, would enhance fat oxidative capacity and “spare” muscle glycogen stores, with the potential for optimizing exercise performance.

Therefore, the aims of the present investigation were (a) to study the effects of a 10-day high-fat, low-CHO diet, followed by 3 days of CHO-loading, on substrate utilization during constant-load, submaximal exercise; and (b) to determine the effects of such a diet on subsequent high-intensity cycling performance.
Materials and Methods

Subjects

Five male endurance-trained cyclists participated in this study, 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-14C] glucose were ingested and venous blood samples were taken, the risks were carefully explained to each subject before their written consent was obtained. All subjects were healthy, had been cycling on a regular basis for more than 3 years, and consumed habitual diets that contained less than 40% of total energy intake from fat. The characteristics of the subjects are presented in Table 1.

Preliminary Testing

All subjects were tested for peak oxygen consumption (\(\dot{V}O_{2\text{peak}}\)) and peak sustained power output (PPO) on an electronically braked cycle ergometer (Lode, Groningen, Holland) modified with clip-in pedals or toe clips and racing handle bars. The incremental cycle test to exhaustion, the determination of PPO, and the accompanying gas collection procedures have been described in detail previously (17). Briefly, each subject began cycling at a workload of 3.33 W · kg\(^{-1}\) · min\(^{-1}\) body mass for 150 s. This workload was then increased by 50 W for a further 150 s and, thereafter, the exercise intensity was increased by 25 W for 150 s until the subject was exhausted. Exhaustion was defined as a reduction in pedaling frequency of > 10 rpm and/or a respiratory exchange ratio (RER) > 1.10. PPO was defined as the last completed work rate plus the fraction of time spent in the final non-completed work rate multiplied by the 25 W work rate increase.

During the progressive exercise test, subjects wore a nose-clip and inspired air via a Hans Rudolph 2700 one-way valve (Vacumed, Ventura, CA) connected to a dry gas meter. The expired air was passed though a 15-L baffled mixing chamber and a condensation coil to an Ametek S-3A/O\(_2\) analyzer and a CD-3A CO\(_2\) analyzer (Scientific Instruments, Pittsburgh, PA). Before each test, the gas meters were calibrated with a Hans Rudolph 5530 3-L syringe, room air, and a 16% CO\(_2\) : 4% O\(_2\) :
80% N2 gas mixture. The instrument outputs were processed by an on-line computer that calculated $L \cdot \text{min}^{-1}$ in ventilation ($V_e$), $\dot{V}O_2$, and expired CO2 ($\dot{V}CO_2$) values using conventional equations. The results of the initial maximal test were used to determine the workload, which corresponded to ~63% of each subject’s PPO (~70% of $\dot{V}O_2_{\text{peak}}$), for the subsequently described experimental trials.

**Dietary Manipulations**

The subjects’ habitual dietary intake was assessed using a 3-day weighed dietary record, consisting of 2 week days and 1 weekend day. The diets were analyzed using the Food Finder program (Medtech Ltd., Medical Research Council, Tygerberg, South Africa) to determine energy intake and macronutrient composition (Table 2). From the analysis of each subject’s habitual diet, isoenergetic high fat (HFD) and high CHO diets were formulated for each subject (Table 2). Prior to each experimental trial, subjects underwent two randomly assigned, 10-day periods of either HFD (>65% energy from fat) followed by 3 days of a high-CHO diet (>65% energy from CHO)(HFD-CHO), or 10 days of their habitual diet (CTL), followed by 3 days of a high-CHO diet (CTL-CHO). To aid compliance to the diets, palatable, individualized menus were formulated for each subject by a registered dietician. All food was purchased for the subjects; however, subjects were free-living and were instructed to follow the diets to the best of their ability. A sample high fat menu, including the energy intake and macronutrient composition, is provided in Table 3.

The prescribed diets aimed at providing an isocaloric intake compared with the habitual diet, with similar protein intakes (20% by energy). There were no reported deviations from the prescribed diets and purchased food items. However, if subjects did not adhere strictly to the diets, then the effects may have actually been underestimated. Compliance was indirectly confirmed by the high initial rates of total fat oxidation after exposure to the high-fat diet.

**Experimental Trial**

Prior to the first trial, subjects underwent a familiarization ride in order to minimize any possible learning effect of the trial. Trials were separated by a 2-week period, during which time the subjects continued with their normal training and reverted

**Table 2** Nutrient Composition (by Energy) of the Dietary Interventions

<table>
<thead>
<tr>
<th>Variable</th>
<th>Habitual diet</th>
<th>High-fat diet</th>
<th>High-CHO diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (MJ)</td>
<td>14.3 ± 0.7</td>
<td>14.00</td>
<td>14.00</td>
</tr>
<tr>
<td>CHO (%)</td>
<td>52.6 ± 5.8</td>
<td>&lt;15%</td>
<td>&gt;65%</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>29.9 ± 4.5</td>
<td>&gt;65%</td>
<td>&lt;15%</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>12.8 ± 0.9</td>
<td>20%</td>
<td>20%</td>
</tr>
<tr>
<td>Alcohol (%)</td>
<td>2.1 ± 1.1</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

*Note.* Values are mean ± SEM.
back to the consumption of their habitual diets. Subjects were asked to refrain from riding on the day preceding the experimental trial.

Each trial consisted of a 150-min cycle at 63% of PPO (~70% of $\dot{V}O_2\text{peak}$) on the electronically-braked ergometer, followed immediately by a simulated 20-km time-trial (TT). The 20-km TT was performed on the subject’s own bicycle mounted on a Kingcycle Trainer (EDS Portaprompt, Ltd., UK). The calibration and reliability of the Kingcycle has been described in detail previously (29). The coefficient of variation for 6 subjects who undertook three 20-km TT was 1.1 ± 0.9%. Throughout each experimental trial, power output and cadence were monitored every 60 s, but the only feedback given to subjects was their elapsed distance (as a percentage of the total distance). Subjects were cooled with an electronic fan, and the laboratory was maintained at a constant temperature of 20 °C and approximately 55% relative humidity.

Experimental trials were conducted between 0700–1100 hours. Upon arrival at the laboratory, each subject’s height and body mass were recorded, and percentage body fat was estimated using the sum of four skinfolds (10). Thereafter, a flexible 18-gauge cannula (Johnson and Johnson, Halfway House, South Africa) was attached

<table>
<thead>
<tr>
<th>Table 3 Sample Menu of a High-Fat Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Breakfast:</strong></td>
</tr>
<tr>
<td>60 g fried South African sausage (boerewors)</td>
</tr>
<tr>
<td>70 g avocado</td>
</tr>
<tr>
<td>50 g fried tomato</td>
</tr>
<tr>
<td>50 g fried onion</td>
</tr>
<tr>
<td><strong>Snack:</strong></td>
</tr>
<tr>
<td>50 g peanuts</td>
</tr>
<tr>
<td><strong>Lunch:</strong></td>
</tr>
<tr>
<td>100 g green salad</td>
</tr>
<tr>
<td>23 g mayonnaise</td>
</tr>
<tr>
<td>90 g tuna in oil</td>
</tr>
<tr>
<td>50 g cheese</td>
</tr>
<tr>
<td>50 g bread roll</td>
</tr>
<tr>
<td>10 g margarine</td>
</tr>
<tr>
<td>10 g peanut butter</td>
</tr>
<tr>
<td><strong>Snack:</strong></td>
</tr>
<tr>
<td>30 g crisps</td>
</tr>
<tr>
<td><strong>Dinner:</strong></td>
</tr>
<tr>
<td>100 g green salad</td>
</tr>
<tr>
<td>23 g salad dressing</td>
</tr>
<tr>
<td>120 g fried pork sausage</td>
</tr>
<tr>
<td>100 g fried potatoes</td>
</tr>
<tr>
<td>50 g fried onions</td>
</tr>
<tr>
<td><strong>Snack:</strong></td>
</tr>
<tr>
<td>50 g peanuts</td>
</tr>
</tbody>
</table>

20 g cream to be added to 2 cups of coffee

<table>
<thead>
<tr>
<th>Nutrient content:</th>
<th>Energy (MJ)</th>
<th>CHO (%)</th>
<th>Fat (%)</th>
<th>Protein (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>13.5</td>
<td>13.5</td>
<td>71.1</td>
<td>13.9</td>
</tr>
</tbody>
</table>
to a 3-way stop-cock (Industries Palex Plc, Barcelona, Spain) and positioned in a forearm antecubital vein for the collection of blood samples at rest and at 20-min intervals throughout each trial. After a blood sample was taken, the cannula was immediately flushed with 2–3 ml of sterile saline plus 5 μL of heparin.

One hour before the start of both trials, subjects consumed 400 ml of a 3.44% (g · 100⁻¹ ml) MCT (C₈₋₁₀): 0.86% long-chain triacylglycerol (LCT) solution (Liprocil, Adcock Ingram, Critical Care Ltd., Johannesburg, South Africa). After 60 min, subjects mounted the cycle ergometer and began a 5-min warm-up at a workload of 100 W. The constant-load ride at 63% of PPO was then commenced, with subjects ingesting 100 ml of a combined 10% glucose polymer + 3.44% MCT solution every 10 min until completion of the trial. The MCT and CHO solution was given to subjects in order to maximize potential fat and CHO oxidation during exercise (22, 38). All drinks were flavored with a low calorie orange concentrate and labeled with trace amounts of [U-¹⁴C] glucose (Amersham International, Buckinghamshire, UK) so that rates of plasma and ingested glucose oxidation could be calculated from drink and plasma ¹⁴C-glucose specific activities and expired ¹⁴CO₂ specific activity.

**VO₂, VCO₂, and ¹⁴CO₂ Measurements**

Steady-state gas exchange was measured for 3–4 min every 20 min until the completion of the ride. Expired ¹⁴CO₂ was trapped by passing air from the CO₂ gas analyzer vent through a solution containing 1 ml of 1 N hyamine hydroxide in methanol (United Technologies, Packard, IL), 1 ml of 96% ethanol (SAARCHEM, Krugersdorp, RSA), and 1–2 drops of phenolphthalein (SAARCHEM). The expired air was bubbled through the trapping mixture until the solution became clear, at which point 1 mmol of CO₂ had been absorbed (33). Liquid scintillation cocktail (Ready Gel, Beckman, Fullerton, CA) was then added, and ¹⁴CO₂ radioactivity was counted in a liquid scintillation counter (Packard Tri-Carb 1500, Downers Grove, IL). All counts were corrected for differences in quench and background disintegration’s min⁻¹ (dpm).

**Blood Sampling and Analysis**

Blood samples (~12 ml) were collected at rest, and then after 40, 80, 100, 120, 140 min during the steady-state ride; at 160 min; and within 1 min of completing the TT. One aliquot (5 ml) was placed into a tube containing potassium oxalate and sodium fluoride for the subsequent analyses of plasma glucose, lactate, and plasma ¹⁴C-glucose concentrations. Another aliquot (3 ml) was placed in a tube containing lithium heparin for the subsequent analysis of plasma insulin concentrations. The remaining aliquot (3 ml) was placed in a tube containing gel and clot activator for determinations of serum free (non-esterified) fatty acids (FFA), glycerol and β-hydroxybutyrate concentrations. All samples were kept on ice, centrifuged at 3,000 rpm at 4 °C for 10 min at the end of the trial, and supernatants were then stored at −20 °C for later analyses.

Plasma glucose concentrations were determined in duplicate using an automated glucose analyzer (Glucose Analyser II, Beckman Instruments, Fullerton, CA), while plasma insulin concentrations were measured by radio-immunoassay (Count-A-Coat Insulin, Diagnostic Products, Los Angeles, CA). Plasma lactate concentrations (Bio Merieux, Marcy-L Etiole, France) and total plasma FFA concentration (Half-micro test, Boehringer Mannheim, Germany) were determined by enzymatic
spectrophotometric assays. Serum glycerol and β-hydroxybutyrate were measured enzymatically according to the methods of Bergmeyer (1).

On days 0, 5, and 10 of the HFD-CHO, a 5-ml blood sample was drawn from each subject and placed in a tube containing EDTA for the subsequent enzymatic analysis of serum cholesterol (Boehringer Manneheim, Germany). HDL cholesterol concentrations were determined after selective precipitation of the apoB-containing lipoproteins with manganese chloride and heparin (14).

**Plasma Glucose and Lactate Specific Activity**

A 1-ml aliquot of the plasma, which was collected during the steady-state ride, was added to a tube containing 70 μL of 50% PCA for the separation of glucose and lactate. After the sample was mixed, and centrifuged at 5,000 rpm for 10 min at 4 °C, the protein-free supernatant was removed and stored at 3 °C. The precipitate was then re-suspended in 0.78 ml of 1.9% PCA and re-centrifuged at 5,000 rpm for 10 min at 4 °C, and the supernatant was added to that previously saved. This step was repeated before the pellet was discarded, and the combined supernatants were adjusted to pH 7–8 with ~200 μL of MK₂CO₃ (3 mol · L⁻¹), made in 0.01 M TRIS-HCl buffer (pH 8.0). The solution was then centrifuged at 5,000 rpm for 20 min at 4 °C. The supernatant was subsequently passed through anion exchange columns containing Sephadex (Bakerbond SAX, Cape Town, South Africa) that had been conditioned with 20 ml of ethanol followed by 20 ml of distilled water adjusted to pH ~8 with trace amounts of NaOH. Glucose was fully eluted into a scintillation vial with 3 × 1 ml of distilled water (pH ~8). Lactate was subsequently eluted into a second scintillation vial with 2 × 1 ml of CaCl₂ (1 mol · L⁻¹), adjusted to pH ~2 with HCl. After collection, the eluates were evaporated to near dryness at 70 °C for ~20 hours before liquid scintillation cocktail (Ready Gel, Beckman, Fullerton, CA) was added for ¹⁴C-counting as described earlier.

Each time glucose and lactate samples were separated, a non-labeled plasma sample was spiked with a known quantity of [U-¹⁴C] glucose and run simultaneously to correct the measured dpm values for the percent recovery. Such recoveries exceeded 90% for all samples. Since there were negligible counts in the lactate fraction, no corrections were made for the contribution to V¹⁴CO₂ from ¹⁴C-lactate oxidation.

**Glucose Oxidation**

The rates of plasma glucose oxidation (Gluox) in grams per minute were determined from the equation:

\[ \text{GLU}_{\text{ox}} = \left( \frac{\text{SA CO}_2}{\text{SA glu}} \right) \cdot \overline{\text{VCO}_2} \]

In this equation, GLUox is the rate of plasma glucose oxidation in mmol · min⁻¹, SA CO₂ is the specific (radio) activity of expired ¹⁴CO₂ in dpm · mmol⁻¹, SA glu is the corresponding specific (radio) activity of the plasma glucose in dpm · mmol⁻¹, \( \overline{\text{VCO}_2} \) is the volume of expired CO₂ in mmol · min⁻¹, calculated from the L · min⁻¹ VCO₂ and the 22.4 ml · mmol⁻¹ gas volume. Since the complete conversion of one molecule of U-¹⁴C glucose to six molecules of ¹⁴CO₂ decreases the dpm · mmol⁻¹ specific radioactivity by a factor of six, the \( \overline{\text{VCO}_2} \) values did not need to be divided by six to allow for six CO₂ molecules arising from oxidation of one glucose molecule.
The same equation was used to determine the rates of ingested CHO oxidation. However, in this case, SA glu was the specific radioactivity of the drink in dpm · mmol\(^{-1}\) glucose equivalents. It should be noted that this formula does not take into account the time taken to equilibrate \(^{14}\)CO\(_2\) with the HCO\(_3\) pool, which has been reported to vary between 5 min (8) and 90 min (6). However, it can be predicted from the flux of CO\(_2\) through the body HCO\(_3\) stores that equilibration is essentially complete in 20–30 min (3). More to the point, any systematic lag in the appearance of \(^{14}\)CO\(_2\) in the breath would have been similar from trial to trial.

**Total CHO and Fat Oxidation**

The overall rates of CHO and fat oxidation (grams per minute) were calculated from the formulae of Frayn (12), assuming a non-protein RER. Differences between the g/min rates of total CHO oxidation and plasma glucose oxidation provided an estimate of the direct and/or indirect (via lactate) oxidation of muscle glycogen.

**Statistical Analyses**

All data are expressed as means ± SEM. We estimated that the minimal sample size required to detect mean differences in fat oxidation during exercise between different dietary regimens of 0.30 g/min (with a standard deviation of 0.15 g/min) was 5 persons per group (or treatment) at an alpha level of .05, with 80% power. Factorial analysis of variance was used to compare differences between treatments over time. The Greenhouse-Geisser adjustment was used to correct for the maximal violation of the assumption of sphericity in this repeated measures study design (39). Where significant F-ratios were found, least squares post hoc analysis was performed to determine differences at specific time points. An alpha level of \(p < .05\) was considered to be statistically significant. TT performance was compared using a paired \(t\) test with two-tailed \(p\) values.

**Results**

**Body Composition and Blood Lipid Profiles**

Exposure to the HFD-CHO or the CTL-CHO diets did not alter body mass or estimated body composition (Table 4). Nor were there any systematic changes in plasma cholesterol or triacylglycerol concentrations in response to either dietary intervention (Table 5).

**Table 4 Variations in Body Composition With the CTL-CHO and HFD-CHO Trials**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pre</th>
<th>CTL-CHO</th>
<th>HFD-CHO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>77.5 ± 1.8</td>
<td>78.2 ± 2.0</td>
<td>77.5 ± 2.1</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>13.7 ± 1.4</td>
<td>14.3 ± 1.6</td>
<td>13.5 ± 1.5</td>
</tr>
</tbody>
</table>

*Note.* Values are mean ± SEM. PRE = pre-trial values.
Serum-Free Fatty Acids (FFA), Glycerol, and β-Hydroxybutyrate

Figure 1 displays the effects of the two dietary manipulations on the products of triacylglycerol metabolism during the constant-load cycle and subsequent 20-km TT. Mean serum glycerol concentrations were significantly higher in the HFD-CHO trial compared to the CTL-CHO trial ($p < .05$). However, there were no differences between dietary treatments for serum FFA or β-hydroxybutyrate concentrations.

Serum FFA concentrations rose in both trials from 0.15–0.25 mmol · L$^{-1}$ at the start of exercise to ~0.45 mmol · L$^{-1}$ at the end of the TT, which were significantly different from resting after 120 min of steady-state exercise ($p < .01$).

Plasma Glucose, Blood Lactate, and Insulin Concentrations

The different dietary treatments also did not affect the plasma glucose and lactate concentrations during the constant-load ride and TT. During both rides euglycaemia was maintained, with an average plasma glucose concentration of between 4.5–5.5 mmol · L$^{-1}$ (Figure 2). In both trials, mean plasma lactate concentrations rose from ~2 mmol · L$^{-1}$ during the constant-load ride to ~4 mmol · L$^{-1}$ at the end of the TT (Figure 2; $p < .07$). However, this response was variable between individuals.

Although plasma insulin concentrations during the constant-load ride tended to be slightly higher following HFD-CHO diet, this difference did not reach statistical significance (Figure 2). However, the area under the insulin curve (AUC) was significantly greater following the HFD-CHO compared to the CTL-CHO trial ($779 ± 129$ mU · L$^{-1}$ AUC vs. $682 ± 129$ mU · L$^{-1}$ AUC, $p < .001$).

Total CHO, Plasma Glucose, Muscle Glycogen, and Total Fat Oxidation Rates

Table 5 Variations in Lipid Profile With the CTL-CHO and HFD-CHO Trials

<table>
<thead>
<tr>
<th>Trial</th>
<th>Day 5</th>
<th>Day 10</th>
<th>Day 13</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum cholesterol (mmol · L$^{-1}$):</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PRE: 3.7 ± 0.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTL-CHO</td>
<td>3.36 ± 0.32</td>
<td>3.38 ± 0.33</td>
<td>3.31 ± 0.34</td>
</tr>
<tr>
<td>HFD-CHO</td>
<td>3.78 ± 0.39</td>
<td>3.69 ± 0.38</td>
<td>3.04 ± 0.42</td>
</tr>
<tr>
<td>Serum HDL-cholesterol (mmol · L$^{-1}$): PRE: 1.33 ± 0.08</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTL-CHO</td>
<td>1.25 ± 0.03</td>
<td>1.25 ± 0.04</td>
<td>1.10 ± 0.05</td>
</tr>
<tr>
<td>HFD-CHO</td>
<td>1.43 ± 0.07</td>
<td>1.38 ± 0.08</td>
<td>1.16 ± 0.09</td>
</tr>
<tr>
<td>Serum triglyceride (mmol · L$^{-1}$): PRE: 1.3 ± 0.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTL-CHO</td>
<td>1.21 ± 0.25</td>
<td>1.44 ± 0.22</td>
<td>1.07 ± 0.32</td>
</tr>
<tr>
<td>HFD-CHO</td>
<td>1.10 ± 0.23</td>
<td>1.16 ± 0.27</td>
<td>1.07 ± 0.17</td>
</tr>
</tbody>
</table>

Note. Values are mean ± SEM. HDL-cholesterol = high-density lipoprotein cholesterol; PRE = pre-trial blood sample.
Figure 1 — Serum free fatty acid (FFA), glycerol, and β-hydroxybutyrate concentrations during constant-load exercise and subsequent 20-km time trial. Serum FFA concentrations rose significantly in both trials and were significantly higher than resting values after 120 min (p < .01). Mean serum glycerol concentrations were significantly higher in the HFD-CHO trial than in the CTL-CHO trial (p < .05).

Total rates of CHO oxidation during exercise were significantly lower (p < .01) and total fat oxidation was significantly higher (p < .05) in the HFD-CHO versus the CTL-CHO trials. The average rates of CHO-oxidation in the CTL-CHO trial were ~1.7 g · min⁻¹ compared to ~1.1 g · min⁻¹ in the HFD-CHO trial.

In both trials, plasma glucose oxidation rose from ~0.35 g · min⁻¹ after 60 min to ~0.8 g · min⁻¹ at the end of the TT (Figure 4). Differences between rates of total CHO oxidation and rates of plasma glucose oxidation suggested that the direct and/or indirect (via lactate) oxidation of muscle glycogen were significantly greater throughout the CTL-CHO trial compared to the HFD-CHO trial (Figure 4; p < .05). The average calculated rates of direct and/or indirect (via lactate) muscle glycogen oxidation were 1.08 ± 0.06 g · min⁻¹ in the CTL-CHO trial and 0.63 ± 0.06 g · min⁻¹ in the HFD-CHO trial.
Figure 2 — Plasma glucose, insulin, and lactate concentrations during constant-load exercise and subsequent 20-km time trial. Plasma glucose levels were similar between trials, and euglycaemia was maintained. Plasma lactate increase in both trials during the time trial ($p = .07$). Plasma insulin decreased significantly in both trials after 60 min of steady-state exercise ($p < .01$).

**Cycling Time Trial Performances**

Individual TT performances for the CTL-CHO and HFD-CHO trials are presented in Figure 5. Time to complete the 20-km TT, following 2.5 hours of constant-load exercise, were significantly shorter in the HFD-CHO trials compared to the CTL-CHO trial ($29.35 \pm 1.25$ min vs. $30.68 \pm 1.55$ min; $p < .05$). Improved TT performances in the HFD-CHO trial were not associated with differences in either the subjective ratings of perceived exertion or heart rates measured during the two trials.

**Discussion**
In the present study, 10 days of dietary fat-adaptation followed by a 3-day CHO-loading regimen improved 20-km cycling TT performance, which was preceded by 150 min of constant-load exercise. In addition, we specifically fed subjects an MCT-CHO suspension during exercise, as a readily oxidizable source of fat, used in this study to maximize the rates of fat oxidation (38). The enhanced performance under the HFD conditions in the present study was associated with a reduction in the estimated rates of muscle glycogen utilization after the HFD compared to the habitual diet. To the best of our knowledge, this is the first study that has specifically examined the effect of a prolonged (>7 days) fat-adaptation regime followed by CHO-loading in already well-trained subjects.

The only other study to examine the effects of a prolonged (7-week) period of fat-adaptation followed by CHO-loading (1 week) was performed on previously untrained subjects who undertook a 7-week endurance training program, followed by 1 week of a high CHO diet (18). This rigorously controlled study showed that prolonged exposure to a HFD attenuated some of the training-induced adaptations normally associated with endurance exercise in subjects consuming a mixed diet. This is despite the finding that there were similar rates of muscle glycogen depletion during a submaximal ride to exhaustion after the two different dietary regimens. Helge et al. (18) suggested that the earlier onset of fatigue following the high-fat
diet may be linked to changes in muscle membrane phospholipid composition and to changes in calcium transport in the cytoplasm.

In the present study, CHO loading after the HFD effectively prevented the expected increase in serum FFA concentrations normally found in studies with high-fat, low-CHO diets (21, 27, 30, 37). Therefore, differences in CHO oxidation between trials cannot be explained on the basis of increased FFA availability. However, the HFD-CHO diet may have resulted in an increased utilization of muscle triacylglycerol, as evidenced by increased serum glycerol concentrations. Previous studies in humans have shown an increase in intramuscular triacylglycerol stores following both short-term [5 days, (21)] and long term [≥2 weeks, (19)] consumption of a HFD. Furthermore, Conlee et al. (7) have shown both increased storage and utilization of intramuscular triacylglycerol during exercise in rats following 3–4 weeks of a HFD. This adaptation, where overall fat oxidation is increased due to increased muscle triacylglycerol oxidation, is similar to that seen with adaptation to endurance training (20, 26).

Differences in exercise performance and intermediary metabolism between the present study and previous studies using short-term “glycogen-depletion” regimens, combining several days of a high-fat diet with exhaustive exercise (2, 5, 13, 23, 27, 35), may be attributable, in part, to the metabolic sequelae in response to high fat feeding. Short-term exposure to a high-fat diet (<7 days) results in impaired glucose

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**Figure 4** — Rates of plasma glucose oxidation and direct and/or indirect (via lactate) oxidation of muscle glycogen during constant-load exercise and subsequent 20-km time trial. Direct and/or indirect (via lactate) oxidation of muscle glycogen during exercise was significantly lower in the HFD-CHO trial compared to the CTL-CHO trial ($p < .05$).
tolerance (37), insulin resistance in the liver, and consequent decreased liver glycogen synthesis (24). More recently, Schrauwen et al. (34) have demonstrated that short-term exposure to a high-fat diet increases the oxidation of plasma triglyceride derived fatty acid metabolism.

More prolonged high-fat feeding (≥2 weeks), however, has been shown to increase carnitine-acyl transferase (11, 15) and decrease hexokinase (11) activities. These changes are accompanied by a decrease in insulin-stimulated muscle glucose uptake (24, 32) decreased oxidative glucose disposal by the muscle and a fall in skeletal muscle pyruvate dehydrogenase activity in exercise-trained humans (9).

Although the effects of HFDs on insulin sensitivity and glucose metabolism in rats have been shown to be partially and rapidly reversed by reducing the fat content of the diet for 3 days (16), we found that the area under the insulin curve during 180 min of exercise was significantly higher after the HFD-CHO trial, despite the 3 days of CHO-loading. These results suggest that there was some residual effect of the HFD on insulin sensitivity, which might partially explain the attenuation of endogenous CHO oxidation. The fact that there were no differences in the rates of plasma glucose oxidation between HFD-CHO and CTL-CHO trials provides support that the site of reduced insulin sensitivity in this study was in the muscle and not the liver. Failure to show differences in blood glucose oxidation in the present study contrasts with the findings of Phinney et al. (30). They showed that blood glucose oxidation was three-fold lower after 4 weeks of a ketogenic diet. Such differences may be due to either the CHO-loading regime undertaken by the subjects in the current study or the ingestion of CHO during exercise.

Furthermore, in exercise-trained subjects, 3 weeks of dietary fat adaptation has been shown to enhance insulin-stimulated, non-oxidative glucose disposal at rest, and increase glycogen synthase activity (9). Sherman et al. (35) demonstrated that CHO-loading following a period of CHO-depletion actually enhanced glycogen storage capacity in the skeletal muscle. Although not measured in this study, it is possible that the starting muscle glycogen concentrations were higher after the HFD-CHO than the CTL-CHO dietary regimen, and that this was associated with improved time trial performance.

Figure 5 — 20-km time trial cycling performance following HFD-CHO and CTL-CHO dietary interventions. Time to complete a 20-km time trial was significantly shorter after the HFD-CHO diet compared to the CTL-CHO diet (p < .05).
One of the cautions often raised when studies of the effect of high-fat feeding are undertaken is that this type of dietary regimen is potentially atherogenic (36). However, in the present study, we found no systematic effect of high-fat feeding for 10 days on serum total cholesterol, HDL-cholesterol and triacylglycerol concentrations. Furthermore, we cannot exclude the role of possible motivational factors associated with the diets on exercise performance, despite efforts to introduce the feeding regimes to subjects in a way that would not favor one diet over the other. All future studies of this nature should employ covert manipulation of the nutrient content of the diet to minimize this possible source of bias.

In summary, the results of this study suggest that dietary fat adaptation (10 days, fat > 65% by energy) followed by CHO-loading (3 days, CHO > 65% by energy) is associated with improved cycle time trial performance after prolonged constant-load exercise. These data provide some evidence that “nutritional periodization” may be a useful strategy for prolonged endurance performance, and that high-fat feeding prior to CHO loading may result in persistent adaptations favoring fat oxidation and sparing of endogenous CHO, even during high-intensity exercise.

References


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