Metabolic and appetite responses to prolonged walking under three isoenergetic diets

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1Research Institute for Sport and Exercise Sciences, Liverpool John Moores University, Liverpool L3 2ET; 2University Department of Anaesthesia, University Hospitals of South Manchester, Withington Hospital, Manchester M20 2LR; 3Oxford Lipid Metabolism Group, Radcliffe Infirmary, Oxford OX2 6HE; and 4Department of Physiology and Pharmacology, University of Nottingham Medical School, Nottingham NG7 2UH, United Kingdom

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Ainslie, P. N., K. Abbas, I. T. Campbell, K. N. Frayn, M. Harvie, M. A. Keegan, D. P. M. MacLaren, I. A. Macdonald, K. Paramesh, and T. Reilly. Metabolic and appetite responses to prolonged walking under three isoenergetic diets. J Appl Physiol 92: 2061–2070, 2002. First published December 7, 2001; 10.1152/japplphysiol.01049.2001.—The effects of three isoenergetic diets on metabolic and appetite responses to prolonged intermittent walking were investigated. Eight men undertook three 450-min walks at intensities varying between 25–30 and 50–55% of maximal O2 uptake. In a balanced design, the subjects were given breakfast, snacks, and lunch containing total carbohydrate (CHO), protein (P), and fat (F) in the following amounts (g/70 kg body mass): mixed diet, 302 CHO, 50 P, 84 F; high-CHO diet, 438 CHO, 46 P, 35 F; high-fat diet, 63 CHO, 44 P, 196 F. Substrate balance was calculated by indirect calorimetry over the 450-min exercise period. Blood samples were taken before exercise and every 45 min during the exercise period. The high-fat diet resulted in a negative total CHO balance (−140 ± 1 g) and a lower negative fat balance (−110 ± 33 g) than the other two diets (P < 0.05). Plasma glucagon, nonesterified fatty acids, glycerol, and 3-hydroxybutyrate were higher with the high-fat diet (P < 0.05 vs. high CHO), whereas plasma insulin was lower after high fat (P < 0.05 vs. mixed and high CHO). Subjective ratings of fatigue and appetite showed no differences between the three trials. Although diet influenced the degree of total CHO and fat oxidation, fat was the main source of energy in all trials. Therefore, the present study was designed with three primary aims: 1) to investigate the effect of isoenergetic dietary manipulation on substrate balance and oxidation during prolonged walking, 2) to identify the extent to which these dietary manipulations will alter the metabolic and hormonal milieu, and 3) the extent to which the dietary strategies may affect indicators of performance [heart rate and ratings of fatigue and perceived exertion (RPE)] and perceptions of appetite and satiety.

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Table 1. Total composition of test meals

<table>
<thead>
<tr>
<th></th>
<th>High-Fat Meal</th>
<th>Mixed Meal</th>
<th>High-CHO Meal</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fat</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>g</td>
<td>196</td>
<td>84</td>
<td>35</td>
</tr>
<tr>
<td>% Saturated</td>
<td>40</td>
<td>39</td>
<td>37</td>
</tr>
<tr>
<td>% Energy</td>
<td>81</td>
<td>36</td>
<td>14</td>
</tr>
<tr>
<td><strong>CHO</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>g</td>
<td>63</td>
<td>302</td>
<td>438</td>
</tr>
<tr>
<td>% Simple</td>
<td>48</td>
<td>56</td>
<td>54</td>
</tr>
<tr>
<td>% Energy</td>
<td>11</td>
<td>54</td>
<td>77</td>
</tr>
<tr>
<td><strong>Protein</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>g</td>
<td>44</td>
<td>50</td>
<td>46</td>
</tr>
<tr>
<td>% Energy</td>
<td>8</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td><strong>Total energy, kJ</strong></td>
<td>9,113</td>
<td>8,743</td>
<td>8,963</td>
</tr>
</tbody>
</table>

All meals are based on a 70-kg reference person and were adjusted for each individual according to body mass. Subjects were given a cup of decaffeinated tea or coffee and water with each meal. All the meals had similar proportions of simple sugars relative to total carbohydrate (CHO) and similar saturated-to-unsaturated fatty acid ratio values. For further details about individual constituents for breakfast, snacks, and lunch, see Table 2.

It was hypothesized that, because of the prolonged relatively low-intensity exercise (7 h of walking between 25 and 55% of \( \dot{V}_{O2\ max} \)), the metabolic response is susceptible to dietary manipulations. We further hypothesized that, at this intensity of exercise, a high-fat diet is associated with decreased rating of fatigue and RPE mediated indirectly through the sparing of CHO and an enhanced fat utilization.

METHODS

**Subjects.** Eight moderately trained male subjects participated in this study. All the subjects were active hill walkers with two of the subjects being “club” level runners. Subjects were given both verbal and written instructions outlining the experimental procedure, and written informed consent was obtained. The study was approved by the Ethics Committee of Liverpool John Moores University and the South Manchester Medical Research Ethics Committee. The subjects’ physical characteristics were as follows: age, 26 ± 3 (SD) yr; height, 1.8 ± 0.1 m; body mass, 74 ± 4 kg; body mass index 20 ± 2 kg/m\(^2\); body fat 17 ± 2%; and peak oxygen uptake (\( \dot{V}_{O2\ peak} \)), 60 ± 4 ml·kg\(^{-1}\)·min\(^{-1}\).

**Experimental design.** Subjects made an initial visit to the laboratory for familiarization with the testing equipment. During this visit, percentage of body fat (%fat) was estimated from skinfold thicknesses over the biceps, triceps, subscapular, and suprailiac areas (18). Fitness level was established by using a continuous, incremental treadmill running test to exhaustion (4). A plateau in the \( \dot{V}_{O2\to work} \) relationship was not reached in all subjects; therefore, the highest aerobic power was expressed as \( \dot{V}_{O2\ peak} \) and not \( \dot{V}_{O2\ max} \). Criteria for maximal aerobic performance were forced ventilation, leveling off of \( \dot{V}_{O2\ min} \), respiratory exchange ratio (RER) above 1.15, and RPE of 20 or heart rate at age-predicted maximal values. In a balanced design, the subjects then attended the laboratory on three occasions. All subjects were studied 1 day/wk during 1 calendar month. Subjects completed a 2-day dietary and physical activity diary recorded before each of the three trials, and they were asked to keep their diet and activity the same before each test day. In this way, variations in diet and exercise before the three trials were minimized.

**Protocol.** In a balanced design, subjects were asked to fast from 2000 and then to consume one of the three test diets. The test diets encompassed breakfast, two snacks, and lunch, containing total CHO, protein, and fat in the following amounts, respectively (g/70 kg body mass): mixed diet, 302 CHO, 50 protein, 84 fat; high-CHO diet, 438 CHO, 46 protein, 35 fat; high-fat diet, 63 CHO, 44 protein, 196 fat (Table 1). All diets were isoenergetic, containing 8,940 ± 128 kJ/70 kg body mass, and were of similar appearance. Food was consumed at breakfast 90 min before the exercise, during a 5-min rest at 90 and 355 min into the exercise protocol, and also during a 45-min rest for lunch at 235 min (Fig. 1).

The subjects rested in the laboratory from 0700 to 0730 and then consumed one of the isoenergetic diets at 0730. All subjects consumed breakfast within 20 min. Similarly, the isoenergetic snacks were consumed during a 5-min rest at 90 and 355 min into the exercise protocol and also during a 45-min rest for lunch at 235 min. Lunch was consumed within 25 min, by all subjects. The composition of the breakfast, snacks and lunch is displayed in Table 2.

Thirty minutes after the consumption of breakfast, a retrograde cannula was placed in a large vein draining the hand. The hand was then warmed throughout the study in a heated box to provide arterialized blood (32). The cannula was kept patent with slow saline infusion (0.9% NaCl). Indirect calorimetry was performed by using an on-line automated gas analyzer (Exercise Tester, P. K. Morgan, Chatham, Kent, UK). At 0900, subjects began the intermittent walking protocol consisting of nine 45-min walking stages at either moderate or low intensity. The low- and moderate-intensity walks were calculated to correspond to 25–30 and 50–55%, respectively, of \( \dot{V}_{O2\ peak} \). Subjects completed a moderate-intensity walk followed by a low-intensity walk and then rested for 5 min, during which time an isoenergetic snack was consumed. This was again repeated before another 5-min rest period, with no food intake. One final moderate-intensity walk was completed before a 45-min lunch break. The following exercise consisted of a low- and moderate-intensity walk, a 5-min rest in which an isoenergetic snack was consumed (same as previous), and then by a final low- and moderate-intensity walk (Fig. 1). The total distance walked was 35 km.

Blood samples (10 ml), indirect calorimetry, heart rate (Polar Sports Tester, Polar Electro, Kempele, Finland), and RPE (7) were obtained at rest, between every 35 and 45 min of the nine walking stages, and at the rest break for lunch. Furthermore, ratings of fatigue and various appetite ratings (see Subjective measurements) were recorded before breakfast, immediately after breakfast, during all the rest breaks, before and immediately after lunch, and immediately after exercise.

![Fig. 1. Test protocol for each exercise trial. Subjects consumed water ad libitum. RPE, ratings of perceived exertion; M, moderate-intensity walking at 50–55% maximal oxygen uptake; L, low-intensity walking at 25–30% maximal oxygen uptake. *Administration of appetite questionnaires.](https://jap.physiology.org/doi/10.1152/jappl.2002.92.5.MAY2002.00016)
Design of diets. The constituents for the high-CHO diet were chosen to typify an athlete’s breakfast, comprising cornflakes, puffed rice, skimmed milk, banana, white toast, jam, flavored low-fat yogurt, and orange juice. The snacks included high-CHO products such as raisins and apricots. Lunch comprised bread, jam, banana, flavored low-fat yogurt, and orange juice. The constituents of the high-fat diet were chosen to typify a breakfast cereal, comprising oats, coconut, almonds, raisins, honey, sunflower oil, banana, double cream, and milk. Snacks during the high-fat manipulation comprised products such as coconut and almonds; lunch included bread and cheese sandwiches with additional margarine and ice cream with a small amount (50 ml/70 kg body mass) of long-chain triacylglycerol emulsion drink (Calogen, Scientific Hospital Supplies Group, Liverpool, UK).

The mixed meal incorporated the same isonenergetic nature of the high-CHO and high-fat diets. The macronutrient intake for the mixed diet was within the normative values for the general population (36). All the meals had similar proportions of simple sugars relative to total CHO and similar saturated-to-unsaturated fatty acid ratio values.

Calculation of energy and substrate balances. The percentage contributions of the CHO and fat oxidation were estimated from nonprotein \( \dot{V}
O_2 \) and nonprotein RER data, by using the following formulas: 

\[
\% CHO = \frac{\text{nonprotein } \dot{V}
O_2 \cdot (1 - 0.707)}{(1 - \text{nonprotein RER}) / (1 - 0.707)}
\]

\[
\% fat = 100 - \% \text{CHO}
\]

It was assumed that protein oxidation contributed 12.5% of energy expenditure at rest and that exercise did not alter this relative rate of protein utilization (36). The respiratory quotients of CHO, fat, and protein were taken as 1.00, 0.707, and 0.81, respectively. Oxidation rates (g/min) were estimated for CHO, fat, and protein, respectively, assuming 0.829, 2.019, and 0.966 liter of oxygen was consumed per gram of substrate oxidized (31). Before exercise, during lunch, and during each 45 min of walking, CHO and fat oxidation rates were determined as a mean for a 5-min period from 35 to 40 min into each exercise stage in accordance with the other measurements. From each 45-min block, total oxidation rates were then averaged for the exercise protocol. Energy expenditure was calculated from the averaged \( \dot{V}
O_2 \) and carbon dioxide production from the whole protocol by using the formulas of Elia and Livesey (19).

Before use and at every 2 h, the on-line system was calibrated by using both calibrated gas and ambient air, and the volume transducer was calibrated by using a 3-liter syringe.

Subjective measurements. Various subjective ratings were recorded before breakfast, immediately after breakfast, during all the rest breaks, before and immediately after lunch, and immediately after exercise. During these time points, subjects were asked to complete ratings of “hunger,” “fullness,” “satiety,” “thirst,” “nausea,” “strength of appetite,” “desire to eat,” and “fatigue.” These ratings were assessed by a 100-mm visual analog rating scale labeled from “not at all” to “extremely.” The nature of these rating scales, their manner of use, and their validity in relation to food consumption have been described previously (15, 26).

Analytic methods. Blood samples were drawn into 10-ml heparinized syringes. A portion (20 \( \mu \)l) was used immediately for the measurement of Hb in duplicate (Hemocue B-hemoglobin photometer, Hemocue, Sheffield, UK) and packed cell volume (conventional microhematocrit method). Plasma volume changes were calculated from changes in Hb and packed cell volume relative to initial resting values as described by Dill and Costill (16). From the remaining blood, plasma was separated rapidly at 4°C and frozen for later determination of plasma glucose, nonesterified fatty acids (NEFA) and triacylglycerol (TAG) concentrations by enzymatic methods by using kits (glucose, TAG: Randox Laboratories, Crumlin, UK; NEFA, WAKO, Alpha Laboratories, Eastleigh, UK). In addition, a portion of the plasma was deproteinized with perchloric acid (7% wt/vol) in preparation for plasma glyceraldehyde, lactate, and 3-hydroxybutyrate (3-OHB) determination by enzymatic methods (10). All enzymatic methods were adapted to an IL Monarch centrifugal analyzer (Instrumentation Laboratory, Warrington, UK). Plasma insulin concentrations were determined with a double-antibody radioimmunoassay, and plasma growth hormone concentration was determined by using a two-site immunoradiometric assay (Pharmacia and Upjohn, Milton Keynes, UK). Plasma cortisol concentrations were determined by using a solid-phase radioimmunoassay (Diagnostic Products, Llanberis, Wales, UK), and plasma epinephrine and norepinephrine concentrations were analyzed by using high-performance liquid chromatography with electrochemical detection. Blood for glucagon (DPC, Llanberis, UK) was collected into potassium EDTA-containing tubes with 200 kallikrein-inhibiting units aprotinin/ml blood (Trasylol, Byer PLC, Newbury, UK) and analyzed by using a double-antibody polyclonal antibody precipitation method. All samples for the hormone analysis were frozen according to the instructions of the manufacturers of the kit and then batch analyzed; the inter- and intra-assay coefficient of variation was <10%.

Statistical procedures. Variables are presented as means ± SD. Data were initially tested for normality, before being analyzed by repeated-measures ANOVA. The ANOVA results were corrected by the Huynh-Feldt \( \varepsilon \)-adjusted degrees
differences between the high-fat diet and the high-CHO diet: *Values are means ± SD; n = 8 subjects for all 3 meals. Significant differences between the high-fat diet and the high-CHO diet: *P < 0.05; **P < 0.01; ***P < 0.001. **Significant differences between the high-CHO and the mixed diet, P < 0.05. Significant differences between the high-fat and the mixed diet: *P < 0.05; **P < 0.01; ***P < 0.001.

of freedom when the violation to sphericity was minimal (>0.75), and the Greenhouse-Geisser correction was used when sphericity was violated (<0.75) and significant condition and condition-time interactions were identified (20). To summarize the data not shown graphically, and to obtain post hoc comparisons between the dietary conditions, responses were assessed as total area under the curve over the 450-min protocol. The area under the curve was divided by the total exercise time to give an average value for the 450-min protocol. The area under the curve was divided by the total exercise time to give an average value for the 450-min protocol. The area under the curve was divided by the total exercise time to give an average value for the 450-min protocol. The area under the curve was divided by the total exercise time to give an average value for the 450-min protocol.

RESULTS

Energy intake. Mean values and SDs for the three macronutrients during the 2 days before each trial (expressed as percentage of total energy intake) were 60.6 ± 8.4% CHO, 12.1 ± 4.1% protein, and 27.3 ± 8.1% fat. Mean daily energy intake was 11,146 ± 1,130 kJ/day. There were no significant differences in both the macronutrient and daily energy intake among the three trials. Furthermore, all subjects reported low levels of physical activity before each trial.

Energy expenditure substrate oxidation and balances. For all trials, energy expenditure exceeded energy intake, leading to a marked negative energy balance, which was the same throughout the trials (Fig. 2). In the high-fat trial, RER was significantly lower both before and during exercise compared with the other two diets, reflecting an increase in the proportion of fat oxidized (Fig. 2, Table 3).

When the three diets are compared, fat balance was least negative in the high-fat trial (−110 ± 33 g) and mixed trials (−164 ± 14 g), and most negative in the high-CHO (−158 ± 10 g). In contrast, CHO balance was positive in the high-CHO (42 ± 36 g) and mixed trials (5 ± 36 g) but negative in the high-fat trial (−140 ± 31 g). Furthermore, the high-fat diet resulted in a higher total fat oxidation compared with the CHO diet (306 ± 63 vs. 221 ± 34 g; P < 0.05), whereas the high-CHO diet resulted in an enhanced CHO oxidation compared with the high-fat trial (396 ± 26 g vs. 203 ± 10 g; P < 0.05, respectively (Table 3). However, as shown in Fig. 3, when the total oxidation rates were expressed as a percentage of nonprotein energy expenditure, after the high-CHO diet, CHO and fat oxidation represented 44 ± 17 and 56 ± 15%, respectively, of the non-protein-derived energy expenditure; for the mixed diet oxidation of these substrates were 35 ± 23 and 65 ± 16% of the energy. Finally, in the high-fat diet, CHO and fat oxidation accounted for 23 ± 13 and 77 ± 34% of the non-protein-derived energy expenditure. Taking the observations collectively, the mixed diet showed a metabolic response in between that of the high-CHO and high-fat diets.

Blood glucose and plasma insulin concentrations. Before exercise and during the lunch break after ingestion of the large CHO intakes, blood glucose and plasma insulin concentrations were higher on both the mixed and high-CHO diets compared with the high-fat diet (P < 0.05; Table 4; statistics in Fig. 4). For all trials, the blood glucose concentration showed a gradual decrease in the subsequent 225 min of exercise before lunch. The snack at 90 min did not produce any

Table 3. Substrate oxidation during the 450-min protocol

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Mixed Diet</th>
<th>High-CHO Diet</th>
<th>High-Fat Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHO oxidation, g/min</td>
<td>0.66 ± 0.11</td>
<td>0.88 ± 0.13</td>
<td>0.45 ± 0.09†</td>
</tr>
<tr>
<td>Fat oxidation, g/min</td>
<td>0.55 ± 0.08</td>
<td>0.49 ± 0.06†</td>
<td>0.68 ± 0.10*</td>
</tr>
<tr>
<td>Energy expenditure, kJ/min</td>
<td>30.9 ± 1.9</td>
<td>32.2 ± 3.8</td>
<td>32.4 ± 5.4</td>
</tr>
</tbody>
</table>

Values are means ± SD for 8 subjects for all 3 meals. *Significant differences between the high-fat diet and the high-CHO diet, P < 0.05. †Significant differences between the high-CHO and the mixed diet, P < 0.05. ‡Significant differences between the high-fat and the mixed diet, P < 0.05.
centrations (Fig. 4) were significant before a gradual decline during the exercise protocol. Values are percents ± SD; n = 8 subjects for all 3 meals. There were no significant differences between the 3 diets of the contribution of fat oxidation or CHO oxidation to NP energy expenditure.

Plasma metabolite concentration. Plasma NEFA concentrations (Fig. 4) were significantly greater both before exercise and during exercise with the high-fat diet than with the high-CHO diet (statistics in Fig. 4). Similarly, the NEFA concentrations were higher on the mixed diet than on the high-CHO diet for the majority of the time points except at rest, 360 min, and 450 min (Fig. 4). The higher NEFA concentrations in both the high-fat and mixed diets were reflected in the higher area under the curves (P < 0.05; Table 4). Plasma TAG concentrations were lower at rest and during the first 90 min of exercise in the high-CHO diet compared with the high-fat and mixed diets. After this time point, there were no significant differences in TAG concentrations among the diets. When the area under the curves among the three diets are compared (Table 3), there were trends for lower plasma TAG in the high-CHO trial (P = 0.058), although the differences did not reach statistical significance, probably as a result of the considerable between-subject variability (Fig. 5). The areas under the curve showed higher concentration of 3-OHB in both the mixed and the high-fat diets compared with that of the high-CHO diet (Table 3). Apart from at rest and at 45 min, these higher concentrations of 3-OHB are reflected throughout the 450 min of exercise (statistics in Fig. 5). Similarly, the areas under the curve showed higher concentrations of plasma glycerol in the high-fat diet compared with the high-CHO diet (P < 0.05), despite the relatively large between-subject variability (Table 3). The higher concentrations of glycerol in the high-fat diet were evident at 90, 135, 180, 315, 405, and 450 min (Fig. 5).

Plasma hormone concentrations. The high-fat diet resulted in a significantly higher area under the curve for glucagon concentration compared with the high-CHO diet, although a large within-subject variability was evident (Table 3). The increases in glucagon concentrations were most apparent before exercise, at 225 min, and during the last 180 min of exercise (statistics in Fig. 4). Epinephrine, growth hormone, and cortisol showed significant changes over time (statistics in Fig. 6), with no differences between the different diets (Table 3, Fig. 6). This interaction was most pronounced at the sample just before lunch at 225 min, where a surge in the hormones was evident in a marked stress response (statistics in Fig. 6). Plasma cortisol concentrations remained similar throughout the exercise protocol, exhibiting a normal circadian variation in concentrations, with, as mentioned, a significant surge on all diets before the rest for lunch (Fig. 6).

Heart rate and RPE. Heart rate and RPE increased significantly above preexercise values for all trials. Both the heart rate and RPE values were significantly higher during the high-intensity walking compared with low intensity. Despite the change in heart rate and RPE in accordance with the exercise intensities, there were no significant differences observed among the three trials at any point (data not shown).

Subjective measurements. Although there were significant effects of time (P < 0.001) on ratings of hunger, fullness, and satiety over the protocol, there was no significant effect of meal type among the three conditions. Similarly, although there was a gradual increase in ratings of fatigue throughout the exercise, differences were not significant (Fig. 7). Furthermore, there were no differences in ratings of thirst, nausea, strength of appetite, or desire to eat (data not shown) between the experimental trials.

Table 4. Areas under the curve for metabolite and hormonal data

<table>
<thead>
<tr>
<th>Metabolites</th>
<th>Mixed Diet</th>
<th>High-CHO Diet</th>
<th>High-Fat Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>NEFA, µmol/l</td>
<td>540 ± 86</td>
<td>265 ± 57†‡</td>
<td>935 ± 137†‡§</td>
</tr>
<tr>
<td>Glycerol, µmol/l</td>
<td>147 ± 40</td>
<td>125 ± 24</td>
<td>182 ± 49*</td>
</tr>
<tr>
<td>TAG, µmol/l</td>
<td>1,316 ± 789</td>
<td>920 ± 340</td>
<td>1,328 ± 327</td>
</tr>
<tr>
<td>3-OHB, mmol/l</td>
<td>99 ± 4</td>
<td>125 ± 62</td>
<td>234 ± 100*</td>
</tr>
<tr>
<td>Lactate, mmol/l</td>
<td>0.6 ± 0.1</td>
<td>0.8 ± 0.1</td>
<td>0.5 ± 0.1</td>
</tr>
<tr>
<td>Glucose, mmol/l</td>
<td>5.1 ± 0.3</td>
<td>5.0 ± 0.3</td>
<td>4.6 ± 0.2*§</td>
</tr>
<tr>
<td>Hormones</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GH, ng/ml</td>
<td>2.3 ± 1.4</td>
<td>2.9 ± 2.5</td>
<td>3.3 ± 1.9</td>
</tr>
<tr>
<td>Glucagon, pg/ml</td>
<td>141 ± 27</td>
<td>125 ± 28</td>
<td>200 ± 60*</td>
</tr>
<tr>
<td>Insulin, mIU/l</td>
<td>9.1 ± 4.5</td>
<td>9.2 ± 1.9</td>
<td>3.1 ± 1.0*</td>
</tr>
<tr>
<td>Cortisol, nmol/l</td>
<td>360 ± 54</td>
<td>330 ± 65</td>
<td>342 ± 66</td>
</tr>
<tr>
<td>Norepinephrine, nmol/l</td>
<td>3.7 ± 1.2</td>
<td>3.8 ± 1.8</td>
<td>3.5 ± 1.4</td>
</tr>
<tr>
<td>Epinephrine, nmol/l</td>
<td>0.5 ± 0.6</td>
<td>0.5 ± 0.2</td>
<td>1.2 ± 1.7</td>
</tr>
</tbody>
</table>

Values are means ± SD for 8 subjects for all 3 meals. Areas under the curve were divided by time baseline to represent average value over the respective 450-min protocol. NEFA, nonesterified fatty acids; TAG, triacylglycerol; 3-OHB, 3-hydroxybutyrate; GH, growth hormone. †Significant differences between the high-fat diet and the high-CHO diet, P < 0.05. ‡Significant differences between the high-CHO diet and the mixed diet, P < 0.05. §Significant differences between the high-fat and the mixed diet, P < 0.05.
DISCUSSION

The present study has yielded two important findings. First, the metabolic responses were, to an extent, susceptible to dietary manipulations. After each diet, although the total fat and CHO oxidation corresponded to the amount of each substrate administered, the main source of energy in all trials was fat oxidation. However, the dietary manipulation did significantly alter the metabolic and hormonal milieu. Second, the absence of any change in heart rate, RPE, or subjective ratings of fatigue between the dietary manipulations during prolonged exercise is an important, rather than an uninteresting, observation. This suggests that dietary composition will not adversely affect physiological and subjective factors over 1 day. However, the high-fat diet resulted in a negative CHO balance over the exercise period. In accordance with previous studies that have involved higher intensity exercise (11, 49), high-fat diets might not be so good for further exercise, even at low to moderate intensities. Decreases in the glycogen stores (11) and/or muscle TAG concentrations (49), especially if continued over a few days of walking, would be detrimental to the ability to sustain the activity.

Substrate oxidation and balances. In resting conditions, there is a clear hierarchy in the maintenance of macronutrient balances, with CHO and protein having the highest priority (1, 45). Fat oxidation, on the other hand, is only marginally influenced by fat intake during resting conditions. Prolonged exercise will generally lead to a negative energy balance because of difficulties in matching sufficient energy intake to the high-energy turnover as a consequence of the exercise. Because fat oxidation is determined mainly by the difference between energy expenditure and CHO and protein oxidation, fat balance is strongly correlated with energy balance (45). The relationship between fat oxidation and energy balance becomes apparent when the substrate balances are considered, i.e., the amounts of substrates ingested minus the amounts oxidized (Fig. 2). Although there were no differences in the negative energy balance among the three trials, the CHO balance was more negative on the CHO diet than in the mixed and fat trials. This highlights the fact that, despite the negative fat balance in all trials, the CHO intake can, to a certain extent, decrease the amount of fat oxidized. However, the failure of the dietary CHO to promote CHO oxidation to the extent shown in resting studies (6, 56) is most probably both a consequence of the large negative energy balance (45) and a result of the hormonal and metabolic state that favors fat oxidation at low to moderate intensities (27, 28). These results suggest that high amounts of dietary CHO during prolonged walking decrease the contribution of fat oxidation but only to a limited extent.

The high-fat diet led to an increased fat oxidation that reduced the magnitude of the negative fat balance but produced a greater negative CHO balance. We believe this to be important, because the high-fat diet was low in CHO, suggesting some use of muscle and or liver glycogen stores. Previous studies have suggested that glycogen stores are important in the rate at which fat oxidation is adapted to fat intake (42–44). Because fat oxidation does not adapt rapidly to the increased fat intake with a high-fat diet, subjects will be in a negative CHO balance. This means that high-fat diets, in the present study, will lead to a reduction in glycogen.

Fig. 4. Plasma insulin (A), glucose (C), glucagon (B), and nonesterified fatty acid (NEFA; D) concentrations during the 450-min exercise protocol after the 3 different diets. Values are means ± SD; n = 8 subjects for all 3 meals. Significant differences between the high-fat diet and the high-CHO diet: *P < 0.05; ***P < 0.001. Significant differences between the high-CHO and the mixed diet: #P < 0.05; ##P < 0.01; ###P < 0.001. Significant differences between the high-fat and the mixed diet: $P < 0.05; $$P < 0.01; $$$P < 0.001. #F, #M, #C, significant change over time in the high-fat, mixed, and high-CHO diets, respectively (P < 0.05).
stores, and an increase in fat oxidation (21). This question the benefit of high-fat strategies during prolonged exercise, which may entail high-energy deficits in relation to the high-fat strategy. For example, high-fat diets may actually promote glycogen utilization as opposed to glycogen sparing, potentially leading to early fatigue.

Previous exercise studies in which subjects were fed isoenergetic diets (55) or Intralipid and heparin infusion (39) have shown that, despite marked alterations in substrate availability in plasma, the pattern of substrate oxidation during exercise is remarkably resistant to alteration by dietary means. These contrast with the results of the present study where the relative rate of fat and CHO oxidation varied with the different diets. The disparity of results is most likely explained by the differing diets and differing intensities used, which would affect the substrate oxidative response (13). For example, a number of the studies have used isoenergetic diets that have entailed a lower fat intake (expressed as percentage of total energy intake) and higher exercise intensities (8, 9, 55), both of which would decrease the substrate oxidative response (13).

There was an enhanced fat oxidation during the high-fat trial. Recent studies have suggested that the increase in fat oxidation after a high-fat diet can be accounted for by both adipose derived fatty acids oxidation and from TAG-derived fatty acid oxidation (very-low-density lipoproteins and/or intramuscular TAG) (45, 47, 51). However, the relative contribution of plasma TAG to energy production during exercise remains unclear (27). Because fat ingestion acutely increases plasma TAG, as demonstrated in the present study, quantifying the contribution of this energy source during exercise will resolve whether fat inges-

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Fig. 5. Triacylglycerol (TAG; A), 3-hydroxybutyrate (3-OHB; B), and glycerol (C) concentrations during the 450-min exercise protocol after the 3 different diets. Values are means ± SD; n = 8 subjects for all 3 meals. Significant differences between the high-fat diet and the high-CHO diet: *P < 0.05; **P < 0.01; ***P < 0.001. Significant differences between the high-CHO and the mixed diet: +P < 0.05; ++P < 0.01; +++P < 0.001. Significant differences between the high-fat and the mixed diet: #P < 0.05; ##P < 0.01; ###P < 0.001. #F, #M, #C, significant change over time in the high-fat, mixed, and high-CHO diets, respectively (P < 0.05).

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Fig. 6. Growth hormone (A), cortisol (B), and epinephrine (C) concentrations during the 450-min exercise protocol after the 3 different diets. Values are means ± SD; n = 8 subjects for all 3 meals. Repeated-measures ANOVA for all hormones showed significant effects of time (P < 0.05) but not meal type. #F, #M, #C, significant change over time, at the 225-min time point, in the high-fat, mixed, and high-CHO diets, respectively (P < 0.05). Arrow denotes surge in the stress hormones before the 45-min rest for lunch.
tion can contribute substantially to energy metabolism during exercise.

The greater increase in fat utilization is not, apparently, without its limitations. The oxygen requirement for the oxidation of fat can be up to 16% greater than that required to produce the same amount of ATP from the oxidation of CHO. One liter of oxygen can oxidize glycogen and produce $6.5 \text{ mol of ATP}$ compared with $5.6 \text{ mol}$ when palmitate is oxidized (5). Consequently, a change toward fat oxidation should produce a higher cardiovascular stress (34, 46). However, in previous studies in which the plasma NEFA has been elevated acutely by either a high-fat meal or by Intralipid-heparin infusion, no effect on $V_{\text{O2}}$ or heart rate during exercise has been reported (23, 35, 53). In agreement with those findings, the present study showed no differences in $V_{\text{O2}}$, heart rate, or RPE between the three trials.

**Metabolic and hormonal responses.** The present study confirms the well-established observation that meals both preexercise and during exercise can profoundly affect the pattern of substrate availability in the plasma. In the present study, a high-CHO meal (438 g CHO and 35 g fat) that included breakfast, snacks, and lunch resulted in a significant suppression of plasma NEFA, 3-OHB, and glyceral during the 450-min exercise protocol compared with an enhanced plasma NEFA, 3-OHB, and glyceral concentration after an isonenergetic high-fat meal (63 g CHO and 196 g fat) and isonenergetic mixed meal (302 g CHO and 84 g fat). This is similar to previous work by Coyle et al. (14) and Montain et al. (33), who reported a suppression of plasma NEFA concentrations during submaximal exercise in endurance-trained cyclists after ingestion of CHO loads several hours before exercise. These observations highlight the potential for manipulating fatty acid supply by dietary means.

It was notable, however, that there was a marked stress response as shown by marked elevations in the concentrations of epinephrine, cortisol, and growth hormone just before the lunch break. This occurred in all three dietary conditions (Fig. 6), and the point coincided with the longest period without ingestion of food while the subjects were still walking. This suggests that snacks, in combination with the breakfast and lunch, may confer some protection against a marked increase in stress hormones. Apart from the spike just before lunch, plasma cortisol showed an actual decline throughout the prolonged exercise protocol, probably as a result of increased clearance (48) and the circadian variation (54).

**Subjective measurements.** To our knowledge, the effects of dietary manipulation during prolonged exercise on hunger responses have not been quantified previously. However, short-term studies that have sought to measure satiating efficiency have produced equivocal results. Indirect evidence from controlled weight loss studies have suggested diets high in CHO suppress appetite and subsequent energy intake (17, 50, 25). The suggested mechanism(s) for this suppression of appetite was that of insulin, which exerts this anorexic effect (25). In the present study, a significant decrease in circulating insulin levels in the high-fat trial was evident, suggesting that, during prolonged exercise, insulin plays no role in modulating any of the subjective ratings of hunger, satiety, or fullness. However, some other resting studies appear to have demonstrated that fat has a satiating action equivalent to CHO (22, 40, 52). In the present study, despite the differing rates of digestion and absorption of CHO and fat, we did not detect any differences in ratings of appetite during the protocol. Further research into prolonged exercise and appetite is clearly warranted.

The results from this study clearly show that the differing dietary manipulations resulted in a similar energy deficient. The similar negative energy balance suggests that a wide range of dietary patterns may be acceptable for those trying to lose weight by incorporating moderate-intensity exercise into their routine.
Prolonged walking may be considered a useful adjunct in a weight loss program.

In summary, the availability of fatty acids, and of other substrates, and the pattern of substrate oxidation and balance, during prolonged walking are altered by dietary means. The main source of energy in all trials was predominantly fat oxidation, although diet influenced the degree of total CHO and fat oxidation. These results emphasize that the close relationship between fat and CHO metabolism after isoenergetic meals can be somewhat displaced, most probably because of the prolonged low to moderate intensity of the exercise and subsequent negative energy balance. In accordance with previous studies that have involved more high-intensity exercise (11, 49), high-fat diets might not be so good for further exercise even at low to moderate intensities. Decreases in the glycogen stores (11) and/or muscle TAG concentrations (49), especially if continued over a few days of walking, would be detrimental to the ability to sustain the activity.

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