Partial restoration of dietary fat induced metabolic adaptations to training by 7 days of carbohydrate diet

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Abstract

The aim of the present study was to investigate whether glucose uptake may be attenuated under such circumstances.

In a recent study, Burke and colleagues (5) demonstrated that 5 days of fat adaptation followed by 1 day of carbohydrate-rich diet resulted in muscle glycogen sparing and a higher fat oxidation during submaximal exercise compared with subjects continuously consuming a carbohydrate-rich diet. In contrast, neither a higher fat oxidation nor a difference in muscle glycogen breakdown rate was observed in our laboratory’s previous study after a dietary switch to a carbohydrate-rich diet for 1 wk after 7 wk on a fat-rich diet, compared with subjects consuming a carbohydrate-rich diet all along (16). Still, because higher preexercise muscle glycogen stores in the group switching from fat to carbohydrate diet by itself would be expected to increase muscle glycogen breakdown (15), it could be argued that identical muscle glycogen breakdown rates in the two groups in fact represent a relative impairment of breakdown after the dietary switch. However, in neither of these latter studies was a detailed characterization of muscle metabolism during exercise made.

The aim of the present study was to investigate substrate metabolism after prolonged adaptation to...
training and a fat-rich diet followed by a carbohydrate-rich diet and to compare muscle metabolism to that observed when a carbohydrate-rich diet was taken throughout the period.

METHODS AND MATERIALS

Subjects. Thirteen healthy, untrained male subjects, age 27 ± 1 yr, height 182 ± 2 cm, weight 87 ± 3 kg and maximal oxygen uptake 3.9 ± 1 liters O2/min participated in the study. The fiber type composition in the vastus lateralis muscle was 55 ± 3% type I, 34 ± 2% type IIA, and 11 ± 2% type IIX fibers (type IIB fibers of old nomenclature). Subjects were fully informed of the nature and the possible risks associated with the study before they volunteered to participate. The study was approved by the Copenhagen Ethics Committee, and all subjects gave written consent.

Design. The experiment was a longitudinal diet-training intervention study. Initially, subjects were randomized into two groups. Over the first 7 wk, one group consumed a fat-rich diet and the other group a carbohydrate-rich diet (see below for details). During the last week, both groups consumed a carbohydrate-rich diet. Through the remainder of this paper, the group that switched diet will be referred to as Fat-Carbohydrate (Fat-CHO in tables and figures) and the other group as Carbohydrate (CHO in tables and figures). After 8 wk of the diet and training regimen, substrate metabolism was investigated in a 60-min exercise bout performed at ~70% of maximal oxygen uptake on a modified Krog bicycle ergometer. Over the experimental period, the subject's maximal oxygen uptake was determined before diet change or training and after 3.5 and 6.5 wk of the experiment. An exercise test was also performed after 7 wk, the results of which have been published separately (17).

Experimental diets. The experimental diets are similar to those applied and described in our laboratory’s previous studies (16). In brief, the energy composition of the fat-rich diet was 21% carbohydrate, 17% protein, and 62% fat, and the carbohydrate-rich diet was 65% carbohydrate, 15% protein, and 20% fat. Thus the diets had a markedly different fat and carbohydrate content but a similar protein content. The habitual diet and energy intake were determined from 4-day diet records, and, in addition, individual energy intakes were estimated from the World Health Organization’s equation for calculation of energy needs (34). On the days of training, the calculated energy expenditure during training was added to the daily energy intake and consumed immediately after training. During the experimental period all food intake was strictly controlled and weighed to within 1 g. The subjects weighed themselves every morning, and the individual dietary energy intake was adjusted such that body weight changes were minimized.

Materials. [1-13C]Palmitate (99% enriched) and NaH13CO3 were purchased from Tracer Technologies (Newton, MA). The palmitic acid tracer in solution was added to methanolic potassium hydroxide to form the potassium salt, which was then dried under nitrogen, redissolved in sterile water, and passed through a 0.22-μm sterile filter. It was then mixed with sterile 20% (wt/vol) human albumin (State Serum Institute, Copenhagen, Denmark), to which it was bound.

Experimental protocol. Subjects were asked to refrain from physical activity 2 days before the start of the studies. The subjects reported to the laboratory in the morning after a 12-h fast after traveling to the laboratories either by car or bus. After subjects spent 30 min in a supine position, a needle biopsy was taken with suction from the vastus lateralis muscle by using local anesthesia with 5 ml of 1% lidocaine (3). After this, the training and diet regimen was begun. Over 8 wk, both dietary groups followed an identical, supervised training program using a bicycle ergometer. During the whole period, physical training was performed four times a week. Each training session lasted between 60 and 75 min, and exercise intensity, which was carefully controlled, varied from 50 to 85% of maximal oxygen uptake. The training program consisted of four different protocols with exercise intervals varying in length and duration interspersed with breaks of active recovery. Training intensity was adjusted to changes in maximal oxygen uptake measured after 3.5 wk of the training period. At every training session, heart rate was monitored; pulmonary oxygen uptake was measured frequently. Thus the training regime could be monitored and adapted as required.

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PE 3000 Sports Tester (Polar Electro). Water intake during exercise was standardized with subjects drinking 200 ml of water every 20 min. Immediately after 60 min of exercise, a further biopsy was taken from the vastus lateralis muscle of the opposite leg through a new incision, suitably anesthetized.

Analyses. Fatty acids (FA) were extracted from plasma, isolated by thin-layer chromatography, and converted to their methyl esters. The arterial and venous isotopic enrichment of plasma $[^{13}C]_{\text{palmitate}}$ was determined by gas chromatography-mass spectrometry (GC-MS, INCOS XL, Finnigan Mat, Hemel Hempstead, UK) by selected ion monitoring of ions at mass-to-charge ratio of 270 and 271. Heptadecanoate (C17) was used as an internal standard for quantification of total palmitate. Enrichment of $[^{13}C]_{\text{CO2}}$ in expired air was analyzed by isotope ratio-mass spectrometry (Europa Scientific 2020 IRMS) as previously described (28). The concentration of isotope in the infusate was determined, so that the exact infusion rate could be calculated.

Blood glucose and lactate were analyzed on a glucose and lactate analyzer (Yellow Springs Instruments, Yellow Springs, OH). Plasma glycerol was analyzed as described by Wieland (33). Total plasma FA was measured fluorometrically as described by Kiens et al. (19). VLDL-TG in serum was isolated by ultracentrifugation at a density of 1.006 g/cm³ and then analyzed as described by Kiens et al. (20). Insulin in arterial plasma was determined by use of a radioimmunoassay kit (Insulin RIA100, Pharmacia, Sweden), and catecholamines in arterial plasma were determined by a radio enzymatic procedure (8). Blood oxygen saturation was measured on an OSM-2 hemoximeter (Radiometer Copenhagen). Hemoglobin was determined spectrophotometrically on the Astrup technique (ABL 30, Radiometer, Copenhagen, Denmark). Hematocrit was determined in triplicate from microcapillary tubes.

The biopsies were frozen in liquid nitrogen within 10–15 s of sampling. Before freezing, a section of the samples was cut off, mounted in embedding medium, and frozen in isopentane cooled to its freezing point in liquid nitrogen. Both parts of the biopsy were stored at −80°C until further analysis. Before biochemical analysis, muscle biopsy samples were freeze-dried and dissected free of connective tissue, visible fat, and blood with the use of a stereomicroscope. Muscle glycogen concentration was determined as glucose residues after hydrolysis of the muscle sample in 1 M HCl at 100°C for 2 h (22). Muscle triacylglycerol was determined as previously described (21). In brief, 20 mg wet wt of muscle tissue were freeze-dried and dissected free of all visible adipose tissue, connective tissue, and blood by the use of a stereomicroscope, leaving the muscle fibers for further analysis. The muscle fibers were mixed, and ~1 mg dry wt of the pooled fibers was used for measurement of the intramyocellular triacylglycerol concentration. Glycogen from the degraded triacylglycerol was assayed fluorometrically as described previously (21). Serial transverse muscle sections were stained for myofibrillar ATPase to identify fiber type composition (4). Total muscle GLUT-4 protein content was assayed by Western blotting using a primary antibody against the 13 COOH-terminal amino acids of GLUT-4.

Whole body oxygen uptake and carbon dioxide excretion at rest and during exercise were determined by collection of expired air in Douglas bags. The volume of air was measured in a Collins bell-spirometer (Tissot principle, Collins W.E., Braintree, MA), and the fractions of oxygen and carbon dioxide were determined with paramagnetic (Servomex) and infrared (Beckmann LB-2) systems, respectively. Gases of known composition were used to calibrate each system regularly.

Calculations. Uptake and release of substrates and metabolites across the leg were calculated from femoral arterial and venous differences multiplied by plasma or blood flow, according to Fick’s principle. Indirect calorimetry calculations were performed according to the stoichiometric equations given by Frayn (12). Substrate balance across the leg was calculated using an active muscle mass of 4.6 kg for each leg, which was estimated as the difference between total carbohydrate oxidation and measured glucose uptake and lactate release divided by the measured muscle glycogen breakdown. To assess the contribution of protein oxidation to exercise, a nitrogen excretion rate of 135 μg·kg⁻¹·min⁻¹ was used, as described by Romijn et al. (27). FA oxidation was determined by converting the rate of triacylglycerol oxidation (in g·kg⁻¹·min⁻¹) to its molar equivalent, with an average molecular weight of triacylglycerol assumed to be 860 g/mol (12).

The calculations of rate of appearance (Ra) and disappearance (Rd), were performed by the use of Stele’s non-steady-state equations (31) modified for the use of stable isotope tracer infusion (9, 27).

\[
Ra = \frac{F - V}{(C_{\text{palm art}}) - (C_{\text{palm vein}})} \left( 1 - \frac{t_2 - t_1}{t_2 - t_1} \right)
\]

\[
Rd = Ra - V (C_{\text{palm art}} - C_{\text{palm vein}}) \frac{t_2 - t_1}{t_2 - t_1}
\]

where F is infusion rate, \( \frac{13CO_2}{H_2O} \) is the content of $^{13}$CO$_2$ in the breath, V the effective volume of distribution (40 ml/kg), \( C_{\text{palm art}} \) and $C_{\text{palm vein}}$ are the concentrations of plasma palmitate at time $t_1$ and $t_2$, and $E_{\text{art}}$ and $E_{\text{vein}}$ are the palmitate enrichments, respectively. The Ra of FAs was determined as the product of the fractional contribution of palmitate to total FA concentration and the Ra palmitate. The percentage of the tracer infused that was oxidized was calculated as the

\[
\%\text{Tracer oxidized} = \frac{\text{FCO}_2 \times (C^{-1} \times (F^{-1}) \times 100}{C}
\]

where C is the acetate correction factor (C = 0.9) as reported by Sidossis and colleagues (29). In the present experiment, it was not ethically feasible to do acetate correction trials for each individual; thus we choose to apply an exercise correction at 0.9 as described and utilized previously (29).

The plasma FA oxidation was determined as

\[
\text{Plasma FA oxidation} = Rd \times \% \text{tracer oxidized}
\]

The estimated oxidation of FAs originating from sources other than plasma FA was calculated as

\[
\text{Oxidation of nonplasma FA} = \frac{\text{Rd}}{\% \text{ tracer oxidized}} - \text{plasma FA oxidation}
\]

Across-the-leg extraction can be calculated as

\[
\text{Fractional extraction} = \frac{[C_{\text{palm art}} \times E_{\text{palm art}} - C_{\text{palm vein}} \times E_{\text{palm vein}}]}{C_{\text{palm art}} \times E_{\text{palm art}}}
\]

The calculated tracer-derived fractional extraction was used to calculate actual tracer determined FA uptake over the leg as the measured arterial FA delivery multiplied by the fractional extraction.

Statistics. Results are given as means ± SE. Two-way ANOVA with repeated measures for the time factor was
performed to test for changes due to diet and/or time. In the case of significant main effects or interactions, a Student-Newman-Keuls post hoc test was performed to discern statistical differences. In all cases, an α of 0.05 was taken as the level of significance.

RESULTS

The habitual dietary energy and nutrient intake were similar in the two groups (Table 1). Over the experimental period, energy intake was also similar in the two groups and significantly higher than the habitual daily energy intake. Throughout the 8 wk, the subjects adhered to the prescribed dietary intake both during the first 7 wk (17) and during the 8th week, as evidenced by the close resemblance between the actual intakes and the prescribed dietary contents (Table 1). After the switch from a high-fat to a high-carbohydrate diet, the Fat-Carbohydrate group increased (P < 0.05) their daily intake of carbohydrates (in g) by 221% and vice versa decreased (P < 0.05) their fat intake by 65%. Protein intake was slightly lower (P < 0.05) in the Fat-Carbohydrate group than in the Carbohydrate group. Over the experimental period body weight decreased (P < 0.05) similarly in both groups from 87.4 ± 2.9 to 86.1 ± 2.9 kg.

Subjects trained under supervision a total of 31 ± 1 times. Before the experimental period, maximal oxygen uptake was similar in the two groups at 3.8 ± 0.1 and 4.1 ± 0.2 l/min, and after the training period it was similarly increased (P < 0.05) to 4.1 ± 0.1 and 4.4 ± 0.2 l/min in the Fat-Carbohydrate group and in the Carbohydrate group, respectively. After 8 wk, subjects exercised at an oxygen uptake of 2.9 ± 0.1 l/min, which was equivalent to a workload of 72 ± 3 and 70 ± 2% of postraining maximal oxygen uptake in the Fat-Carbohydrate group and in the Carbohydrate group, respectively. During exercise, respiratory exchange ratio (RER) values remained constant throughout the exercise period in both Fat-Carbohydrate (0.94 ± 0.01) and Carbohydrate (0.92 ± 0.02) groups, and RER was similar between the groups. During exercise, whole body fat oxidation (Table 2) and carbohydrate oxidation (175 ± 4 and 186 ± 5 μmol·min⁻¹·kg⁻¹ in the Fat-Carbohydrate and the Carbohydrate group, respectively) were similar between groups.

**Blood samples.** Resting leg blood flow was similar in the two groups: 0.29 ± 0.04 and 0.36 ± 0.04 liters blood/min in the Fat-Carbohydrate group and in the Carbohydrate group, respectively. The blood flow increased (P < 0.05) similarly during the first 15 min of
exercise to 6.3 ± 0.3 and 6.0 ± 0.2 liters blood/min in the Fat-Carbohydrate and Carbohydrate group, respectively, after which no further increases were observed. In the Fat-Carbohydrate group, arterial blood glucose concentrations increased \((P < 0.05)\) progressively and continuously through exercise, whereas in the Carbohydrate group the arterial glucose increased \((P < 0.05)\) until 30 min, after which it decreased \((P < 0.05)\) at the termination of exercise down to the initial value (Fig. 1A). After 60 min, arterial glucose concentration was borderline significantly higher \((P < 0.07)\) in the Fat-Carbohydrate group than in the Carbohydrate group. Arterial blood lactate concentrations increased \((P < 0.05)\) similarly from rest to 15 min to 2.3 ± 0.4 mmol/l in both groups, and no further changes were observed. Throughout the exercise, lactate release was similar between the groups, and, after an initial increase to 0.72 ± 0.24 mmol/min after 15 min \((P < 0.05)\), a continuous decrease \((P < 0.05)\) was observed across the rest of the exercise bout to 0.30 ± 0.11 mmol/min.

Arterial plasma FA concentrations were not significantly different during exercise in the two groups (Fig. 2A). After 15 min of exercise, arterial plasma FA concentrations were at a nadir, after which a continuous increase \((P < 0.05)\) was observed until the end of exercise in both groups (Fig. 2A). During the 60 min of exercise, FA delivery was not significantly different between groups, averaging 1.18 ± 0.24 and 1.52 ± 0.22 mmol/min in the Fat-Carbohydrate group and the Car-
bohydrate group, respectively. During exercise, plasma net FA uptake across the leg was lower ($P < 0.05$) in the Fat-Carbohydrate group than in the Carbohydrate group, averaging $99 \pm 24$ and $166 \pm 28$ mmol/min, respectively. The FA clearance was not significantly different between groups and was on average $0.24 \pm 0.07$ l/min in the Fat-Carbohydrate group and $0.34 \pm 0.04$ l/min in the Carbohydrate group during exercise. During the first 30 min of exercise, the average arterial serum VLDL-TG concentration was $1.06 \pm 0.08$ and $0.76 \pm 0.15$ mmol/l in the Fat-Carbohydrate and Carbohydrate groups, respectively, and a significant decrease, to $0.96 \pm 0.13$ and $0.70 \pm 0.29$ mmol/l for Fat-Carbohydrate and Carbohydrate, respectively, was observed at 60 min. No measurable VLDL-TG uptake was observed across the leg during the exercise, $-0.02 \pm 0.07$ and $-0.02 \pm 0.06$ mmol/min in the Fat-Carbohydrate group and in the Carbohydrate group, respectively. The arterial plasma glycerol concentration was similar in the two groups and increased ($P < 0.05$) continuously from $40 \pm 4$ mmol/l at rest to $136 \pm 20$ mmol/l at the end of exercise. During exercise, plasma glycerol release across the leg was similar between groups and increased ($P < 0.05$) from rest $17 \pm 2$ to $84 \pm 23$ mmol/min after 15 min. After 30 min of exercise, a small decrease ($P < 0.05$) was observed to $31 \pm 17$ mmol/min, whereafter plasma glycerol release was not further changed.

The arterial epinephrine concentration increased ($P < 0.05$) similarly from $0.71 \pm 0.21$ and $0.52 \pm 0.09$ nmol/l at rest to $1.79 \pm 0.21$ and $1.93 \pm 0.41$ nmol/l after 60 min in the Fat-Carbohydrate group and in the Carbohydrate group, respectively. Likewise, the arterial norepinephrine concentration increased ($P < 0.05$) similarly from $4.2 \pm 1.6$ and $3.7 \pm 1.5$ nmol/l at rest to $14.4 \pm 1.3$ and $15.8 \pm 2.3$ nmol/l after 60 min of exercise in Fat-Carbohydrate and Carbohydrate, respectively. The arterial plasma insulin concentration decreased ($P < 0.05$) from $10.7 \pm 3.7$ and $6.2 \pm 1.1$ μU/ml at rest to $6.8 \pm 1.3$ and $3.5 \pm 0.4$ μU/ml after 15 min of exercise in Fat-Carbohydrate and Carbohydrate, respectively, after which no further changes were observed. At rest, the plasma insulin concentration was significantly higher in the Fat-Carbohydrate group than in the Carbohydrate group; however, during exercise no significant difference between groups was discernable.

**Muscle samples.** Muscle glycogen concentration before training was similar in the two groups (Table 3). After 7 wk, the glycogen concentration was unchanged after fat diet adaptation and significantly lower ($P < 0.05$) than after 7 wk of carbohydrate diet, whereas after the 7-wk carbohydrate diet the glycogen concentration was increased ($P < 0.05$) by $46\%$ compared with the initial values (reported in Ref. 17). After 8 wk, the glycogen concentration was significantly higher ($P < 0.05$) in the Fat-Carbohydrate group than in the Carbohydrate group and higher ($P < 0.05$) than the initial values (Table 3). In the Carbohydrate group, glycogen storage remained at the level observed after 7 wk. Muscle glycogen breakdown was similar in the Fat-Carbohydrate group and in the Carbohydrate group across the 60 min of exercise (Table 3). After 8 wk, muscle triacylglycerol concentrations were similar to the initial values in both groups, and in the Fat-Carbohydrate group the values were significantly lower ($P < 0.05$) than those reported after 7 wk (reported in Ref. 17). This finding clearly implies that muscle triacylglycerol stores are prone to rather large fluctuations when nutrient composition and physical activity are markedly altered. The total muscle GLUT-4 protein content was significantly increased ($P < 0.05$) with training after 7 wk in both groups (Table 3). After 8 wk, total muscle GLUT-4 protein content was not further changed from the 7-wk values.

**Stable isotopes and substrate kinetics.** The enrichment of plasma $[^{13}$C$]$palmitate to $[^{12}$C$]$palmitate, the tracer-to-trace ratio, decreased ($P < 0.05$) from rest to 15 min into the exercise bout, after which a stable plateau was maintained until the termination of exercise (Table 2). During exercise, the arterial palmitate enrichment was similar between groups, and, as expected, the arterial enrichments were higher ($P < 0.05$) than the venous enrichments during exercise. This resulted in an average fractional extraction over the exercise calculated to $28 \pm 14$ and $22 \pm 3\%$ and a net extraction of $9 \pm 2$ and $10 \pm 1\%$ in the Fat-Carbohydrate group and the Carbohydrate group, respectively. The tracer-derived total leg FA-uptake during exercise from 30 min and onward was not different between

Table 3. Muscle substrate storage and utilization

<table>
<thead>
<tr>
<th></th>
<th>Before Rest</th>
<th>After 7 wk Rest</th>
<th>After 8 wk Rest</th>
<th>60 min</th>
<th>Breakdown, mmol·min⁻¹·kg dry wt⁻¹</th>
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<tbody>
<tr>
<td>Muscle glycogen, mmol/kg dry wt</td>
<td></td>
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<tr>
<td>Fat-CHO</td>
<td>518 ± 74</td>
<td>480 ± 29</td>
<td>872 ± 59†‡</td>
<td>609 ± 38†</td>
<td>4.4 ± 0.5</td>
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<tr>
<td>CHO</td>
<td>467 ± 37</td>
<td>683 ± 46†‡</td>
<td>688 ± 43‡</td>
<td>435 ± 33</td>
<td>4.2 ± 0.7</td>
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<tr>
<td>Muscle triacylglycerol, mmol/kg dry wt</td>
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<tr>
<td>Fat-CHO</td>
<td>44 ± 7</td>
<td>69 ± 8‡</td>
<td>38 ± 4‡</td>
<td>37 ± 4</td>
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<tr>
<td>CHO</td>
<td>36 ± 7</td>
<td>40 ± 6</td>
<td>45 ± 9</td>
<td>45 ± 12</td>
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<tr>
<td>Muscle GLUT-4 protein content, arbitrary units</td>
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<tr>
<td>Fat-CHO</td>
<td>53 ± 6</td>
<td>77 ± 10‡</td>
<td>72 ± 15‡</td>
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<td></td>
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<tr>
<td>CHO</td>
<td>52 ± 13</td>
<td>60 ± 10‡</td>
<td>68 ± 20‡</td>
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</tr>
</tbody>
</table>

Values are means ± SE. †$P < 0.05$, values higher than before training; ‡$P < 0.05$, Fat-CHO vs. CHO; †‡$P < 0.05$, values different from 7-wk values.
and lactate was calculated from blood stable isotope tracer methodology, and contribution of blood glucose uptake multiplied by the whole body %FA oxidized assessed by (4.6 kg). Plasma FA contribution was calculated as the leg total FA breakdown multiplied by the estimated active muscle mass of the leg CHO diets. The contribution from glycogen was determined as net fasted state after 8 wk of training and adaptation to Fat-CHO or Fig. 3. Leg substrate utilization during 60 min bicycle exercise in the carbohydrate diet after the fat adaptation (Fat-Carbohydrate). The tracer-determined uptake of plasma long chain FAs (LCFA) across the leg was not significantly different between the groups and neither was whole body tracer determined $R_d$ of plasma LCFA for palmitate. The RER values demonstrated that the overall proportions of carbohydrate and lipid utilization were similar in the two groups during exercise.

The similar RER values during exercise in the two groups are in agreement with our laboratory’s previous findings (16), in which a switch to a carbohydrate-rich diet after prolonged fat diet adaptation completely abolished the increased fat oxidation during exercise that was observed after prolonged fat adaptation (17). Burke and colleagues (5) found that RER values during 2 h cycling at 70% of peak oxygen uptake in well-trained athletes were lower after short-term fat adaptation (5 days) followed by 1 day of carbohydrate loading (70–75% energy % carbohydrate) compared with 6 days of adaptation to the carbohydrate-rich diet. In addition, they reported that tracer-determined whole body glucose uptake during exercise was similar in the two groups. Thus Burke and colleagues demonstrated that the observed decrease in carbohydrate oxidation was solely due to muscle glycogen sparing in the fat-adapted group. Intake of a fat-rich diet followed by carbohydrate loading for 1 day also lowered carbohydrate oxidation during a 4-h bicycle exercise trial at 65% of peak oxygen uptake compared with when carbohydrates were ingested during the whole period (6). In contrast, we found that muscle glycogen utilization in absolute numbers was similar between groups, but blood glucose uptake across the leg was lower during exercise in Fat-Carbohydrate than in Carbohydrate. The latter finding is most likely due to the increased muscle glycogen concentration in the Fat-Carbohydrate group (Table 3) because studies have demonstrated that glucose uptake during muscle contractions is inversely related to the muscle glycogen level (11, 13). The molecular mechanism behind the effect of glycogen on glucose uptake has been shown to involve impaired GLUT-4 translocation to the surface membrane (11). Increased muscle glycogen stores have also been demonstrated to be associated with decreased activation of 5’-AMP-activated protein kinase during contractions (10, 25), but whether this is related to decreased muscle glucose uptake during exercise is uncertain (10, 23, 25). The difference in muscle glucose uptake between the two groups could not be ascribed to differences in total muscle GLUT-4 protein content because this was similar in the two groups (Table 3). However, it does not rule out the possibility of either recruitment differences in GLUT-4 transporters or differential distribution of the pools of transporters between the muscle membrane and intracellular stores. High plasma concentrations of LCFA have been shown to decrease muscle glucose uptake during exercise (14), but, because these were similar in the two groups during exercise (Fig. 2), group differences in leg glucose

DISCUSSION

In the present study, fuel utilization in the exercising leg was studied in two groups after prolonged adaptation to either fat- or carbohydrate-rich diet combined with training, followed by a carbohydrate-rich diet for an additional week in both groups. The main findings were that preexercise muscle glycogen stores were increased and leg glucose uptake during submaximal exercise decreased in the group that switched to a carbohydrate diet after the fat adaptation (Fat-Carbohydrate) compared with the group that remained on the carbohydrate-rich diet (Carbohydrate). The tracer-determined uptake of plasma long chain FAs (LCFA) across the leg was not significantly different between the groups and neither was whole body tracer determined $R_d$ of plasma LCFA for palmitate. The RER values demonstrated that the overall proportions of carbohydrate and lipid utilization were similar in the two groups during exercise.

The similar RER values during exercise in the two groups are in agreement with our laboratory’s previous findings (16), in which a switch to a carbohydrate-rich diet after prolonged fat diet adaptation completely abolished the increased fat oxidation during exercise that was observed after prolonged fat adaptation (17). Burke and colleagues (5) found that RER values during 2 h cycling at 70% of peak oxygen uptake in well-trained athletes were lower after short-term fat adaptation (5 days) followed by 1 day of carbohydrate loading (70–75% energy % carbohydrate) compared with 6 days of adaptation to the carbohydrate-rich diet. In addition, they reported that tracer-determined whole body glucose uptake during exercise was similar in the two groups. Thus Burke and colleagues demonstrated that the observed decrease in carbohydrate oxidation was solely due to muscle glycogen sparing in the fat-adapted group. Intake of a fat-rich diet followed by carbohydrate loading for 1 day also lowered carbohydrate oxidation during a 4-h bicycle exercise trial at 65% of peak oxygen uptake compared with when carbohydrates were ingested during the whole period (6). In contrast, we found that muscle glycogen utilization in absolute numbers was similar between groups, but blood glucose uptake across the leg was lower during exercise in Fat-Carbohydrate than in Carbohydrate. The latter finding is most likely due to the increased muscle glycogen concentration in the Fat-Carbohydrate group (Table 3) because studies have demonstrated that glucose uptake during muscle contractions is inversely related to the muscle glycogen level (11, 13). The molecular mechanism behind the effect of glycogen on glucose uptake has been shown to involve impaired GLUT-4 translocation to the surface membrane (11). Increased muscle glycogen stores have also been demonstrated to be associated with decreased activation of 5’-AMP-activated protein kinase during contractions (10, 25), but whether this is related to decreased muscle glucose uptake during exercise is uncertain (10, 23, 25). The difference in muscle glucose uptake between the two groups could not be ascribed to differences in total muscle GLUT-4 protein content because this was similar in the two groups (Table 3). However, it does not rule out the possibility of either recruitment differences in GLUT-4 transporters or differential distribution of the pools of transporters between the muscle membrane and intracellular stores. High plasma concentrations of LCFA have been shown to decrease muscle glucose uptake during exercise (14), but, because these were similar in the two groups during exercise (Fig. 2), group differences in leg glucose

![Fig. 3. Leg substrate utilization during 60 min bicycle exercise in the fasted state after 8 wk of training and adaptation to Fat-CHO or CHO diets. The contribution from glycogen was determined as net breakdown multiplied by the estimated active muscle mass of the leg (4.6 kg). Plasma FA contribution was calculated as the leg total FA uptake multiplied by the whole body %FA oxidized assessed by stable isotope tracer methodology, and contribution of blood glucose and lactate was calculated from blood flow multiplied by the arteriovenous difference through the exercise. The partition between non-protein fat-carbohydrate oxidation calculated from whole body respiratory exchange ratio is indicated on the right side of each dietary treatment. Values are means. *P < 0.05 Fat vs. CHO.](image-url)
uptake in the present study could not be ascribed to differences in plasma LCFA.

Despite the higher resting muscle glycogen concentration in the Fat-Carbohydrate group, the actual muscle glycogen breakdown during exercise was not significantly different between groups. It has been demonstrated that muscle glycogen breakdown rate normally is related to preexercise muscle glycogen concentrations both in vitro (18, 24) and in vivo (15). Thus the absence of increased muscle glycogen breakdown in the Fat-Carbohydrate group suggests that muscle glycogen breakdown may be attenuated after fat diet adaptation followed by carbohydrate loading. This interpretation is supported by our laboratory’s previous study (16) in which exhaustion in subjects on a similar diet as the present Fat-Carbohydrate group occurred in spite of very high (~500 μmol/g dry wt) muscle glycogen levels. It thus appears that prolonged adaptation to a fat-rich diet, even when switching to a carbohydrate-rich diet for an additional week, affects muscle metabolism during exercise in such a way that muscle glycogen breakdown is impaired. The molecular mechanisms behind this phenomenon remain to be established.

It might be argued that if glucose utilization is decreased and the overall oxidation of carbohydrates is unchanged in the Fat-Carbohydrate group compared with the Carbohydrate group, then glycogen breakdown must be increased. This was not found. However, the decrease in leg glucose uptake in terms of energy is rather small (Fig. 3), and it is quite possible that a similarly small increase in muscle glycogenolysis is missed. Furthermore, the overall combustion of carbohydrates and fat is calculated from whole body RER values that may not be exactly the same as respiratory quotient in the muscle and therefore small changes in the balance of carbohydrate vs. fat combustion in the leg may not necessarily be picked up at the level of pulmonary gas exchange. However, the similar isotope-derived calculated leg and whole body uptake of LCFA and absence of measurable breakdown of intramyocellular triacylglycerol in both groups supports the concept of no significant difference in overall carbohydrate and fat utilization in the two groups.

In our previous study (17), breakdown of VLDL-TG across the leg during exercise could account for a significant part of the lipid utilization across the leg after prolonged fat diet adaptation. However, in the present study there was no measurable breakdown of VLDL-TG across the leg in either group. Apparently, 1 wk of carbohydrate-rich diet after the fat adaptation is enough to abolish the significant contribution of VLDL-TG to energy provision during exercise in the fat-adapted state. Intramyocellular triacylglycerol breakdown during exercise was not measurable in the present and several previous studies performed in men (2, 19, 26, 30, 32). When calculating the relative contribution of oxidized substrates in the two groups, there is an apparent lack of substrates (Fig. 3). The extent to which this may be accounted for by uptake of VLDL-TG or breakdown of intramyocellular triacylglycerol, which were both too small to be measurable, or by FAs liberated from adipocytes adherent to muscle cells, as suggested by Kiens et al. (19), is not possible to determine from the available data. In any case, the contribution of these potential sources of energy substrates to oxidation is likely to be minor. Finally, it should be considered that because the pulmonary RER may not be exactly the same as leg respiratory quotient, the balance between carbohydrate and lipid combustion may not be entirely correct and therefore the apparent missing substrate may not necessarily be lipid.

In conclusion, when switching to a carbohydrate diet for a week after prolonged fat adaptation, overall fat and carbohydrate utilization during submaximal exercise was not different compared with the group that consumed the carbohydrate-rich diet all along. However, muscle glycogen concentration was increased by 27% on average in the group switching from fat adaptation to a carbohydrate-rich diet. This did not lead to increased muscle glycogen utilization during exercise but to a decrease in utilization of blood glucose. Thus it seems as if prolonged fat adaptation leads to impaired muscle glycogen utilization, which is not remedied by 1 wk of carbohydrate-rich diet.

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