Reduced oxidation of dietary fat after a short term high-carbohydrate diet\textsuperscript{1–3}

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ABSTRACT

Background: Short-term high-carbohydrate (HC) diets induce metabolic alterations, including hypertriacylglycerolemia, in both the fasting and postprandial states. The underlying tissue-specific alterations in fatty acid metabolism are not well understood.

Objective: We investigated alterations in exogenous and endogenous fatty acid metabolism by using stable isotope tracers to label meal triacylglycerol and plasma fatty acids.

Design: Eight healthy subjects consumed isocaloric diets containing a high percentage of energy from carbohydrates or a higher percentage of energy from fat for 3 d in a randomized crossover dietary intervention study. A test meal containing \([U-^{13}C]\)palmitate was combined with intravenous infusion of \([^{2}H_{2}]\)palmitate to label plasma fatty acids and VLDL triacylglycerol. Blood and breath samples were taken before the meal and for 6 h postprandially. Blood samples were drawn from the femoral artery and from veins draining subcutaneous adipose tissue and forearm muscle for monitoring of tissue-specific metabolic substrate partitioning.

Results: Systemic triacylglycerol concentrations were increased in both fasting (\(P = 0.02\)) and postprandial (\(P = 0.02\)) periods, and a greater amount of infused labeled fatty acid appeared in VLDL triacylglycerol after the HC diet than after the higher-fat diet (\(P = 0.05\)). Significantly less \(^{13}CO_{2}\) was exhaled after the HC diet (\(P = 0.04\)) and significantly less production of \(^{13}CO_{2}\) was seen across forearm muscle (\(P = 0.04\)). Systemic 3-hydroxybutyrate was significantly lower, postprandially, after the HC diet (\(P = 0.02\)).

Conclusion: Metabolic alterations suggestive of repartitioning of fatty acids away from oxidation toward esterification in both liver and muscle occur in response to short-term adaptation to a HC diet.

INTRODUCTION

Low-fat, high-carbohydrate (HC) diets induce numerous changes in metabolism, the most prominent of which is hypertriacylglycerolemia, both fasting and postprandial. Carbohydrate-induced hypertriacylglycerolemia may in principle result from triacylglycerol overproduction, reduced triacylglycerol removal, or both. There is evidence in favor of each of these mechanisms (1–3). However, underlying these whole-body responses must be alterations of fatty acid (FA) metabolism at a tissue level. These alterations have not been intensively investigated. Mittendorfer and Sidossis (2) identified the splanchnic tissues as a site of triacylglycerol overproduction after a 2-wk HC diet. Removal of triacylglycerol from the circulation occurs mainly in tissues expressing lipoprotein lipase, especially skeletal muscle and adipose tissue, but the role of these tissues in the metabolic alterations induced by HC diets has not been specifically explored. Because insulin induces lipoprotein lipase activity in adipose tissue but suppresses it in muscle (4), a plausible hypothesis would be that the relative roles of these tissues in triacylglycerol removal would change with HC feeding. Many of the changes induced by HC feeding could be interpreted as a shift in the intracellular partitioning of FAs toward esterification and away from oxidation. For instance, elevated insulin concentrations on an HC diet may stimulate the pathway of fatty synthesis, raising the malonyl-CoA concentrations, which would divert FAs from oxidation into triacylglycerol synthesis. That possibility may explain increased triacylglycerol production by the liver but may also affect FA oxidation by muscle.

We have sought to investigate some of these tissue-specific aspects of FA metabolism after a short-term HC diet rich in sugars, a diet that is known to induce marked metabolic alterations (5). We have used state-of-the-art stable-isotope tracer techniques to label dietary and endogenous FA pools (6) and specific catheterization techniques to investigate metabolic alterations in adipose tissue and skeletal muscle. Our hypothesis was that an HC diet would alter FA partitioning, which would be observed as changes in circulating triacylglycerol concentration and in fat oxidation in the fasting and postprandial states.

SUBJECTS AND METHODS

Subjects

Eight healthy nonobese persons (6 F, 2 M) were recruited to take part in a randomized-order crossover dietary intervention...
study, in which subjects consumed either a high-fat, low-carbohydrate (HF) diet or an isocaloric HC diet for 3 d before a metabolic investigation. A 6-wk washout period, during which subjects resumed their usual dietary habits, took place between diets.

The clinical characteristics of the subjects are summarized in Table 1. These characteristics did not change significantly over the 6-wk course of the study. Subjects were asked to refrain from strenuous exercise and alcohol consumption for 24 h before the study. In addition, subjects were asked to avoid foods containing corn (naturally enriched in 13C) for 48 h before the metabolic intervention.

All subjects gave written informed consent. The study was approved by the Oxfordshire Clinical Research Ethics Committee.

Diet

The HC diet consisted of 10% of energy from fat and 75% of energy from carbohydrate, and the HF diet consisted of 40% of energy from fat and 45% of energy from carbohydrate. Both diets contained 15% of energy from protein. In both cases, the ratio of the percentage of starch and sugar in carbohydrate was 30%: 70%. Fiber content was kept below 15 g/d (7). The food items consisted of white bread, potatoes, tuna, chicken, carrots, canned fruit, fruit juices, cola, jam, marmalade, sweets, and sugar cubes. The fat component of the diet was given in the form of a fat spread, which consisted of a mixture of lard, palm oil, olive oil, and corn oil (the ratio of polysaturated to saturated fat was 0.5). The subjects were allocated to standardized daily meal plans with 1 of 4 levels of energy contents—9450, 10500, 11550, or 12600 kJ—according to their estimated energy requirements, which were determined on the basis of a diet history taken by a dietitian. Physical activity was also taken into account to estimate energy needs. Nutritional analysis of the diets was done with the aid of MICRODIET nutritional software (version 1.0; Downlee Systems Limited, Salford, United Kingdom). All foods were provided and packed in weighed portions and labeled appropriately for each meal. Three meals and 2 snacks were provided each day. Subjects refrained from consuming other foods and drinking alcohol during the 3-d diet.

Study protocol

On the day of the metabolic investigation, serial blood samples were taken at the time-points shown in the figures in the fasting state and for 6 h after consumption of a mixed test meal [40 g fat, 40 g carbohydrate, and 100 mg [U-13C]palmitic acid (isotope purity: 97%; CK Gas Products Ltd) complexed to human albumin (Blood Transfusion Service, John Radcliffe Hospital, Oxford, United Kingdom)]. The infusion was begun ≥60 min before fasting blood sampling to allow equilibration of tracer in plasma, and it was continued throughout the study period.

Arterial blood was sampled from a catheter in the femoral artery. The superficial epigastric vein was cannulated, as previously described (8), to sample the venous effluent of subcutaneous abdominal adipose tissue. Venous blood from forearm muscle was obtained from a cannula placed retrogradely in a deep antecubital vein to reflect skeletal muscle drainage. To avoid contamination of the blood from the forearm with blood from the hand, a wrist cuff was inflated to 200 mm Hg for 2 min before samples were taken. All catheters were kept patent by continuous saline infusion. Blood sampling was performed simultaneously from all 3 sites. At each blood sampling time-point, a breath sample was taken and a sample was injected into an Extetainer tube (Labco Ltd, High Wycombe, United Kingdom) until atmospheric pressure was reached.

Oxygen consumption and carbon dioxide production (VCO2) were measured by using a ventilated-hood indirect calorimeter (Deltatrac; Datex, Helsinki, Finland) to calculate the respiratory exchange ratio (ie, the ratio of VCO2 to oxygen consumption) in the fasting and postprandial periods. A 20-min reading was taken before the meal and then 20-min readings were taken hourly after the test meal.

Subcutaneous abdominal adipose tissue blood flow was measured by xenon (133Xe) washout (9). Forearm muscle blood flow was assessed by using occlusion strain-gauge plethysmography (10) while the blood flow from the hand was occluded. Blood flow measurements were made immediately after blood sampling.

Sample analyses

Biochemical analyses

Whole blood was collected into heparinized syringes (Sarstedt, Leicester, United Kingdom) for measurement of metabolite, gas, and insulin concentrations. A sample of whole blood was added to perchloric acid for later analysis of 3-hydroxybutyrate (3-OHB) as described previously (11). Plasma glucose, triacylglycerol, and nonesterified FA (NEFA) concentrations were measured enzymatically (glucose and triacylglycerol: Instrumentation Laboratory, Milan, Italy; NEFA: NEFA C kit; Wako Chemicals, Neuss, Germany) with the use of a multianalyzer (ILab 600; Instrumentation Laboratory, Warrington, United Kingdom). Plasma insulin concentrations were measured by radioimmunoassay using a commercially available kit (Linco Research, St Charles, MO).

Fatty acid analyses

To determine FA composition and isotopic enrichment, total lipids were extracted from plasma, and methyl esters were prepared from NEFA and triacylglycerol fractions as described previously (12). FA composition (μmol/100 μmol total FAs) in plasma fractions was measured by using gas chromatography (12), and palmitate concentrations were calculated by multiplying the proportion of palmitate by the corresponding plasma concentration of NEFA or triacylglycerol, measured enzymatically. We measured [U-13C] and [2H2]palmitate enrichments simultaneously with gas chromatography–mass spectrometry
using a gas chromatograph (model 5890; Agilent Technologies, West Lothian, United Kingdom). The gas chromatograph was equipped with a capillary column (DB-Wax 30-m; internal diameter: 0.25 mm; film thickness: 0.25 μm; Agilent Technologies), and ions with mass-to-charge ratios (m/z) of 270 (M + 0), 272 (M + 2), and 286 (M + 16) were determined by using selected ion monitoring. Dwell time was 100 ms. Tracer-to-trace ratios ([TTRs] henceforth simply called “enrichment”) for \[^{13}\text{C}\]palmitate (M + 16):(M + 0) and \[^{2}\text{H}_2\]palmitate (M + 2):(M + 0) were multiplied by the corresponding palmitate-NFA or palmitate-triacylglycerol concentrations to give plasma tracer concentrations.

**Blood and breath gas analysis**

Total carbon dioxide saturation of heparinized blood was measured by using a blood gas analyzer (ABL-700 Series; Radiometer, Copenhagen, Denmark). At each time-point, 1 mL blood from each site was injected into a 10-mL Exetainer tube for measurement of \(^{13}\text{CO}_2\) enrichment, and 1 mL of 1 mol sulfuric acid/L was added to liberate total carbon dioxide (13). Nitrogen was then injected until atmospheric pressure was reached. Samples were analyzed for the ratio of \(^{13}\text{CO}_2\) to \(^{12}\text{CO}_2\) on a gas chromatograph–isotope ratio mass spectrometer (Delta Plus XP GC-IRMS; Thermo Electron, Bremen, Germany). Breath and liberated blood carbon dioxide were resolved from the presence of other gases by using a capillary column with dimensions of 27.5 m × 0.32 mm × 10 μm (CP-PoraPLOTQ; Varian Ltd, Oxford, United Kingdom). Splitless injection mode was used, with an injection volume of 40 μL and injector temperature of 110 °C. The oven temperature was kept constant at 35 °C with a total runtime of 10 min. The column flow was held constant at 1.2 mL/min. Allowance was made for natural enrichment by subtracting a baseline value from each sample enrichment.

**Calculations and statistical analysis**

Arteriovenous difference calculations allow determination of tissue-specific substrate flux (8). Concentrations of lipids (measured in plasma) were converted to whole blood values by using the hematocrit values to calculate tissue exchange (12). Arteriovenous (A-V) and vеноarterial (V-A) differences in metabolite concentrations (labeled and unlabeled) were calculated across both adipose tissue and skeletal muscle. Absolute flux was calculated as the product of A-V or V-A difference and tissue blood flow. A positive A-V difference in metabolite concentrations implies uptake, or extraction, across a tissue, whereas a positive V-A difference implies release from a tissue. Fractional triacylglycerol extraction was calculated as the A-V difference divided by the arterial concentration of triacylglycerol and expressed as a percentage (14). Clearance was calculated as the fractional triacylglycerol extraction multiplied by blood flow.

The whole-body rate of appearance (Ra) of NEFA (Ra\(_{\text{NEFA}}\)) was derived from arterial enrichments of \[^{2}\text{H}_2\]palmitate. To calculate Ra\(_{\text{NEFA}}\), Steele’s equation for steady-state conditions modified for use with stable isotopes (15) was used in the fasting state, and the equations modified for non-steady-state conditions (16) were used after feeding.

Concentrations of \(^{13}\text{C}\)- and \(^{2}\text{H}_2\)-labeled triacylglycerol were calculated by multiplying plasma concentrations of palmitate by the enrichment of \[^{13}\text{C}\]palmitate or \[^{2}\text{H}_2\]palmitate, respectively, in triacylglycerol. Total carbon dioxide content in blood was calculated from the partial pressure of carbon dioxide, and pH was measured by using the blood gas analyzer as described by Douglas et al (17). The \(^{13}\text{CO}_2\) content in blood was calculated by multiplying the total carbon dioxide in blood by the enrichment of the blood carbon dioxide pool. The rate of expiration of \(^{13}\text{CO}_2\) in breath was calculated by multiplying the \(\text{VCO}_2\) by the enrichment of the breath carbon dioxide pool.

Because of the difficulty of obtaining sufficient blood volume, some values were missing for total blood \(^{13}\text{CO}_2\) content. Suitable samples were available from only 5 subjects. One subject had missing values at 120 min and (on both diets) at 300 and 360 min. The value at 120 min was interpolated from the values on either side, and the values for 300 and 360 min were simply taken to be equal to the 240-min value before statistical analysis as described below.

Data were analyzed by using SPSS for WINDOWS software (version 11; SPSS UK, Chertsey, United Kingdom). Statistical significance was set at \(P<0.05\). Repeated-measures analysis of variance (ANOVA) with time and diet as within-subject effects was used to identify time effects, differences between diets, and time × diet interactions. Areas under the curve (AUCs) were calculated by using the trapezoid method and compared by using Wilcoxon’s signed-rank test for 2 related samples. AUCs have been divided by the relevant period to give a time-averaged value. Correlation coefficients were tested by using Spearman’s rank correlation.

**RESULTS**

**Arterial concentrations and whole-body responses**

Systemic (arterial) triacylglycerol concentrations increased after the test meal no matter which diet subjects were following \((P<0.001; \text{Figure 1A})\). Elevation of plasma triacylglycerol concentrations with the HC diet occurred in 7 of 8 subjects (1 male subject did not respond). Compared with the HF diet, plasma triacylglycerol concentrations were, on average, 90% higher in the fasted state and 70% higher postprandially with the HC diet \((n=8; \text{Figure 1A})\). The increase in postprandial triacylglycerol concentration reflected the difference in fasting triacylglycerol because there was no significant \((P=0.3)\) difference between the diets in incremental AUCs: 882 ± 291 μmol/L for the HC diet and 738 ± 150 μmol/L for the HF diet. The induced rise in triacylglycerol concentrations over the whole time period was correlated to fasting triacylglycerol concentrations, taken as an average from both diets \((r=0.75, P=0.05 \text{ (data not shown)})\).

\[^{13}\text{C}\]palmitate-labeled triacylglycerol appeared in the circulation 60 min after the test meal, and the concentration peaked at 240 min after the meal (HC diet) and at 180 min (HF diet) \((P=0.01 \text{ for time × diet interaction; ANOVA}; \text{Figure 1C})\). The enrichment of \[^{13}\text{C}\]palmitate-labeled triacylglycerol was, however, consistently lower after the HC diet than after the HF diet \((P=0.01, \text{ANOVA}; \text{Table 2})\). \[^{2}\text{H}_2\]palmitate in triacylglycerol, indicating incorporation of labeled NEFA into liver-derived VLDL-triacylglycerol, was detected in the fasting blood samples, but no significant difference was seen between diets. The concentration of \[^{2}\text{H}_2\]palmitate in triacylglycerol increased consistently throughout the study \((P<0.001 \text{ for the time effect for each diet; ANOVA}; \text{Figure 1D})\, and a significantly higher concentration of \[^{2}\text{H}_2\]palmitate in triacylglycerol was detected postprandially in arterial blood with the HC diet than with the HF
diet (Table 2). The enrichment of $[^{2}H_{2}]$palmitate in triacylglycerol, however, was consistently lower over the whole time period with the HC diet ($P < 0.05$, ANOVA; Table 2).

After the meal, arterial plasma glucose and insulin concentrations reached a peak simultaneously at 30 (HC diet) and 60 (HF diet) min. There was no significant effect of diet on glucose or insulin concentrations in the fasting or postprandial state (Figure 2A and B).

Systemic (arterial) NEFA concentrations fell during the early postprandial period, before rising again toward the end of the study after both diets. There was no significant effect of diet on NEFA concentration in either the fasting or postprandial state (Figure 2C). There also was no significant effect of diet on the whole-body RaNEFA (Table 2).

Arterial 3-OHB concentrations fell during the early postprandial period before rising again toward the end of the study period. Arterial blood 3-OHB concentrations were not significantly affected by diet in the fasting state but were significantly lower postprandially with the HC diet (Figure 2D), which suggests reduced hepatic FA oxidation.

There was no significant effect of diet on whole-body respiratory exchange ratio (Table 2) or relative substrate oxidation (data not shown). Significant increases in $^{13}$CO$_{2}$ were detected in breath samples after both diets 60 min after the test meal, which reflected whole-body oxidation of exogenous FAs. Significantly more $^{13}$CO$_{2}$ was exhaled postprandially with the HF diet than with the HC diet ($P = 0.04$ for main effect of diet, ANOVA; Figure 3A).

**Adipose tissue and forearm muscle responses**

Adipose tissue blood flow increased by a factor of 1.6 (HC diet) ($P = 0.04$) and 1.7 (HF diet) ($P = 0.05$) from the fasting to the postprandial period (AUCs are given in Table 3). NEFA release was suppressed and triacylglycerol extraction was increased after the meal. There was, however, no effect of diet on blood flow or on FA or triacylglycerol metabolism in adipose tissue (Table 3).
There was a significant rise in forearm muscle blood flow in response to the test meal (Table 3) but no significant difference between diets. There was net NEFA uptake across muscle in the fasting state (A-V difference multiplied by blood flow), which fell to a nadir 60 min after the test meal and rose later in the postprandial period ($P < 0.001$ for each diet; data not shown). There was, however, no difference in either the fasting or post-prandial period between diets.

### TABLE 2
Summary of derived whole-body responses to the 2 diets

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Mean</th>
<th>Postprandial period</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R_{A\text{NEFA}}$ ($\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$)</td>
<td>8.20 ± 0.81</td>
<td>7.36 ± 0.41</td>
</tr>
<tr>
<td>RER</td>
<td>0.78 ± 0.01</td>
<td>0.77 ± 0.01</td>
</tr>
<tr>
<td>$[^{13}C]_{\text{Palmitate enrichment in plasma TG}} \times 10^{-3}$</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>$[^{13}C]_{\text{Palmitate in plasma TG}}$ ($\mu\text{mol/L}$)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>$[^{13}C]<em>{\text{[H]</em>{2}Palmitate enrichment in plasma TG}} \times 10^{-3}$</td>
<td>1.97 ± 0.45</td>
<td>2.73 ± 0.49</td>
</tr>
<tr>
<td>$[^{13}C]<em>{\text{[H]</em>{2}Palmitate in plasma TG}}$ ($\mu\text{mol/L}$)</td>
<td>1.11 ± 0.43</td>
<td>0.61 ± 0.06</td>
</tr>
<tr>
<td>$[^{13}C]<em>{\text{CO</em>{2}}}$ in breath ($\mu\text{mol/min}$)</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

$^1 n = 8$. HC, low-fat, high-carbohydrate; HF, high-fat, low carbohydrate; AUC, area under the curve; $R_{A\text{NEFA}}$, whole-body rate of appearance of nonesterified fatty acids; RER, respiratory exchange ratio; TG, triacylglycerol. Enrichment refers to tracer-to-tracee ratio.

$^2$ Wilcoxon’s signed-rank test was used for 2 related samples to compare differences in AUCs between diets.

$^3$ Values were divided by time for the postprandial study period.

$^4 \bar{x} \pm \text{SEM}$ (all such values).

FIGURE 2. Mean (±SEM) systemic changes after a mixed meal, given at time 0, following either a 3-d low-fat, high-carbohydrate diet (●; HC) or a high-fat, low-carbohydrate diet (○; HF), in concentrations of plasma glucose (A), plasma insulin (B), plasma nonesterified fatty acids [NEFA (C)], and 3-hydroxybutyrate [3-OHB (D)]. The 3-OHB concentrations after the HF diet were significantly higher over the whole time period than those after the HC diet ($P = 0.03$ for main effect of diet, ANOVA).
Triacylglycerol absolute extraction across forearm muscle was not affected by diet in either the fasting or the postprandial state. Triacylglycerol clearance, however, which makes allowances for systemic triacylglycerol concentrations, was significantly lower with the HC diet than with the HF diet in either the fasting or postprandial state (Table 3, Figure 1B). Clearance of forearm [U-13C]palmitate-labeled triacylglycerol and [2H2]palmitate-labeled triacylglycerol was unaffected by

### TABLE 3
Summary of derived tissue-specific responses to the 2 diets

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Fasting</th>
<th>Postprandial period</th>
<th>P2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>AUC</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HC diet</td>
<td>HF diet</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>AUC</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HC diet</td>
<td>HF diet</td>
<td></td>
</tr>
<tr>
<td>Muscle</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Forearm BF (mL·100 mL⁻¹·min⁻¹)</td>
<td>1.43 ± 0.09</td>
<td>1.45 ± 0.14</td>
<td>0.89</td>
</tr>
<tr>
<td>TG absolute extraction across forearm muscle (nMol·100 mL⁻¹·min⁻¹)</td>
<td>17.7 ± 14.2</td>
<td>292 ± 9.9</td>
<td>0.09</td>
</tr>
<tr>
<td>TG clearance across muscle (mL·100 mL⁻¹·min⁻¹)</td>
<td>0.02 ± 0.01</td>
<td>0.05 ± 0.01</td>
<td>0.02</td>
</tr>
<tr>
<td>[13C]Palmitate in TG clearance across muscle (mL·100 mL⁻¹·min⁻¹)</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>[13C]Palmitate in TG clearance across muscle (mL·100 mL⁻¹·min⁻¹)</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>[13C]Palmitate in TG clearance across muscle (mL·100 mL⁻¹·min⁻¹)</td>
<td>0.04 ± 0.10</td>
<td>0.05 ± 0.23</td>
<td>0.89</td>
</tr>
<tr>
<td>[13CO2] production across muscle (nMol·100 mL⁻¹·min⁻¹)</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Adipose tissue</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adipose tissue BF (mL·100 g⁻¹·min⁻¹)</td>
<td>2.81 ± 0.44</td>
<td>2.83 ± 0.28</td>
<td>0.61</td>
</tr>
<tr>
<td>TG absolute extraction across adipose tissue (nMol·100 g⁻¹·min⁻¹)</td>
<td>164 ± 559</td>
<td>148 ± 38</td>
<td>0.58</td>
</tr>
<tr>
<td>TG clearance across adipose tissue (mL·100 g⁻¹·min⁻¹)</td>
<td>0.14 ± 0.04</td>
<td>0.21 ± 0.04</td>
<td>0.23</td>
</tr>
<tr>
<td>[13C]Palmitate in TG clearance across adipose tissue (mL·100 g⁻¹·min⁻¹)</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>NEFA output from adipose tissue (nMol·100 g⁻¹·min⁻¹)</td>
<td>1080 ± 269</td>
<td>592 ± 121</td>
<td>0.33</td>
</tr>
</tbody>
</table>

1 n = 8. HC, low-fat, high-carbohydrate; HF, high-fat, low-carbohydrate; AUC, area under the curve; BF, blood flow; TG, triacylglycerol; NEFA, nonesterified fatty acid.

2 Wilcoxon’s signed-rank test was used for 2 related samples to compare differences in AUCs between diets.

3 Values were divided by time for the postprandial study period.

4 ± SEM (all such values).
diet. It is noteworthy that the value of forearm \([^{13}C]\) palmitate-labeled triacylglycerol (representing the chylomicron fraction) clearance was significantly higher than that of \([^{2}H_{2}]\) palmitate-labeled triacylglycerol (representing VLDL) \((P = 0.01)\) for both the HC and HF diets, Wilcoxon’s signed-rank test for 2 related samples; Table 3).

With the HC diet, no significant production of \(^{12}CO_{2}\) across the forearm muscle was seen during the course of the study. We did, however, observe significantly higher production with the HF diet, which reflected the oxidation of meal-derived FAs \((P = 0.02)\) for main effect of diet, ANOVA; Figure 3B).

**DISCUSSION**

We investigated the effects of alterations in FA metabolism after an HC diet on whole-body and tissue-specific bases. The expected hypertriglycerolemia induced by the HC diet was accompanied at a whole-body level by a reduction, specifically, in dietary FA oxidation. At a tissue level, lower dietary FA oxidation was also observed in skeletal muscle after the HC diet, and there was suggestive evidence of an altered partitioning of FAs away from oxidation and toward esterification in the liver. It is surprising that we found no evidence for metabolic alterations in adipose tissue.

In agreement with numerous published studies \((1–3, 5, 18)\), the findings of the present study showed that consumption of an HC diet by healthy subjects increases plasma triacylglycerol concentrations. The higher concentration of fasting triacylglycerol observed after the HC diet in 7 of 8 subjects must reflect good compliance with the diets. The increase in fasting triacylglycerol concentrations during an HC diet varies considerably between persons, and it is dependent on the baseline triacylglycerol concentration \((2, 19)\)—findings that are in agreement with those in the present study. The fasting hypertriglycerolemia seen after the HC diet may be an acute result of the difference in the evening meal on the night before the metabolic investigation \((20)\). An HC evening meal has been shown to elevate plasma triacylglycerol after a 12-h fast, although the rise was much less pronounced than in the present study, despite a more extreme composition of the evening meal.

The increase in plasma triacylglycerol concentrations in the fasting state has been attributed to an increase in the "production" of VLDL \((2)\). However, Mancini et al \((1)\) found an increase in the plasma Svedberg flotation rate \((S_{f}) >400\) (chylomicron) fraction after an HC diet, and Parks et al \((3)\) found an increase in lipoproteins containing B48 (chylomicrons), possibly because of altered secretion from the intestine. In the present study, there was a greater contribution of liver-derived triacylglycerol to the hypertriglycerolemia after the HC diet than after the HF diet, as indicated by the higher systemic concentration of \(^{2}H_{2}\)-labeled triacylglycerol. The lower enrichment of \(^{2}H_{2}\)-labeled triacylglycerol emphasizes the dilution of the newly synthesized triacylglycerol with greater amounts of unlabeled triacylglycerol that are present in the circulation after the HC diet. A similar argument can be made for the \(^{13}C\) tracer. These unlabeled triacylglycerol FAs may arise either from VLDL that was secreted before the tracer infusions were started and that remains in the circulation or from sources within the liver.

De novo lipogenesis in the liver could contribute to greater VLDL production, because de novo lipogenesis can be elevated during an HC diet when the content of starch is <50% of that of sugars \((21)\). Specific methodology is required for the quantification of de novo lipogenesis \([\text{which we have reported separately (22)}]\), showing greater de novo lipogenesis after the HC diet intervention. A similar study conducted after a 5-d HC diet resulted in a fractional de novo lipogenesis of \(\approx 13\%\) \((23)\). However, it seems likely that this pathway may be key in altering the partitioning of FAs between oxidation toward esterification in the liver; this possibility is discussed below.

Various studies have reported lower whole-body removal of triacylglycerol after an HC diet \((2, 3)\). Through our use of A-V difference measurements, we were able to evaluate tissue-specific removal of triacylglycerol by muscle and adipose tissue. The absolute rate of triacylglycerol extraction in these tissues was not altered by the HC diet, although there was a significant reduction in muscle triacylglycerol clearance—ie, removal in relation to plasma concentration—after the HC diet in both the fasting and fed states. Because of the lack of change of absolute triacylglycerol extraction, it is difficult to conclude that this reduction was a cause of the hypertriglycerolemia. We saw a greater postprandial clearance of \(^{13}C\) labeled triacylglycerol \((\text{representing the chylomicron fraction})\) than of \(^{2}H_{2}\)-labeled triacylglycerol \((\text{representing VLDL})\) across muscle. The clearance of VLDL-triacylglycerol is suppressed by the presence of chylomicrons in the postprandial state, because chylomicrons are the better substrate for lipoprotein lipase \((6, 24)\).

We expected to observe metabolic alterations in adipose tissue after the HC diet, but we found no detectable differences. Our data suggest that liver and muscle are the tissues most highly involved in the response to short-term HC feeding. It is quite possible that longer-term studies would show greater effects in adipose tissue.

We observed significantly lower \(^{13}CO_{2}\) excretion in breath after the HC diet than after the HF diet, which reflected the HC diet’s lower whole-body oxidation of meal-derived fat. Lower FA oxidation in skeletal muscle is likely to contribute to this effect; we observed lower \(^{13}CO_{2}\) production across muscle. We propose that the HC diet may alter partitioning of FAs toward re-esterification rather than toward oxidation in muscle, which is a novel finding. Altered FA metabolic partitioning may also occur in the liver, as indicated by the significant decrease in systemic 3-OHB concentration after the HC diet. Lower FA oxidation in the liver has been noted previously, with lower plasma 3-OHB concentrations after a 14-d isocaloric HC diet \((2)\). In addition, in that study, the oxidation of plasma-derived FAs in the splanchnic region was lower after the HC diet than after the HF diet, a change that is unrelated to a decline in FA availability. In the present study, the greater appearance of \(^{2}H_{2}\)-labeled triacylglycerol also implies an altered partitioning of FA handling in the liver, away from oxidation toward triacylglycerol assembly. Hepatic de novo lipogenesis may increase VLDL secretion directly by contributing additional FAs and also by inhibition of FA oxidation due to increased intrahepatic malonyl-CoA, an inhibitor of CPT-1 and hence of FA oxidation \((23)\). A malonyl-CoA–dependent mechanism also may alter the partitioning of FAs in skeletal muscle after an HC diet, because more glucose is made available to skeletal muscle \((25)\), despite an almost identical insulin response to the test meal after both diets.

In the present study, we used a short dietary intervention of 3 d, because that is a sufficient period in which to bring about metabolic changes \((5)\) without adaptation to the HC diet, which may
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happen after several months (26). Thus, we accept that our findings may not have relevance to the long-term consumption of low-fat diets. The HC diet was rich in simple sugars to provoke more profound metabolic changes (27). Fiber in both diets was kept low because plant fibers lower fasting triacylglycerol concentrations and attenuate the postprandial rise in chylomicron concentrations after HC feeding (28). In conclusion, metabolic alterations seen in response to a short-term HC diet are suggestive of lower postprandial dietary FA oxidation in muscle and liver and altered partitioning of FAs toward esterification in these tissues.

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