PUFAs acutely affect triacylglycerol-derived skeletal muscle fatty acid uptake and increase postprandial insulin sensitivity

Anneke Jans, Ellen Konings, Gijs H Goossens, Freek G Bouwman, Chantalle C Moors, Mark V Boekschoten, Lydia A Afman, Michael Müller, Edwin C Mariman, and Ellen E Blaak

ABSTRACT

Background: Dietary fat quality may influence skeletal muscle lipid processing and fat accumulation, thereby modulating insulin sensitivity.

Objective: The objective was to examine the acute effects of meals with various fatty acid (FA) compositions on skeletal muscle FA processing and postprandial insulin sensitivity in obese, insulin-resistant men.

Design: In a single-blind, randomized, crossover study, 10 insulin-resistant men consumed 3 high-fat mixed meals (2.6 MJ), which were high in SFAs, MUFAs, or PUFAs. Fasting and postprandial skeletal muscle FA processing was examined by measuring differences in arteriovenous concentrations across the forearm muscle. [{3H}_2]Palmitate was infused intravenously to label endogenous triacylglycerol and FFAs in the circulation, and [U-{13C}]palmitate was added to the meal to label chylomicron-triacylglycerol. Skeletal muscle biopsy samples were taken to assess intramuscular lipid metabolism and gene expression.

Results: Insulin and glucose responses (AUC) after the SFA meal were significantly higher than those after the PUFA meal (P = 0.006 and 0.033, respectively). Uptake of triacylglycerol-derived FAs was lower in the postprandial phase after the PUFA meal than after the other meals (AUC_{postprandial} = 0.02). The fractional synthetic rate of the triacylglycerol, diacylglycerol, and phospholipid pool was higher after the MUFA meal than after the SFA meal. PUFA induced less transcriptional downregulation of oxidative pathways than did the other meals.

Conclusion: PUFAs reduced triacylglycerol-derived skeletal muscle FA uptake, which was accompanied by higher postprandial insulin sensitivity, a more transcriptional oxidative phenotype, and altered intramyocellular lipid partitioning and may therefore be protective against the development of insulin resistance. This trial was registered at clinicaltrials.gov as NCT01466816.

INTRODUCTION

Increased fat storage in nonadipose tissues such as skeletal muscle (ectopic fat accumulation) is a strong indicator of insulin resistance (1). Conditions characterized by insulin resistance are often accompanied by adipose tissue dysfunction and a reduced lipid-buffering capacity, which may lead to an increased supply of triacylglycerols and FFAs to nonadipose tissues (lipid overflow hypothesis) (1, 2). Together with an impaired capacity of skeletal muscle to oxidize fat (3–6), lipid overflow may cause excess fat storage in skeletal muscle. Increased lipid accumulation in skeletal muscle, particularly the accumulation of lipid metabolites, such as diacylglycerol, long-chain fatty acyl-CoA, and ceramides, may reduce insulin action by interference with insulin signaling (7–10).

Dietary intervention, particularly a reduction in SFAs, may improve insulin sensitivity (11–13), possibly through effects on lipid overflow and skeletal muscle FA processing. PUFAs may reduce lipid overflow in the circulation by increasing lipid uptake and storage in adipose tissue by inducing adipocyte differentiation (14). Furthermore, it has been found in human muscle cell lines that SFAs accumulate preferentially as diacylglycerol, thereby activating protein kinase C and inhibiting insulin signaling, whereas unsaturated FAs are readily converted to triacylglycerol (15). Furthermore, oleic acid accumulates to a lesser extent as diacylglycerol or triacylglycerol than as palmitic acid and is more directed toward oxidation (16). The underlying mechanisms for the differential effect of FA on metabolism may relate to intracellular pathways that are differently tuned by FA subtypes. It has been shown that PUFAs may regulate gene transcription within minutes, acting as agonists of peroxisome proliferator–activated receptors and sterol regulatory element-binding protein 1c (17). However, human in vivo studies investigating the effects of meals with different FA composition on skeletal muscle FA processing are lacking.

Recently, a unique dual-stable-isotope tracer approach was validated to study skeletal muscle FA metabolism in detail. This approach allows for the quantification of lipid flux within the muscle compartment and the study of lipid metabolism in response to meals with different FA composition. To date, there are only a few studies that investigated the effects of meals with different FA composition on skeletal muscle FA processing. PUFAs were shown to reduce lipid overflow and skeletal muscle FA processing. PUFAs may improve insulin sensitivity, thereby activating protein kinase C and inhibiting insulin signaling, whereas unsaturated FAs are readily converted to triacylglycerol (15). Furthermore, oleic acid accumulates to a lesser extent as diacylglycerol or triacylglycerol than as palmitic acid and is more directed toward oxidation (16). The underlying mechanisms for the differential effect of FA on metabolism may relate to intracellular pathways that are differently tuned by FA subtypes. It has been shown that PUFAs may regulate gene transcription within minutes, acting as agonists of peroxisome proliferator–activated receptors and sterol regulatory element-binding protein 1c (17). However, human in vivo studies investigating the effects of meals with different FA composition on skeletal muscle FA processing are lacking.

Recently, a unique dual-stable-isotope tracer approach was validated to study skeletal muscle FA metabolism in detail. This approach allows for the quantification of lipid flux within the muscle compartment and the study of lipid metabolism in response to meals with different FA composition. To date, there are only a few studies that investigated the effects of meals with different FA composition on skeletal muscle FA processing. PUFAs were shown to reduce lipid overflow and skeletal muscle FA processing. PUFAs may improve insulin sensitivity, thereby activating protein kinase C and inhibiting insulin signaling, whereas unsaturated FAs are readily converted to triacylglycerol (15). Furthermore, oleic acid accumulates to a lesser extent as diacylglycerol or triacylglycerol than as palmitic acid and is more directed toward oxidation (16). The underlying mechanisms for the differential effect of FA on metabolism may relate to intracellular pathways that are differently tuned by FA subtypes. It has been shown that PUFAs may regulate gene transcription within minutes, acting as agonists of peroxisome proliferator–activated receptors and sterol regulatory element-binding protein 1c (17). However, human in vivo studies investigating the effects of meals with different FA composition on skeletal muscle FA processing are lacking.
offers, in combination with measurements of differences in arteriovenous concentrations across the forearm muscle (6, 18), the possibility of differentiation between the metabolic fate of dietary compared with endogenous FAs.

The hypothesis of the current study was that a meal high in unsaturated FAs may acutely improve insulin sensitivity by reducing lipid overflow and shifting FA partitioning within muscle toward oxidation as compared with a meal high in SFAs in obese-insulin resistant subjects. The objective of the current study was to examine the acute effects of meals with various FA compositions on forearm muscle FA processing and postprandial insulin sensitivity in obese insulin-resistant men by using the dual-stable-isotope technique. Furthermore, baseline and postprandial skeletal muscle biopsy samples were collected for assessment of intramuscular FA processing and the transcriptional regulation of FA metabolism.

SUBJECTS AND METHODS

Ten insulin-resistant men aged 50–70 y with a BMI (in kg/m²) of 29–39 (homeostasis model assessment of insulin resistance: >2.5) participated in a single-blind, randomized, crossover study (Table 1). Exclusion criteria were as follows: weight change of >3 kg within the 3 mo before the study, diabetes, chronic inflammatory conditions, kidney or liver dysfunction, use of hypolipidemic or antiinflammatory medication, use of β-blockers, use of aspirin >1 time/wk, highly trained athletes, and alcohol abuse. All subjects were informed about the nature of the study, and written informed consent was obtained before study participation. The local Medical Ethical Committee of Maastricht University Medical Centre approved the study protocol.

Experimental design

The study had a randomized crossover design with 3 different treatments. On 4 occasions, subjects arrived at the university after an overnight fast. On the first day, subjects underwent anthropometric measurements, a fasting blood sample was collected, and a hyperinsulinemic–euglycemic clamp was performed to measure insulin sensitivity by using a modification of the method described by DeFronzo et al (19). On the other 3 d (2–3 wk between measurements, in randomly assigned order), subjects were studied under baseline conditions and for 4 h after the ingestion of a liquid high-fat mixed meal, which was high in SFAs, MUFAs, or PUFAs. A dual-stable-isotope technique was used to study the contribution of dietary triacylglycerol (labeled with [U-13C]palmitate) and endogenous triacylglycerol (ie, VLDL and circulating FFAs, both labeled with [3H2]palmitate) to lipid overflow and skeletal muscle FA processing, as reported previously (6, 18, 20).

Test meal composition

The liquid test meal provided 2.6 MJ, consisting of 61% of energy as fat, 33% of energy as carbohydrates, and 6.3% of energy as protein. The subjects were asked to drink the test meal within 5 min. The FA composition of the test meals is presented in Table 2. The MUFA meal consisted of 40 g olive oil, and the PUFA meal contained 20 g safflower oil and 20 g fish oil (18% EPA and 12% DHA) (Bioriginal).

Tracer infusion study

Subjects arrived at the university after an overnight fast and were asked to refrain from drinking alcohol and to perform no strenuous exercise 24 h before the study day. In addition, they were asked to avoid food products naturally enriched with 13C (eg, corn and pineapple) during the entire study period. Skeletal muscle metabolism was studied in the forearm muscle, by using differences in arteriovenous concentrations corrected for blood flow. Three catheters were inserted before the start of the experiment. One catheter was placed retrogradely into a superficial dorsal hand vein, which was heated in a hot box (60°C) to obtain arterialized blood. In the same arm, another catheter was placed in an antecubital vein for the infusion of the [2H2]palmitate tracer. A third catheter was placed retrogradely in a deep antecubital vein of the contralateral forearm to sample venous blood draining the forearm muscle. After arterialized and deep venous background samples were collected 90 min before meal ingestion, a continuous intravenous infusion of the stable-isotope tracer [2H2]palmitate (97% enrichment; Cambridge Isotope Laboratories) complexed to albumin was started (0.035 μmol · kg body weight−1 · min−1). Baseline blood sampling was started after 1 h of tracer infusion to allow for isotope equilibration to occur. Blood samples were taken simultaneously from the radial artery and the deep muscle vein at 3 time points during fasting (−30, −15, and 0 min), and 6 time points postprandial (30, 60, 90, 120, 180, and 240 min) after consumption of a high-fat mixed meal (0 min) containing 200 mg [U-13C]palmitate (98% enrichment; Cambridge Isotope Laboratories). Forearm blood flow was measured before each blood sampling as described.
previously (21). Skeletal muscle biopsy samples were collected during fasting conditions (after placing the 3 catheters and before the background blood sampling) and at the end of the postprandial measurement period (240 min).

Indirect calorimetry

Energy expenditure and whole-body substrate utilization were measured by using an open-circuit ventilated hood system (Omnical) under fasting conditions and after meal ingestion (30, 60, 90, 120, 180, and 240 min) (22). Gas analyses, which occurred every 15 s, were performed with dual-paramagnetic oxygen analyzers and dual-infrared carbon dioxide analyzers (type 1156, 1507, 1520; Servomex), similar to the analysis system described by Schoffelen et al (23). Nitrogen excretion was calculated on the basis of the assumption that protein oxidation represents ∼15% of total energy expenditure. Energy expenditure was calculated by using the formula of Weir (24).

Biochemical analyses

Blood was collected into EDTA-containing tubes and centrifuged (1000 × g, 10 min, 4°C), and the plasma was immediately frozen in liquid nitrogen and stored at −80°C until analysis. Plasma FFAs were analyzed by using standard enzymatic techniques automated on a Cobas-Fara centrifugal spectrophotometer (Roche Diagnostics). Plasma triacylglycerol, glycerol, glucose, and lactate were analyzed enzymatically on a Cobas Mira automated spectrophotometer. Plasma insulin was measured with a double-antibody radioimmunoassay (Linco Research). Hematocrit was measured in heparin-containing blood by using a microcapillary system.

To determine isotope enrichment of plasma FFAs and triacylglycerol, total lipids were extracted from the plasma by using chloroform-methanol 2:1 (vol:vol). The FFA and triacylglycerol fractions were separated by thin-layer chromatography and derivatized to their methyl esters for the analysis of plasma palmitate. Plasma fractions were analyzed for the ratio of 13C to 12C with a GC continuous-flow isotope ratio mass spectrometer (MAT-252 GC-isotope ratio mass spectrometer; Finnigan) and for enrichment of [2H2] (Incos-XL GC-MS; Finnigan). The methyl ester of palmitate contains 17 carbon atoms; therefore, the TTR of palmitate was corrected for the extra methyl group. Plasma palmitate concentrations (μmol/L) were analyzed on an analytic GC with ion flame detection by using heptadecanoic acid as internal standard.

Skeletal muscle biopsy

Skeletal muscle biopsy samples were collected at baseline (after placement of the 3 catheters and before background blood sampling) and at the end of the postprandial measurement period (240 min). Biopsy samples were obtained from the vastus lateralis muscle under local anesthesia of the skin and fascia by using the Bergström method with suction (25), cleaned from any visible fat and blood, immediately frozen in isopentane at its melting point, and stored at −80°C until analyzed. The biopsy samples were collected to determine the intramuscular triacylglycerol, diacylglycerol, FFA, and phospholipid content and degree of saturation and the FSR of triacylglycerol, diacylglycerol, and phospholipid (see “Skeletal muscle lipids” below). We investigated the gene expression profiles by using microarray.

Skeletal muscle lipids

Skeletal muscle biopsy samples were lyophilized and dissected free of extramyocellular lipid, blood, and connective tissue. Total lipids were extracted from 10- to 20-mg muscle
samples by using chloroform-methanol (2:1, vol:vol) and internal standards and thereafter evaporated under nitrogen at 37°C. The extracted lipids were separated into triacylglycerol, diacylglycerol, FFA, and phospholipid by thin-layer chromatography and transferred into tubes for methylation. The triacylglycerol and diacylglycerol fractions were methylated by adding 1 mL toluene-methanol-(BF3-methanol 14%) (20%, 55%, and 25%, respectively; vol:vol) and incubated in capped tubes for 30 min at 100°C. The phospholipid fraction was methylated by adding 1 mL (BF3-methanol, 14%) and incubated in capped tubes for 90 min at 100°C. The FFA fraction was methylated by adding 1 mL methanol-(BF3-methanol 14%) (50% and 50%, respectively; vol:vol) and incubated in capped tubes at room temperature for 15 min. After incubation, 2 mL pentane was added to the samples, vortex mixed, and centrifuged (1000 x g, 5 min, 20°C), which was followed by isolation of pentane extracts (upper phase) and evaporation under nitrogen at 30°C. Finally, the residues were dissolved in iso-octane, and concentrations of FA in the fractions were measured by using an analytic GC. Stable-isotope enrichment of the lipid fractions was determined by measuring the ratio of 13C to 12C at a GC continuous-flow isotope ratio-mass spectrometer (Finnigan MAT-252).

### Skeletal muscle gene expression

RNA extraction. Total RNA was extracted from frozen skeletal muscle specimens by using TRIzol reagent (Invitrogen). The quantity of RNA was measured with the ND-100 spectrophotometer (Isogen Life Science), and RNA integrity was analyzed.

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**Table 3**: Integrated responses (AUC) of plasma metabolites after the intake of high-fat mixed meals

<table>
<thead>
<tr>
<th></th>
<th>SFA</th>
<th>MUFA</th>
<th>PUFA</th>
<th>ANOVA</th>
<th>SFA vs MUFA</th>
<th>SFA vs PUFA</th>
<th>MUFA vs PUFA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterialized glucose (mmol · min⁻¹)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Postprandial (0–4 h)</td>
<td>5.77 ± 0.13</td>
<td>5.59 ± 0.14</td>
<td>5.48 ± 0.12</td>
<td>0.003</td>
<td>0.139</td>
<td>0.006</td>
<td>0.301</td>
</tr>
<tr>
<td>Early</td>
<td>5.94 ± 0.15</td>
<td>5.79 ± 0.15</td>
<td>5.74 ± 0.13</td>
<td>0.040</td>
<td>0.474</td>
<td>0.013</td>
<td>1.000</td>
</tr>
<tr>
<td>Mid</td>
<td>5.61 ± 0.14</td>
<td>5.39 ± 0.15</td>
<td>5.22 ± 0.15</td>
<td>0.002</td>
<td>0.179</td>
<td>0.011</td>
<td>0.174</td>
</tr>
<tr>
<td>Arterialized insulin (μU · min⁻¹)</td>
<td></td>
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</tr>
<tr>
<td>Postprandial (0–4 h)</td>
<td>38.7 ± 4.5</td>
<td>34.5 ± 4.1</td>
<td>27.0 ± 2.5</td>
<td>0.028</td>
<td>0.681</td>
<td>0.033</td>
<td>0.387</td>
</tr>
<tr>
<td>Early</td>
<td>46.3 ± 6.2</td>
<td>42.2 ± 5.3</td>
<td>34.7 ± 4.3</td>
<td>0.182</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Mid</td>
<td>31.0 ± 3.9</td>
<td>26.8 ± 3.6</td>
<td>19.3 ± 2.0</td>
<td>0.006</td>
<td>0.243</td>
<td>0.021</td>
<td>0.305</td>
</tr>
<tr>
<td>Net glucose flux across forearm muscle (μmol · 100 mL⁻¹ · min⁻¹)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Postprandial (0–4 h)</td>
<td>0.59 ± 0.09</td>
<td>0.52 ± 0.06</td>
<td>0.51 ± 0.11</td>
<td>0.228</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Early</td>
<td>0.70 ± 0.14</td>
<td>0.51 ± 0.06</td>
<td>0.58 ± 0.14</td>
<td>0.075</td>
<td>0.107</td>
<td>0.254</td>
<td>1.000</td>
</tr>
<tr>
<td>Mid</td>
<td>0.48 ± 0.05</td>
<td>0.52 ± 0.09</td>
<td>0.43 ± 0.09</td>
<td>0.289</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Arterialized lactate (mmol · min⁻¹)</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Postprandial (1–4 h)</td>
<td>1.08 ± 0.06</td>
<td>1.00 ± 0.09</td>
<td>1.02 ± 0.08</td>
<td>0.366</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
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<tr>
<td>Early</td>
<td>1.12 ± 0.06</td>
<td>1.14 ± 0.11</td>
<td>1.20 ± 0.09</td>
<td>0.244</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Mid</td>
<td>1.04 ± 0.08</td>
<td>0.87 ± 0.07</td>
<td>0.83 ± 0.09</td>
<td>0.048</td>
<td>0.068</td>
<td>0.145</td>
<td>1.000</td>
</tr>
<tr>
<td>Arterialized FFAs (mmol · min⁻¹)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Postprandial (0–4 h)</td>
<td>336 ± 17</td>
<td>377 ± 33</td>
<td>377 ± 26</td>
<td>0.193</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Early</td>
<td>374 ± 28</td>
<td>360 ± 33</td>
<td>350 ± 29</td>
<td>0.704</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Mid</td>
<td>297 ± 16</td>
<td>393 ± 36</td>
<td>403 ± 29</td>
<td>0.019</td>
<td>0.142</td>
<td>0.086</td>
<td>1.000</td>
</tr>
<tr>
<td>Rate of appearance of FFAs (μmol · kg⁻¹ · min⁻¹)</td>
<td></td>
<td></td>
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<tr>
<td>Postprandial (0–4 h)</td>
<td>4.60 ± 0.40</td>
<td>5.29 ± 0.42</td>
<td>5.62 ± 0.28</td>
<td>0.019</td>
<td>0.202</td>
<td>0.044</td>
<td>1.000</td>
</tr>
<tr>
<td>Early</td>
<td>5.09 ± 0.43</td>
<td>5.71 ± 0.47</td>
<td>5.86 ± 0.36</td>
<td>0.174</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Mid</td>
<td>4.11 ± 0.42</td>
<td>4.88 ± 0.46</td>
<td>5.37 ± 0.25</td>
<td>0.021</td>
<td>0.357</td>
<td>0.029</td>
<td>0.717</td>
</tr>
<tr>
<td>ArterIALIZED TAG (μmol · min⁻¹)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Postprandial (0–4 h)</td>
<td>1716 ± 192</td>
<td>1727 ± 183</td>
<td>1424 ± 180</td>
<td>0.377</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Early</td>
<td>1359 ± 179</td>
<td>1274 ± 162</td>
<td>1125 ± 148</td>
<td>0.656</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Mid</td>
<td>2073 ± 209</td>
<td>2181 ± 208</td>
<td>1724 ± 218</td>
<td>0.141</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
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<tr>
<td>[³H]Palmitate in TAG (μmol · min⁻¹)</td>
<td></td>
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<tr>
<td>Postprandial (0–4 h)</td>
<td>9.8 ± 0.9</td>
<td>8.8 ± 0.8</td>
<td>8.0 ± 0.8</td>
<td>0.036</td>
<td>0.208</td>
<td>0.176</td>
<td>0.496</td>
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<tr>
<td>Early</td>
<td>6.0 ± 0.6</td>
<td>5.5 ± 0.5</td>
<td>5.3 ± 0.6</td>
<td>0.224</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Mid</td>
<td>13.6 ± 1.3</td>
<td>12.0 ± 1.2</td>
<td>10.6 ± 1.1</td>
<td>0.025</td>
<td>0.185</td>
<td>0.125</td>
<td>0.445</td>
</tr>
<tr>
<td>[U-¹³C]Palmitate in TAG (μmol · min⁻¹)</td>
<td></td>
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<tr>
<td>Postprandial (0–4 h)</td>
<td>4.0 ± 0.4</td>
<td>7.0 ± 1.0</td>
<td>3.8 ± 0.5</td>
<td>0.024</td>
<td>0.149</td>
<td>1.000</td>
<td>0.079</td>
</tr>
<tr>
<td>Early (1–2 h)</td>
<td>13.3 ± 3.2</td>
<td>22.2 ± 10.2</td>
<td>7.5 ± 2.5</td>
<td>0.249</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Mid</td>
<td>5.6 ± 0.8</td>
<td>9.9 ± 1.7</td>
<td>5.4 ± 1.0</td>
<td>0.019</td>
<td>0.107</td>
<td>1.000</td>
<td>0.069</td>
</tr>
</tbody>
</table>

1 All values are means ± SEMs; n = 10. Postprandial data are expressed as AUC/min and are based on postprandial period (0–4 h after meal ingestion) unless otherwise stated. “Early” is defined as 0–2 h after meal ingestion, and “Mid” is defined as 2–4 h after meal ingestion. FFAs, free fatty acids; NA, not applicable; TAG, triacylglycerol.

2 Post hoc testing was performed by using the Bonferroni correction.

ANOVA with a GC continuous-flow isotope ratio-mass spectrometer (Finnigan MAT-252).
on an Agilent 2100 BioAnalyzer (Agilent Technologies) by using nanochips according to the manufacturer’s instructions.

Microarray processing. Total RNA (100 ng/sample) was labeled by using Whole Transcript Sense Target Assay and hybridized to human whole-genome Affymetrix Gene 1.0 ST arrays targeting 19,793 unique genes (Affymetrix). Sample labeling, hybridization to chips, and image scanning were performed according to the manufacturers’ instructions.

Microarray data analysis. Quality control was performed and fulfilled the criteria for array hybridization suggested by the Tumor Analysis Best Practices Working Group (26). Microarrays were analyzed by using the reorganized oligonucleotide probes as described by Dai et al (27). All individual probes for a gene were combined, which allowed the possibility of detecting overall transcription activity on the basis of the latest genome and transcriptome information instead of on the basis of the Affymetrix probe set annotation. Expression values were calculated with the Robust Multichip Average method and normalized by using quantile normalization (28, 29). Only probe sets with normalized signals $\geq 20$ on $>4$ arrays were defined as expressed and selected for analysis. Individual genes were defined as changed when comparison of the normalized signal intensities showed a false discovery rate (30) $Q$ value $\leq 0.05$ in a 2-tailed paired $t$ test with Bayesian correction (Limma) (31).

Calculations

The homeostasis model assessment for insulin resistance was calculated according to Matthews et al (33). The $M$-value from the hyperinsulinemic-euglycemic clamp was calculated as described by DeFronzo et al (19). Glucose and insulin AUCs in response to the meal and $\text{AUC}_{\text{glucose}} \times \text{AUC}_{\text{insulin}} \times 10^{-6}$ (PGI) were used as indexes of postprandial insulin sensitivity (34, 35). Lower PGI values indicate better insulin sensitivity. The net flux of metabolites (labeled and unlabeled) across the forearm muscle was calculated by multiplying the arteriovenous concentration difference by forearm plasma flow. Plasma flow was calculated by multiplying forearm blood flow as $(1 - \text{hematocrit})/100$. A positive flux indicates net uptake across forearm muscle, whereas a negative flux indicates net release. Fractional extraction of metabolites (%) was calculated as the arteriovenous concentration difference divided by the arterIALIZED concentration. As a measure

**FIGURE 2.** Postprandial whole-body FFA metabolism. Mean ($\pm$SEM) arterialized plasma concentrations (A), the TTR of [U-13C]palmitate (D), $n = 10$. Repeated-measures ANOVA was performed with Bonferroni post hoc testing by using the integrated responses (AUC) (see Table 3). B and D: Paired Student’s $t$ test was also used to analyze the difference in TTR between arterialized and deep venous plasma. B: The TTR of [U-13C]palmitate in FFAs was higher in arterIALIZED than in deep venous plasma after all high-fat mixed meals (incremental AUC: SFA, $P = 0.004$; MUFA, $P = 0.002$; PUFA, $P = 0.001$). D: The TTR of [U-13C]palmitate was not significantly different between arterIALIZED and deep venous plasma after the 3 high-fat mixed meals. B and D: No significant differences were observed between the 3 high-fat mixed meals. White symbols represent arterialized plasma concentrations, and black symbols represent deep venous plasma concentration. FFA, free fatty acid; $Ra_{FFA}$, rate of appearance of free fatty acid; TTR, tracer-tracee ratio.
of efficiency of substrate removal from the circulation, clearance across the forearm muscle was calculated by multiplying the fractional extraction by forearm plasma flow. Fasting RaFFA (μmol · kg⁻¹ · min⁻¹) was calculated with Steele’s equation for steady state, whereas Steele’s single-pool non–steady state equations adapted for use with stable isotopes was used to calculate RaFFA in the postprandial state (36). Labeled FFA and triacylglycerol concentrations were calculated as the product of fractional extraction by forearm plasma flow. Fasting RaFFA across the forearm muscle was calculated by multiplying the fraction of extraction by forearm plasma flow. A steady state was assumed that a proportion of FAs derived from triacylglycerol hydrolysis may not be taken up by skeletal muscle (6, 18).

The FSR of skeletal muscle triacylglycerol, diacylglycerol, and phospholipid were calculated by using skeletal muscle FFA as the precursor pool for lipid synthesis (37). The increase in TTR...

### TABLE 4

Fasting and postprandial FFA and TAG metabolism and forearm blood flow

<table>
<thead>
<tr>
<th></th>
<th>SFA</th>
<th>MUFA</th>
<th>PUFA</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forearm blood flow (mL · 100 mL⁻¹ · min⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting</td>
<td>3.28 ± 0.53</td>
<td>2.74 ± 0.29</td>
<td>2.65 ± 0.21</td>
<td>0.189</td>
</tr>
<tr>
<td>Postprandial (0–4 h)</td>
<td>3.25 ± 0.41</td>
<td>2.95 ± 0.26</td>
<td>2.59 ± 0.26</td>
<td>0.859</td>
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<tr>
<td>Early</td>
<td>3.28 ± 0.48</td>
<td>2.90 ± 0.27</td>
<td>2.77 ± 0.26</td>
<td>0.725</td>
</tr>
<tr>
<td>Mid</td>
<td>3.10 ± 0.26</td>
<td>2.87 ± 0.23</td>
<td>3.01 ± 0.23</td>
<td>0.628</td>
</tr>
<tr>
<td>Net FFA flux across forearm muscle (nmol · 100 mL⁻¹ · min⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting</td>
<td>14.3 ± 80.9</td>
<td>20.4 ± 61.2</td>
<td>11.5 ± 60.1</td>
<td>0.937</td>
</tr>
<tr>
<td>Postprandial (0–4 h)</td>
<td>26.7 ± 37.2</td>
<td>47.2 ± 29.6</td>
<td>20.0 ± 32.7</td>
<td>0.375</td>
</tr>
<tr>
<td>Early</td>
<td>-14.0 ± 52.2</td>
<td>6.7 ± 39.1</td>
<td>-20.2 ± 35.0</td>
<td>0.195</td>
</tr>
<tr>
<td>Mid</td>
<td>67.3 ± 24.0</td>
<td>87.6 ± 22.0</td>
<td>64.5 ± 37.5</td>
<td>0.601</td>
</tr>
<tr>
<td>[³H₂]Palmitate FFA net flux across forearm muscle (nmol · 100 mL⁻¹ · min⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting</td>
<td>2.19 ± 0.47</td>
<td>1.78 ± 0.40</td>
<td>1.80 ± 0.34</td>
<td>0.504</td>
</tr>
<tr>
<td>Postprandial (0–4 h)</td>
<td>1.80 ± 0.37</td>
<td>1.58 ± 0.23</td>
<td>1.68 ± 0.29</td>
<td>0.703</td>
</tr>
<tr>
<td>Early</td>
<td>1.78 ± 0.40</td>
<td>1.49 ± 0.32</td>
<td>1.48 ± 0.32</td>
<td>0.271</td>
</tr>
<tr>
<td>Mid</td>
<td>1.83 ± 0.38</td>
<td>1.66 ± 0.32</td>
<td>1.89 ± 0.33</td>
<td>0.826</td>
</tr>
<tr>
<td>[¹³C]Palmitate FFA net flux across forearm muscle (nmol · 100 mL⁻¹ · min⁻¹)</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Fasting</td>
<td>7.53 ± 3.64</td>
<td>9.87 ± 3.97</td>
<td>3.69 ± 4.56</td>
<td>0.448</td>
</tr>
<tr>
<td>Postprandial (1–4 h)</td>
<td>0.07 ± 0.10</td>
<td>-0.07 ± 0.12</td>
<td>0.02 ± 0.03</td>
<td>0.505</td>
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<tr>
<td>Mid</td>
<td>0.06 ± 0.03</td>
<td>0.08 ± 0.03</td>
<td>0.03 ± 0.04</td>
<td>0.408</td>
</tr>
<tr>
<td>Net TAG flux across forearm muscle (nmol · 100 mL⁻¹ · min⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting</td>
<td>58.0 ± 35.5</td>
<td>21.6 ± 9.7</td>
<td>44.8 ± 21.5</td>
<td>0.365</td>
</tr>
<tr>
<td>Postprandial (0–4 h)</td>
<td>81.4 ± 9.9</td>
<td>70.3 ± 55.0</td>
<td>48.7 ± 16.3</td>
<td>0.526</td>
</tr>
<tr>
<td>Early</td>
<td>85.6 ± 33.8</td>
<td>47.6 ± 10.8</td>
<td>44.6 ± 16.3</td>
<td>0.904</td>
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<tr>
<td>Mid</td>
<td>74.4 ± 25.2</td>
<td>87.2 ± 24.1</td>
<td>68.9 ± 15.3</td>
<td>0.647</td>
</tr>
<tr>
<td>[³H₂]Palmitate TAG net flux across forearm muscle (nmol · 100 mL⁻¹ · min⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting</td>
<td>0.05 ± 0.27</td>
<td>0.12 ± 0.21</td>
<td>0.38 ± 0.14</td>
<td>0.479</td>
</tr>
<tr>
<td>Postprandial (0–4 h)</td>
<td>-0.08 ± 0.30</td>
<td>-0.35 ± 0.20</td>
<td>0.18 ± 0.18</td>
<td>0.277</td>
</tr>
<tr>
<td>Early</td>
<td>0.13 ± 0.24</td>
<td>0.31 ± 0.19</td>
<td>0.34 ± 0.23</td>
<td>0.678</td>
</tr>
<tr>
<td>Mid</td>
<td>-0.28 ± 0.53</td>
<td>-1.02 ± 0.31</td>
<td>0.02 ± 0.36</td>
<td>0.255</td>
</tr>
<tr>
<td>[¹³C]Palmitate TAG net flux across forearm muscle (nmol · 100 mL⁻¹ · min⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting</td>
<td>0.35 ± 0.18</td>
<td>-0.18 ± 0.18</td>
<td>0.12 ± 0.30</td>
<td>0.256</td>
</tr>
<tr>
<td>Postprandial (0–4 h)</td>
<td>0.19 ± 0.07</td>
<td>0.30 ± 0.10</td>
<td>0.04 ± 0.02</td>
<td>0.103</td>
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<tr>
<td>Early</td>
<td>0.43 ± 0.26</td>
<td>-0.43 ± 0.31</td>
<td>0.16 ± 0.45</td>
<td>0.214</td>
</tr>
</tbody>
</table>

1 All values are means ± SEMs; n = 10. Postprandial data are expressed as AUC/min and are based on the postprandial period (0–4 h after meal ingestion) unless otherwise stated. “Early” is defined as 0–2 h after meal ingestion, and “Mid” is defined as 2–4 h after meal ingestion. Post hoc testing was performed by using the Bonferroni correction. *Significantly different from MUFA, P < 0.1. **Significantly different from SFA, P < 0.05. FFA, free fatty acid; NA, not applicable; TAG, triacylglycerol.
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of $[\text{U-}^{13}\text{C}]$ from fasting to 4 h postprandially was divided by the
enrichment of skeletal muscle FFA and expressed as percentage
per hour (%/h). The degree of saturation of skeletal muscle triacylglycerol, diacylglycerol, phospholipid, and FFA (%/h) was
calculated by dividing the sum of SFAs by the total amount of
FFAs in a fraction. $\Delta^9$-Desaturase activity was estimated as the
proportion of palmitoleic acid (16:1n\(-7\)) to palmitic acid (16:0)
and as the proportion of oleic acid (18:1n\(-9\)) to stearic acid
(18:0). Total skeletal muscle triacylglycerol, diacylglycerol,
FFA, and phospholipid contents were estimated as the sum of
the particular FA content of the assessed fraction. Postprandial
AUCs of metabolites and substrate fluxes were calculated by
using the trapezoidal rule. In addition to the total AUC (0–4 h
after meal ingestion), the “early” (0–2 h) and “mid” (2–4 h)
AUCs were calculated to obtain more detailed information about
the time course of postprandial responses.

Statistical analysis

Differences between the diets were examined by using re-
peated-measures ANOVA (using AUC). When a significant diet \times
time interaction was observed, Bonferroni post hoc testing was
performed. Statistical analyses were performed by using SPSS
19.0 for Mac (SPSS Inc). All data are presented as means ±
SEMs. Significance was set at $P < 0.05$.

RESULTS

Arterialized metabolites, forearm blood flow, and forearm
muscle metabolism

Fasting arterialized plasma glucose (Figure 1A) and insulin
(Figure 1B) concentrations were comparable between the
different meal test days. The postprandial arterialized plasma
glucose and insulin concentrations were higher after the SFA
meal than after the PUFA meal (glucose: $P = 0.006$; insulin: $P =
0.033$; Table 3); values were intermediate after the MUFA meal.
AUC for glucose and insulin, and the product of AUC$_\text{glucose}$
\times
AUC$_\text{insulin}$ \times $10^{-6}$ (PGI), were used as indexes of insulin sensi-
tivity. The PGI was significantly higher after the SFA meal
(12.8 ± 1.5) than after the PUFA meal (8.5 ± 0.8) ($P = 0.02$).
Net glucose flux (Figure 1C), arterialized lactate concentrations
(Figure 1D), and net lactate flux (data not shown) were not
different after intake of the different meals (Table 3).

Whole-body and forearm muscle FFA metabolism

Whole-body FFA metabolism

Postprandially, arterialized FFA concentrations decreased
during the first 2 h and returned to fasting values after 4 h in all
conditions (Figure 2A). FFA concentrations tended to be lower
after the SFA meal in the midphase (2–4 h) than after the PUFA
meal ($P = 0.086$, Table 3). $[\text{H}_2]$Palmitate was infused in-
travenously and mixed with the plasma FFA pool. The TTR
reached a steady state during fasting measurements (Figure 2B).
Consistent with these findings, the Ra$_\text{FFA}$ in the circulation
decreased after the meals, which indicates suppression of whole-
body lipolysis (Figure 2C). The Ra$_\text{FFA}$ was more reduced after the
SFA meal in the postprandial phase than after the PUFA
meal ($P = 0.044$; Table 3). $[\text{U-}^{13}\text{C}]$Palmitate (resulting from
spillover of FA derived from chylomicron-triacylglycerol hy-
drolysis) appeared in the plasma FFA pool from 60 min after
meal ingestion, and concentrations increased throughout the
postprandial period. No differences in arterIALIZED concen-
trations of $[\text{H}_2]$- or $[\text{U-}^{13}\text{C}]$ palmitate in FFA were observed
after the 3 high-fat mixed meals at any time (data not shown).
The TTR of $[\text{H}_2]$ palmitate in FFAs was higher in arterialized
than in deep venous plasma after all of the high-fat mixed meals
(SFA: $P = 0.004$; MUFA: $P = 0.002$; PUFA: $P = 0.001$), which
indicated dilution of the $[\text{H}_2]$ tracer in the plasma FFA pool
across forearm muscle (Figure 2B). The TTR of $[\text{U-}^{13}\text{C}]$pal-
mitate in FFAs was not different between arterialized and deep
venous plasma after the 3 high-fat mixed meals (Figure 2D).

Forearm muscle FFA metabolism

The net flux of plasma FFAs across forearm muscle (arte-
riovenous concentration difference multiplied by forearm plasma
flow) was not different after the high-fat mixed meals (Table 4).
At the same time, a consistent uptake of $[\text{H}_2]$palmitate FFA was
observed after all of the high-fat mixed meals (Table 4), and no
differences were observed between meals.

Whole-body and forearm muscle triacylglycerol
metabolism

Whole-body triacylglycerol metabolism

Arterialized plasma triacylglycerol concentrations were similar
between groups under fasting conditions and after ingestion of
a high-fat mixed meal (Figure 3A and Table 3). The $[\text{H}_2]$pal-
mitate tracer could be measured in plasma triacylglycerol from
the first baseline sample onward, which reflected incorporation of
the intravenously infused tracer into VLDL-triacylglycerol (Fig-
ure 3B). The $[\text{U-}^{13}\text{C}]$palmitate tracer, given with the meal, ap-
ppeared in plasma triacylglycerol from 60 min after meal ingestion,
which represented chylomicron-triacylglycerol in the circulation
(Figure 3B). Both labeled triacylglycerol fractions increased up
to 4 h postprandially, whereas the $[\text{U-}^{13}\text{C}]$palmitate in triacyl-
glycerol tended to increase more pronounced after the MUFA
meal than after the PUFA meal ($P = 0.079$; Figure 3B and Table
3). The increase in $[\text{H}_2]$palmitate in triacylglycerol was most
pronounced after the SFA meal ($P = 0.036$; Figure 3B and Table 3).

Forearm muscle triacylglycerol metabolism

Fractional extraction of $[\text{U-}^{13}\text{C}]$palmitate in triacylglycerol
across forearm muscle was consistently greater than that of
$[\text{H}_2]$palmitate in triacylglycerol throughout the entire post-
prandial period (data not shown). No significant differences in
the extraction of $[\text{H}_2]$palmitate in triacylglycerol were observed
after the 3 high-fat mixed meals (Table 4). Extraction of $[\text{U-}^{13}\text{C}]$
palmitate in triacylglycerol tended to be lower after the PUFA
meal than after the MUFA meal in the early phase ($P = 0.06$, Table
4). The (unidirectional) uptake of FA derived from tri-
acylglycerol hydrolysis across forearm muscle was lower after
the PUFA meal than after the SFA meal ($P = 0.02$; Figure 3C
and Table 4).

Energy expenditure and whole-body substrate metabolism

Whole-body energy expenditure, respiratory quotient, carbo-
hydrate oxidation, and fat oxidation during fasting and after meal
Ingestion were comparable between the different meals (data not shown).

Intramuscular lipid metabolism

Muscle lipid content and composition

The content of skeletal muscle triacylglycerol, diacylglycerol, FFA, and phospholipid did not change after the 3 high-fat mixed meals (data not shown). The saturation of muscle triacylglycerol, diacylglycerol, phospholipid, and FFA was not different after the ingestion of the different meals (data not shown). Also, the individual FAs in the different muscle lipid pools did not change after the 3 meals (data not shown).

Incorporation of [U-13C]palmitate in muscle lipids (FSR)

The FSR of skeletal muscle diacylglycerol \((P = 0.015)\) was higher after the MUFA meal than after the SFA meal and tended to be higher in the triacylglycerol \((P = 0.067)\) and phospholipid \((P = 0.08)\) fractions (Figure 4). After the PUFA meal, FSR values were of similar magnitude after the MUFA meal but were not statistically different from the SFA meal. This may indicate that a higher proportion of palmitate from the intramuscular FFA pool was directed toward the muscle lipid pools after the unsaturated FA meals.

Microarray analysis

Microarray analysis was performed on skeletal muscle tissue before and 4 h after consumption of a high-fat mixed meal consisting of SFA, MUFA, or PUFA. During 2 skeletal muscle biopsy procedures, we could not obtain enough biopsy material for further analysis. From the 19,793 genes present on the array, 14,424 genes were defined as expressed in skeletal muscle (Figure 5A). Genes that showed comparable regulation after the 3 meals (5429 genes) were not taken into account for further analysis (Figure 5B). About 66% of the genes that were changed after the SFA and MUFA meals were downregulated, whereas 60% of the genes were downregulated after the PUFA meal. To obtain further insight into the physiologic relevance of these genes, pathway analysis was performed. The top 10 most significantly regulated genes are shown in Figure 5B.

![Figure 3](https://example.com/figure3.png)

**FIGURE 3.** Postprandial triacylglycerol metabolism. Mean (±SEM) arterialized plasma triacylglycerol concentrations (A), [2H2]palmitate and [U-13C]palmitate in triacylglycerol (B), and total uptake of triacylglycerol-derived FA during fasting (0 min) and after consumption of a high-fat mixed meal. \(n = 10\). Repeated-measures ANOVA was performed with Bonferroni post hoc testing by using the integrated responses (AUC) (see Tables 3 and 4). Black symbols represent [2H2]palmitate, and white symbols represent [13C]palmitate in panel C. FA, fatty acids; palm., palmitate; TAG, triacylglycerol.

![Figure 4](https://example.com/figure4.png)

**FIGURE 4.** Mean (±SEM) FSR in triacylglycerol (A), diacylglycerol (B), and phospholipid (C) after consumption of a high-fat mixed meal. \(n = 8\). Paired Student’s \(t\) tests were performed. DAG, diacylglycerol; FSR, fractional synthetic rate; PL, phospholipid; TAG, triacylglycerol.
pathways after each meal are listed in Figure 6. The pathways that were affected the most after consumption of the SFA and MUFA meals were those involved in ubiquinone biosynthesis, mitochondrial dysfunction, and oxidative phosphorylation, which consisted of genes involved in complex I-V of the respiratory chain. Consumption of the SFA meal resulted in a significantly decreased expression of 33 of 96 oxidative phosphorylation genes, whereas 28 genes were downregulated after the MUFA meal and 7 after the PUFA meal. Thus, fewer genes were significantly downregulated after the PUFA meal, which indicated less downregulation of mitochondrial function (data not shown). Quantitative PCR was used to confirm gene expression changes revealed by microarray analysis. We selected genes involved in complex I-V of the respiratory chain. All genes were significantly downregulated after the SFA meal, which confirmed the microarray analysis. NADH dehydrogenase (ubiquinone) 1α subcomplex, NADH dehydrogenase (ubiquinone) 1β subcomplex 3, cytochrome c oxidase assembly homolog, and ATP synthase H+ transporting, mitochondrial Fo complex, subunit F6 were less downregulated after the PUFA meal, so the change in expression of genes showed the same direction of change as the microarray, but the relatively small differences in expression of individual genes were not statistically significant between meals (data not shown).

DISCUSSION

The current study examined postprandial skeletal muscle FA processing in obese, insulin-resistant men consuming 3 high-fat mixed meals with different FA compositions. Ingestion of the PUFA meal resulted in an improved postprandial insulin sensitivity compared with SFA, which was accompanied by a reduced muscle triacylglycerol-derived FA uptake and a tendency toward increased intramuscular lipid turnover.

Arterialized plasma insulin and glucose concentrations were higher after the SFA meal than after the PUFA meal, with intermediate values for the MUFA meal. The finding of no differences in net glucose flux between meals implies higher postprandial insulin sensitivity after the PUFA meal (as evidenced by data of the PGI). Our data are in line with those of previous studies, showing that a PUFA meal improved insulin sensitivity as compared with an SFA meal (38, 39). We found that the effect of MUFA was intermediate between the SFA meal and the PUFA meal, which agrees with studies that have shown beneficial effects of MUFA on insulin sensitivity as compared with SFAs (39–42).

This differential effect on insulin sensitivity of PUFAs, as compared with SFAs, was accompanied by differences in skeletal muscle FA metabolism. The uptake of triacylglycerol-derived FA by forearm muscle decreased after the PUFA meal in the postprandial period, as compared with the SFA and MUFA meals. Circulating triacylglycerol concentrations were not significantly lower after the PUFA meal than after the other meals, which suggests that a reduced triacylglycerol supply is not responsible for the reduced PUFA-induced triacylglycerol extraction. The reduced triacylglycerol extraction may be related to the decreased postprandial insulin concentrations, which may have resulted in reduced muscle lipoprotein lipase activity (35). Furthermore, the FA transporter CD36 is acutely upregulated during insulin stimulation (34). Thus, reduced CD36 content in skeletal muscle may also have contributed to the lower triacylglycerol extraction after the PUFA meal.

In addition to the reduced uptake of triacylglycerol-derived FAs with PUFA, it seemed that there was a higher FSR of the triacylglycerol, diacylglycerol, and phospholipid pools after the MUFA meal than after the SFA meal. The FSR of the lipid pools was comparable after the PUFA and MUFA meals, but values after the PUFA meal were not significantly different from the SFA meal. These data should be interpreted with caution, because skeletal muscle biopsies were collected 4 h after ingestion of the high-fat mixed meal, when a steady state had not yet been reached. Nevertheless, these data hint toward a higher incorporation of dietary [U-13C]palmitate from the muscle FFA pool in the different lipid fractions in skeletal muscle with unsaturated FAs compared with SFAs. In addition, there was
a lesser downregulation of the mitochondrial oxidative genes after the PUFA than after the SFA or MUFA meal, which suggests that overall lipid turnover was higher after the PUFA meal than after the SFA meal. The finding that a PUFA-induced improvement in postprandial insulin sensitivity was accompanied by a higher intramuscular lipid turnover is consistent with the findings of a recent study, which showed that obese prediabetic insulin-resistant subjects had a lower FSR of intramuscular triacylglycerol than did control subjects (43). Overall, our data support the concept that not the triacylglycerol concentration per se, but rather lipid turnover, may determine insulin sensitivity. Human studies that examined the short-term effect of dietary FAs on the postprandial lipid profile suggest that the FA composition of the meal may influence the absorption, synthesis, and secretion of dietary triacylglycerol as well as the size of the chylomicron particles (44–46). This study showed that postprandial circulating chylomicron concentrations were highest after the MUFA meal. Other studies also found increased triacylglycerol concentrations after a MUFA meal (44, 47–49). de Bruin et al (48) found that the removal rate of olive oil chylomicron remnants was lower, which may be explained by reduced endothelial lipoprotein lipase action due to their triacylglycerol content or greater particle number. This may imply that MUFA-chylomicrons have to rely on an alternative route for their removal from the circulation. Indeed, it has been suggested that hepatic lipase plays a significant role in the removal of MUFA-enriched particles (48, 50).

Note that we measured responses in the acute postprandial period. It remains to be established whether an improvement in insulin sensitivity after the PUFA meal, due to lower lipid uptake (and possible less accumulation of fat in the muscle), is relevant in the acute postprandial period. In fact, the increase in insulin sensitivity after the PUFA meal may also be explained by mechanisms other than lower lipid uptake. For example, differences in the secretion of gut hormones (glucagon-like peptide 1 and cholecystokinin) could potentiate differential insulin secretion and insulin sensitivity according to the FA composition of the meals (41, 46). Thus, the exact mechanisms responsible for the differential postprandial insulin sensitivity should be elucidated in future studies. Furthermore, it remains to be established whether these acute effects may contribute to findings after long-term dietary intervention. It has become clear from dietary intervention studies that long-term isocaloric replacement of SFAs has no major effects on insulin sensitivity (51).

In conclusion, the current study showed that dietary FAs have an acute effect on postprandial insulin sensitivity. Replacing SFAs with PUFAs may induce a reduced triacylglycerol-derived FA uptake and a tendency toward a higher lipid turnover, as reflected by a more transcriptional oxidative phenotype and altered intracellular lipid partitioning. These data suggest that the
effects of replacement of SFA by PUFA may contribute to lower uptake of lipids in skeletal muscle and therefore may protect against the development of insulin resistance in humans.

We thank all of the subjects for participation in the current study. We gratefully thank Jos Stegen, Wendy Sluijsmans, Hasibe Aydeniz, Yvonne Essers, and Annemie Gijssen for their excellent analytical support.

The authors’ responsibilities were as follows—EEB: designed the research; AJ, EK, GHG, FGB, and CCM: conducted the research; AJ, EK, MVB, and LAA: analyzed the data and performed the statistical analysis; AJ and EK: wrote the manuscript; EEB: had primary responsibility for the final content; and GHG, FGB, CCM, MVB, LAA, MM, ECM, and EEB: reviewed and edited the manuscript. None of the authors declared a conflict of interest.

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