Differential effect of saturated and polyunsaturated fatty acids on hepatic glucose metabolism in humans

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Clore, John N., Julie S. Stillman, Jing Li, Stephen J. D. O’Keeffe, and James R. Levy. Differential effect of saturated and polyunsaturated fatty acids on hepatic glucose metabolism in humans. Am J Physiol Endocrinol Metab 287:E358–E365, 2004. First published April 13, 2004; 10.1152/ajpendo.00360.2003.—Prolonged infusions of lipid and heparin that achieve high physiological free fatty acid (FFA) concentrations inhibit hepatic (and peripheral) insulin sensitivity in humans. These infusions are composed largely of polyunsaturated fatty acids (PUFA; linoleic and linolenic). It is not known whether fatty acid composition per se affects hepatic glucose metabolism in humans. To address this issue, we examined the impact of enteral infusions of either palm oil (48% palmitic, 35% oleic, and 8% linoleic acids) or safflower oil (6% palmitic, 12% oleic, 74% linoleic acids) in 14 obese nondiabetic subjects. 3H2O was administered to determine the contribution of gluconeogenesis to endogenous glucose production (EGP), and a primed continuous infusion of [6,6-2H]glucose was administered to assess glucose appearance. As a result of the lipid infusions, plasma FFA concentrations increased significantly in both the palm oil (507.5 ± 47.4 to 939.3 ± 61.3 μmol/l, P < 0.01) and safflower oil (588.2 ± 43.0 to 857.8 ± 68.7 μmol/l, P < 0.01) groups after 4 h. EGP was similar at baseline (12.4 ± 1.8 vs. 11.2 ± 1.0 μmol·kg FFM⁻¹·min⁻¹). During a somatostatin-insulin clamp, the glucose infusion rate was significantly lower (AUC glucose infusion rate 195.8 ± 50.7 vs. 377.8 ± 38.0 μmol/kg FFM, P < 0.01), and rates of EGP were significantly higher (10.7 ± 1.4 vs. 6.5 ± 1.5 μmol·kg FFM⁻¹·min⁻¹, P < 0.01) after palm oil compared with safflower oil, respectively. Baseline rates of gluconeogenesis and glycogenolysis were also similar. However, after lipid infusion, rates of glycogenolysis were suppressed by safflower oil but not by palm oil. Thus these studies demonstrate, for the first time in humans, a differential effect of saturated fatty acids and PUFA on hepatic glucose metabolism.

hepatic autoregulation; gluconeogenesis; glycogenolysis

A ROLE FOR FATTY ACIDS in hepatic insulin resistance is well established (22, 45). Experimental elevations in plasma free fatty acids (FFA) are associated with impaired suppression of endogenous glucose production (EGP) by insulin (7, 22). There is also evidence for a link between increased FFA availability and increased gluconeogenesis in humans (13, 17, 48, 52). However, the stimulation or suppression of gluconeogenesis in vivo is not generally associated with changes in EGP. In fact, gluconeogenesis is increased in obese nonobdiabetic individuals without an increase in EGP (21, 25). Thus regulation of hepatic glycogen storage is increasingly recognized as a critical component to the regulation of EGP and fasting hyperglycemia (17, 25, 52). Evidence that fatty acids play an important role in the regulation of liver glycogen is provided by recent studies that examined glycogenolysis directly by nuclear magnetic resonance during infusions of saline or Intralipid. In association with the expected increase in gluconeogenesis without a change in EGP, lipid infusion prevented the depletion of liver glycogen expected during fasting (52). However, it should be pointed out that intravenous infusions of lipid emulsions are composed primarily of linoleic (18:2) and linolenic (18:3) acids, essential polyunsaturated fatty acids (PUFA). It is not known whether infusions of saturated fatty acids (SFA) have the same effect on hepatic glucose metabolism in humans. Plasma SFA appear to predict the development of type 2 diabetes over 4 yr (33) and are increased in patients with type 2 diabetes in association with increased EGP (15). Observations linking regulation of glucose phosphorylation (15, 18), fatty acid oxidation (20), and insulin signaling (49) with differences in fatty acid composition suggest that defects in hepatic glucose disposal may be demonstrated with acute increases in SFA. By use of enteral infusions of safflower oil (PUFA) or palm oil (SFA), the present studies were designed to compare the acute effects of changes in plasma FFA composition on hepatic glucose disposal in humans.

METHODS

Fourteen obese nondiabetic subjects were enrolled in the proposed studies. The studies were approved by the institutional review board at Virginia Commonwealth University (VCU), and informed consent was obtained from each subject before enrollment. All subjects underwent a 75-g oral glucose tolerance test and none had diabetes mellitus (1). Body fat composition was assessed by skinfold measurements and bioimpedance, and usual dietary intake was assessed by analysis of a 3-day diet history using Nutrition Data System for Research, Minneapolis, MN.

Subjects were randomly assigned to one of two groups to receive either palm oil or safflower oil. Subjects were admitted to the General Clinical Research Center for the metabolic studies. They were taken to the endoscopy suite at VCU for placement of an enteral feeding tube. After nasal lubrication and oropharyngeal local anesthetic spray, a fine-bore (8-French) Silastic nasojejunal feeding tube was passed through the nose and guided through the stomach into the duodenum by fluoroscopy. Total fluoroscopy time was <5 min. Thereafter, the subjects received only water and/or ice chips by mouth until completion of the studies. Beginning at 12 midnight, subjects received a total of 5.0 ml H2O/kg total body water to enrich total body water to ~0.5%. Total body water was estimated as 50% of body weight for women and 60% for men, corrected for obesity by dividing the total body weight by the ratio of actual body mass index (BMI, 25 kg/m²). The water was ingested in four equal portions to minimize dizziness, which has been described in some subjects. At 0500, a primed [26.7 μmol/kg fat-free mass (FFM)] continuous (0.33 μmol·kg FFM⁻¹·min⁻¹) infusion of [6,6-2H]glucose was administered via forearm vein for the assessment of overall EGP (Fig. 1). Blood samples were obtained from a heated dorsal hand vein at 0740, 0750, 0800, and 0815 for the assessment of overall EGP (Fig. 1). Blood samples were obtained from a heated dorsal hand vein at 0740, 0750, 0800, and 0815 for the assessment of overall EGP (Fig. 1). Blood samples were obtained from a heated dorsal hand vein at 0740, 0750, 0800, and 0815 for the assessment of overall EGP (Fig. 1).

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Fig. 1. Study design.

cleared from the monoacetone with periodic acid, and the monoacetone was reduced with sodium borohydride. The monoacetone xylose was then hydrolyzed to D-xylose with H₂SO₄, and the hydrolysate was chromatographed over sequential anion (AG 1-X8, formate) and cation (AG 50W-X8 hydrogen) exchange columns. The neutral fraction containing xylose was evaporated to dryness. A hexamethyl-enetetraamine (HMT) derivative of C-5 in xylose was performed by the formation of formaldehyde. Oxidation of the xylose was performed by incubation with periodic acid (0.3 M) and sodium bicarbonate (1 N). After 1 h at room temperature, HCl (1 N) and sodium arsenite (1.2 N) were added, and the formaldehyde was distilled. The distillate was placed in 7 M NH₄OH and evaporated to dryness. Determination of deuterium enrichment in the HMT was performed by GC-MS in electron ionization mode by monitoring at mass 141 (m + 1). Yields with this method were consistently 35–40% of the starting glucose. Enrichment of plasma water was determined by GC-MS from plasma samples using the [U-¹³C]acetate method of Yang et al. (58).

**Fatty acid composition.** Fatty acid composition was analyzed according to Borkman et al. (11) with minor modifications. Total lipids were extracted by the method of Folch et al. (24). FFA were separated by thin-layer chromatography on silica gel G plates (Whatman LK6D) with a solvent system consisting of chloroform-ethanol-triethylamine-water (30;34;30;8, vol/vol/vol/vol) for the first development (SF1) and hexane-dimethyl ether (50;50, vol/vol) for the second development (SF2). The lids were visualized under UV light after the plate was sprayed with rhodamine G. The separated spots were scraped and placed into glass tubes. Fatty acid methyl esters were prepared with 1.5 ml of 1 N methanolic HCl at 80°C overnight. Fatty acid methyl esters were redissolved in 20 µl of hexane, separated, and analyzed on a Hewlett-Packard 5890 gas chromatograph equipped with a 30 × 0.2-mm fused silica capillary column (Omega wax 320; Supelco, Bellefonte, PA) and flame ionization detector. The injection temperature was 250°C and detector temperature 280°C. The initial oven temperature was 140°C. After 5 min, the oven temperature was increased from 140 to 200°C at a rate of 20°C/min, then to 280°C at 5°C/min. Fatty acids were identified by comparing their retention times with those of authentic standards.

Fatty acid standards were obtained from Sigma (St. Louis, MO). High-performance precoated silica gel H-P-K plates (10 × 10 cm) were purchased from Whatman (Clifton, NJ). All other reagents and solvents were of analytical or HPLC grade from Sigma or Fisher (Pittsburgh, PA).

**Calculations.** Baseline rates of glucose appearance (Rₐ) and disappearance (Rₐₑ) were calculated using the steady-state equation Rₐₑ = F/IE – F, where F is the isotopic infusion rate determined by direct measurement of the infused and IE is the steady-state enrichment (expressed as atom percent excess). Glucose Rₐₑ and Rₐ were during the clamp were calculated using non-steady-state equations (19). EGP was calculated as Rₐₑ – exogenous glucose infusion. The fraction of glucose derived from gluconeogenesis was estimated by the ratio of deuterium enrichments of C-5 to that of plasma water. Close agreement between results using the deuterium enrichment of C-2 of glucose [as originally described by Landau et al. (34)] and plasma water has recently been demonstrated (48).

Rates of gluconeogenesis were determined by the product of Rₐₑ and %gluconeogenesis (NGN) to correct for dilution related to infusion of unlabeled glucose (26). Glycogenolysis was defined as EGP – NGN. Absolute concentrations of individual FFA species were determined as the product of the percentage of fatty acid composition and total FFA concentration.

**Statistical analysis.** The primary end points of the study were rates of EGP, gluconeogenesis, and glycogenolysis. Rates are expressed in micromoles per kilogram FFM per minute, and mean values obtained in the two study groups were compared by analysis of variance. Statistical significance was assumed at the P < 0.05 level.
RESULTS

Characteristics of the subjects enrolled in the studies are presented in Table 1. All of the subjects were obese, and there were no differences between the two groups. Based on 3-day food records, the macronutrient composition of the subjects’ habitual diet was 49.5 ± 3.9% carbohydrate, 35.8 ± 3.1% fat, and 15.7 ± 1.3% protein. The composition of the dietary fat (expressed as a percentage of total macronutrients) was 12.9 ± 1.1% SFA, 13.1 ± 1.4% monounsaturated fats (MUFA), and 6.8 ± 0.8% PUFA. There were no differences in the diets of the two groups of subjects (data not shown).

Plasma glucose, insulin, and FFA concentrations at baseline and during the insulin clamp are shown in Table 2. There was no change in plasma insulin during the first 120 min of enteral infusion. By design, plasma insulin fell during the first 120 min of the somatostatin-insulin clamp to maximize the delivery of FFA to the liver. During the second step of the clamp, plasma insulin concentrations increased after the infusion to the liver. The percent change in FFA concentration to be greater in the subjects who received palm oil compared with those who received safflower oil (114.3 ± 8.7 vs. 25.5 ± 8.7 μmol/l, P < 0.01). There was a trend for significantly greater increases in SFA, MUFA, and PUFA were observed in both groups at the 240-min time point. The percent increase in plasma SFA was significantly greater in the palm oil compared with the safflower oil group (101.6 ± 25.5 vs. 25.6 ± 6.5%, P < 0.05). As might be expected on the basis of the greater content of oleic acid in the palm oil (35%) compared with safflower oil (12%), the increase in plasma MUFA in the palm oil group was also greater than that in the safflower group (114.3 ± 26.2 vs. 48.4 ± 6.4%, P < 0.05). Surprisingly, neither the increases in total plasma PUFA nor in linoleic acid (18:2 n-6) were greater in the safflower oil group compared with the palm oil group. However, when the ratio of SFA to PUFA (SFA/PUFA) was examined before and after lipid infusion, the expected decrease in SFA/PUFA was observed after safflower oil (¬7.3 ± 3.1%), whereas an increase was observed after palm oil (12.4 ± 0.7).

Table 2. Plasma glucose, insulin, and FFA at baseline, after enteral infusion of palm oil or safflower oil, and during a 2-step somatostatin, insulin, and glucagon clamp

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>120 min</th>
<th>180 min</th>
<th>240 min</th>
<th>360 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Safflower Oil</td>
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</tr>
<tr>
<td>Glucose, mmol/l</td>
<td>5.4 ± 0.3</td>
<td>5.1 ± 0.2</td>
<td>4.7 ± 0.2</td>
<td>5.4 ± 0.2</td>
<td>5.3 ± 0.39</td>
</tr>
<tr>
<td>Insulin, µU/ml</td>
<td>11.8 ± 2.7</td>
<td>13.2 ± 2.5</td>
<td>13.2 ± 2.5*</td>
<td>4.2 ± 0.4*</td>
<td>18.5 ± 1.9</td>
</tr>
<tr>
<td>FFA, μmol/l</td>
<td>588 ± 43.3</td>
<td>582.2 ± 59.5</td>
<td>778.3 ± 70.2</td>
<td>857.8 ± 68.7†</td>
<td>445.2 ± 118.1</td>
</tr>
<tr>
<td>Palm</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Glucose, mmol/l</td>
<td>5.0 ± 0.1</td>
<td>4.9 ± 0.1</td>
<td>4.7 ± 0.2</td>
<td>5.3 ± 0.2</td>
<td>4.9 ± 0.3</td>
</tr>
<tr>
<td>Insulin, µU/ml</td>
<td>9.1 ± 2.4</td>
<td>8.7 ± 2.0</td>
<td>5.8 ± 1.6*</td>
<td>3.9 ± 1.4*</td>
<td>15.5 ± 2.0</td>
</tr>
<tr>
<td>FFA, µmol/l</td>
<td>507.5 ± 47.4</td>
<td>552.2 ± 45.7</td>
<td>912.1 ± 95.4†</td>
<td>939.3 ± 61.3†</td>
<td>390.4 ± 47.5</td>
</tr>
</tbody>
</table>

Values are means ± SE. FFA, free fatty acids. *P < 0.05, †P < 0.01 compared with baseline within groups.

Table 3. Plasma FFA composition before and after enteral infusion of safflower oil or palm oil

<table>
<thead>
<tr>
<th></th>
<th>Safflower Oil</th>
<th>Palm Oil</th>
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</thead>
<tbody>
<tr>
<td>Fatty acid</td>
<td>Baseline</td>
<td>240 min</td>
</tr>
<tr>
<td>14:0</td>
<td>6.2 ± 1.2</td>
<td>7.5 ± 1.8</td>
</tr>
<tr>
<td>16:0</td>
<td>100.3 ± 8.7</td>
<td>137.8 ± 11.8</td>
</tr>
<tr>
<td>16:1 n-9</td>
<td>26.0 ± 5.2</td>
<td>34.0 ± 5.7</td>
</tr>
<tr>
<td>18:0</td>
<td>58.5 ± 5.0</td>
<td>62.5 ± 3.9</td>
</tr>
<tr>
<td>18:1 n-9</td>
<td>160.5 ± 9.2</td>
<td>243.1 ± 16.2†</td>
</tr>
<tr>
<td>18:2 n-6</td>
<td>88.7 ± 5.2</td>
<td>130.0 ± 9.2†</td>
</tr>
<tr>
<td>20:3 n-6</td>
<td>43.3 ± 3.2</td>
<td>45.7 ± 13.5</td>
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<tr>
<td>22:5</td>
<td>3.8 ± 0.6</td>
<td>3.0 ± 0.3</td>
</tr>
<tr>
<td>22:6 n-3</td>
<td>7.0 ± 0.8</td>
<td>7.8 ± 0.3</td>
</tr>
<tr>
<td>Σ SFA</td>
<td>168.5 ± 10.9</td>
<td>211.5 ± 16.6†</td>
</tr>
<tr>
<td>Σ MUFA</td>
<td>186.5 ± 10.0</td>
<td>277.1 ± 19.9†</td>
</tr>
<tr>
<td>Σ PUFA</td>
<td>121.1 ± 7.4</td>
<td>163.8 ± 10.5†</td>
</tr>
</tbody>
</table>

Results are expressed as means ± SE in μmol/l. SFA, MUFA, and PUFA, saturated, monounsaturated, and polyunsaturated fatty acids, respectively. *P < 0.05, †P < 0.01 compared with baseline within groups.
infusion by using the heavy water method. Mean H\textsubscript{2}O enrich-
ment was 0.48% and, in agreement with the studies of
Gastaldelli et al. (26), did not change during the clamps (coefficient of variation 3.5%). Rates of EGP, gluconeogenesis, and glycogenolysis are shown in Fig. 3. There was no difference in the proportion of gluconeogenesis at baseline or after infusion of lipids (240 min) in the two groups of obese subjects (Fig. 3). Absolute rates of glycogenolysis between the two

<table>
<thead>
<tr>
<th>Time, min</th>
<th>Baseline</th>
<th>120</th>
<th>150</th>
<th>180</th>
<th>210</th>
<th>240</th>
<th>360</th>
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<tbody>
<tr>
<td><strong>Safflower</strong></td>
<td></td>
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<tr>
<td>R\textsubscript{d}</td>
<td>11.2±0.98</td>
<td>9.8±0.8</td>
<td>9.8±0.7*</td>
<td>9.8±0.6*</td>
<td>12.1±0.9</td>
<td>11.6±0.9</td>
<td>10.3±0.7</td>
</tr>
<tr>
<td>EGP</td>
<td>11.2±0.98</td>
<td>9.8±0.8</td>
<td>4.8±1.2*</td>
<td>1.7±1.1†</td>
<td>5.2±1.9†</td>
<td>6.5±1.5*</td>
<td>9.9±0.0</td>
</tr>
<tr>
<td><strong>Palm</strong></td>
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<td></td>
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</tr>
<tr>
<td>R\textsubscript{d}</td>
<td>12.4±1.8</td>
<td>11.9±1.6</td>
<td>11.9±1.5</td>
<td>11.6±1.4</td>
<td>12.3±1.6</td>
<td>12.9±1.9</td>
<td>12.1±1.9</td>
</tr>
<tr>
<td>EGP</td>
<td>12.4±1.8</td>
<td>11.9±1.6</td>
<td>8.8±1.8</td>
<td>6.8±1.6†</td>
<td>9.4±1.5</td>
<td>10.7±1.4</td>
<td>11.6±1.7</td>
</tr>
</tbody>
</table>

Results are means ± SE expressed as μmol·kg fat-free mass\textsuperscript{-1}·min\textsuperscript{-1}. R\textsubscript{d}, rate of disappearance; EGP, endogenous glucose production. Times shown (min) are relative to enteral fat feeding (0–120 min). *P < 0.05, †P < 0.01 compared with baseline within groups.

6.4%). These directional changes in SFA/PUFA were signifi-
cantly different between the two groups (P < 0.05).

Glucose R\textsubscript{d} and EGP are shown in Table 4. Baseline rates of EGP did not differ between subjects in the safflower and palm oil groups (11.2 ± 1.0 vs. 12.4 ± 1.8 μmol·kg FFM\textsuperscript{-1}·min\textsuperscript{-1}, respectively). After enteral infusion of the two oils and during the first 120 min of the somatostatin-insulin-glucagon clamp, exogenous glucose was required to maintain euglycemia in both groups of subjects. However, rates of glucose infusion were significantly greater in subjects receiving safflower oil compared with those receiving palm oil [Fig. 2; area under the curve of glucose infusion rate 377.8 ± 38.0 vs. 195.8 ± 50.7 μmol·kg FFM, P < 0.01] despite similar plasma FFA concentrations. During the final 30 min of the clamp at low insulin infusion, rates of EGP were significantly greater in subjects who received palm oil compared with those receiving safflower oil (10.7 ± 1.4 vs. 6.5 ± 1.5 μmol·kg FFM\textsuperscript{-1}·min\textsuperscript{-1}, P < 0.01). When plasma insulin was increased for an additional 120 min, and despite a fall in plasma FFA (Table 2), minimal exogenous glucose was required in either group. Rates of EGP at completion of the clamp tended to be greater in the subjects who received palm oil compared with those who received safflower oil (11.6 ± 1.7 vs. 9.9 ± 0.9 μmol·kg FFM\textsuperscript{-1}·min\textsuperscript{-1}), but this did not reach statistical significance.

We also examined the effect of fatty acid composition on gluconeogenesis and glycogenolysis at baseline and after lipid infusion by using the heavy water method. Mean \textsuperscript{2}H\textsubscript{2}O enrichment was 0.48 ± 0.01% and, in agreement with the studies of

Fig. 2. Glucose infusion rates during somatostatin-insulin-glucagon clamp (time 120–240 min) following enteral infusion of safflower oil (●) and palm oil (○). Results shown are means ± SE.

Fig. 3. Rates of endogenous glucose production (A), gluconeogenesis (B) and glycogenolysis (C) before (filled bars) and after (240 min, hatched bars) infusion of safflower oil and palm oil. Results shown are means ± SE and are expressed as μmol·kg FFM\textsuperscript{-1}·min\textsuperscript{-1}. *P < 0.05 compared with baseline.
groups were also not statistically significantly different before (3.8 \pm 0.3 \text{ vs. } 4.2 \pm 0.6 \text{ mol-kg FFMM}^{-1} \text{-min}^{-1}) or after (-1.27 \pm 1.3 \text{ vs. } 2.0 \pm 1.0 \text{ mol-kg FFMM}^{-1} \text{-min}^{-1}, \ P = 0.06, \text{ safflower or palm oil, respectively}). However, a statistically significant fall in glycogenolysis was observed in subjects who received safflower oil in association with the fall in EGP at 240 min. This was not observed after palm oil administration. Moreover, the reduction in glycogenolysis observed in the subjects receiving safflower oil was significantly greater than that observed with palm oil (133.6 \pm 35.8 \text{ vs. } 42.6 \pm 19.1\%, \ P < 0.05), accounting for the lower rates of EGP in the former group.

**DISCUSSION**

To our knowledge, the present studies are the first to examine the effects of acute alteration in fatty acid composition on EGP in humans. Our studies demonstrate 1) significantly increased rates of EGP and 2) decreased suppression of glycogenolysis in obese, nondiabetic individuals after infusion of palm oil compared with safflower oil. This difference was observed after 240 min of FFA elevation and with reduced plasma insulin concentrations. Subsequent elevation of plasma insulin did not suppress EGP in either group.

Previous studies that have examined the effects of increased fatty acid levels on EGP have employed intravenous infusions of lipid emulsions that are composed primarily of oleic (18:2) and linolenic (18:3) acids. Lipid emulsions containing primarily long-chain SFA are not available for intravenous infusion. Consequently, in the present studies we infused either palm oil or safflower oil via enteral feeding tube. Because lipid absorption from the proximal small intestine requires CCK, and somatostatin is a potent inhibitor of CCK as well as peristalsis (35), initiation of the somatostatin infusion was delayed for 120 min. This method results in an increase in plasma FFA concentration within the physiological range to levels that have previously been shown to inhibit hepatic insulin action in man (8, 22).

The present studies were designed to maximize our ability to detect potential differences in intrahepatic fatty acid metabolism in response to elevation of the different fatty acids. Plasma insulin concentrations were permitted to fall during the first portion of the clamp to minimize peripheral reesterification and enhance the delivery of the absorbed FFA to the liver. Direct measurement of plasma FFA composition (Table 3) indicates that a significant change in FFA delivery to the liver was achieved. In particular, plasma concentrations of palmitic acid increased to a significantly greater extent in those subjects who received palm oil. Conversely, the change in plasma PUFA was less striking with safflower oil infusion. This could be due to differences in either absorption or clearance. Gastrointestinal absorption of PUFA is, if anything, greater than that of SFA (47) such that one would have predicted increased plasma PUFA with safflower oil compared with palm oil. An alternative mechanism lies in tissue preferences for FFA. Uptake and oxidation of fatty acids follow a hierarchy with more PUFA than SFA preferred over SFA (4, 36). Thus our failure to demonstrate a significant increase in plasma linoleic acid (18:2) in subjects who received safflower oil may reflect preferential uptake of PUFA compared with SFA. Enhanced splanchnic uptake of safflower oil has been suggested recently by others to explain lower-than-expected plasma PUFA following enteral administration before and after heparin administration in healthy volunteers (5). Despite this, we have observed a decrease in the SFA/PUFA ratio after safflower oil in contrast to an increase following palm oil infusion, suggesting that the methods used have achieved a significant difference in plasma FFA composition between the two groups.

In association with these changes in plasma FFA composition, rates of EGP were significantly greater after palm oil compared with safflower oil. As a result, greater exogenous glucose was required during the first 120 min of the somatostatin infusion in those subjects receiving safflower oil (Fig. 2). It is well known that exogenous glucose is required to maintain euglycemia during the first 120–180 min of somatostatin infusion (3, 38). The difference in glucose insulin rates (and EGP) observed in the present study might be explained by differences in plasma insulin concentrations during the clamp. Although insulin concentrations were not significantly different at any time during the studies (Table 2), insulin concentrations observed before the clamp were slightly greater in the subjects who received safflower oil compared with those who received palm oil, and the values are in a steep portion of the insulin dose-response curve for suppression of EGP. With initiation of the somatostatin infusion, a prompt decrease in plasma (and portal) insulin occurs and, on the basis of early studies with somatostatin infusions (38), insulin concentrations at 150 (not measured) and 180 min (Table 2) were likely very similar in the present study. Because the plasma insulin concentrations are virtually identical at 180 min, the apparently greater suppression of plasma insulin in the subjects who received safflower oil might have increased EGP and thereby minimized the fall in rates of EGP observed. Moreover, rates of glucose infusion during the first 30 min of the clamp were not different (Fig. 2). Thus differences in plasma insulin seem an unlikely explanation for our observations. However, we cannot say with certainty that the greater suppression of EGP with safflower oil was not related in part to this putative difference. That exogenous glucose would be required after safflower oil infusion is perhaps surprising, given the known adverse effects of lipid on insulin action. However, the effects of PUFA on EGP are more easily observed with prolonged lipid infusion and with greater elevations in plasma FFA than were observed in the present studies (48). Indeed, a fall in plasma glucose has been observed during the first 180 min of somatostatin despite Intralipid infusion when no exogenous glucose is provided (48). Our studies are also consistent with studies in dogs in which concomitant infusions of Intralipid and heparin did not reverse the suppression of net hepatic glucose production by somatostatin (30).

The lower rates of EGP with safflower oil compared with palm oil do not appear to be related to changes in gluconeogenesis. One might have expected an increase in rates of gluconeogenesis under conditions of reduced portal and peripheral plasma insulin and increased FFA. Intralipid infusions resulting in plasma FFA concentrations >1 mM in lean, nondiabetic individuals have been shown to increase rates of gluconeogenesis (13, 48). However, more modest increases in FFA (similar to those observed in the present study) have no significant effect on rates of gluconeogenesis (48). As reported previously (25), the proportion of EGP from gluconeogenesis is increased in obese compared with lean individuals. Other
than the present study, we are not aware of any studies that have examined the effect of physiological increases in plasma FFA on gluconeogenesis in obese subjects. Our data suggest that, like lean individuals, an increase does not occur. With respect to insulin, Boden et al. (9) have demonstrated that physiological waning of insulin has little effect on gluconeogenesis in patients with type 1 diabetes, whereas tenfold elevations of insulin (to ~113 μU/ml) in nondiabetic subjects are associated with an ~20% reduction in gluconeogenesis (26). Changes in glucagon might also be expected to alter gluconeogenesis (29). Although peripheral glucagon concentrations did not change during the present studies, it is almost certain that portal glucagon levels fell during the clamp (6), and this might have been expected to reduce gluconeogenesis (and glycolysis). However, using a very similar study design, Boden et al. showed that gluconeogenesis was not affected by somatostatin infusion (decreased portal but baseline peripheral glucagon) despite hyperinsulinemia (10). Taken together, these studies suggest that the modulation of gluconeogenesis in vivo by FFA under conditions of decreased portal insulin and glucagon is modest.

Rather, our data suggest that the acute effects of fatty acids on EGP observed in the present studies are related to differential effects on hepatic glycogen release. We and others (16, 17) have suggested that overall EGP is maintained by the reciprocal regulation of gluconeogenesis and glycogenolysis during Intralipid infusion in lean, nondiabetic subjects. Direct evidence for this hypothesis was recently demonstrated in studies using the NMR technique (52). In those studies, liver glycogen depletion occurred at a decreased rate with fasting in the presence of Intralipid and heparin compared with control. Potential mechanisms by which fatty acid composition might affect hepatic glycogen content include activation of glucose-6-phosphatase (G-6-Pase) and direct modulation of glycogen release. Regarding the former, an increase in G-6-Pase activity would be expected to reduce intracellular concentrations of glucose 6-phosphate and relieve the restraint on glycogen phosphorylase. FFA have been shown to increase both the expression and activity of G-6-Pase in vitro and in vivo (39, 55). However, G-6-Pase activity is also inversely related to fatty acid chain length and degree of fatty acid unsaturation (43, 44). Thus greater release of glucose from the liver in the presence of SFA compared with PUFA might be expected. Our previous observations that G-6-Pase activity (18) and plasma SFA (15) are increased in patients with type 2 diabetes in association with increased EGP support this contention.

Fatty acids may also modulate liver glycogen content directly. With regard to methodology, the differences in suppression of glycogenolysis observed in the present study could potentially be explained by differences in glycogen cycling induced by the two oils. Glycogen cycling occurs when plasma glucose derived from the indirect (i.e., gluconeogenic) pathway is stored in glycogen and then released from glycogen. To the extent that glycogen cycling occurred, the apparent suppression of glycogenolysis after safﬂower oil compared with palm oil may have been overestimated. However, glycogen cycling has not been observed under conditions similar to those of the present study (euglycemia and decreased insulin) (46). Boden et al. (9) have recently reported that an infusion of Intralipid sufﬁcient to increase plasma FFA to ~1,200 μM is associated with impaired suppression of glycogenolysis by insulin. However, this effect was bimodal. Suppression of glycogenolysis during the first 120 min was not different from saline control but decreased progressively during the second 120 min of the study. This bimodal response might be explained either by a delay in the uptake of FFA or by a change in intrahepatic fatty acid composition. The former possibility seems unlikely. Using positron emission tomography, Iozzo et al. (28) recently demonstrated that the uptake of FFA into the liver is both rapid and quantitatively signiﬁcant (300 μmol/min). Assuming that the uptake of FFA is independent of composition and that FFA oxidation of PUFA is preferred over SFA (4, 36), then a progressive accumulation of intrahepatic SFA might be expected.

There is little known about the role of fatty acid composition on hepatic glycogen regulation. However, there is increasing appreciation that ceramides derived from SFA (but not from PUFA) are associated with hepatic and peripheral insulin resistance (12, 14). Thus an increase in hepatic SFA (either through preferential oxidation of PUFA or increased delivery of SFA) may impair hepatic autoregulation and increase the release of free glucose derived from either gluconeogenesis or glycolysis. Indeed, a time-effect relationship of FFA and fatty acid composition on the regulation of glycogenolysis is suggested by these and other recent studies. After 5 h of PUFA elevation (51), the expected fall in glycogenolysis with fasting is attenuated, whereas in the present study persistent glycogenolysis is observed after 2 h of SFA elevation.

We believe that the present studies have important implications for both the development and the management of type 2 diabetes mellitus (DM2). Recent prospective data demonstrating an increased risk for DM2 over 4 yr in association with increased plasma SFA and decreased plasma PUFA at baseline (33) suggest that fatty acid composition may be an important independent risk factor. In this study (33), greater plasma PUFA was associated with smaller increases in fasting plasma glucose and insulin concentrations over 4 yr compared with individuals with higher SFA/PUFA ratios. Although EGP was not measured directly in these studies, the strong relation between fasting plasma glucose and EGP would support the hypothesis that fatty acid composition may have important long-term effects on hepatic insulin sensitivity. Increased plasma SFA may be derived either from diet or from endogenous synthesis. Diets higher in SFA would be expected to impair hepatic (and perhaps peripheral) glucose uptake during a meal and contribute to elevations in postprandial glucose. In support of this contention, epidemiological studies in humans have established the association between a diet high in fat and glucose intolerance and DM2, with SFA most closely linked (27, 37, 41). Vessby et al. (56) have shown a strong, positive correlation between fatty acid intake [increased cholesterol ester palmitic (16:0) and palmitoleic acid (16:1) and decreased linoleic acid (18:2 n-6)] and risk for progression to DM2. Finally, Vessby et al. (57) have shown a decrease in peripheral insulin sensitivity with an increase in dietary saturated fat. The observations that SFA accumulation in skeletal muscle inhibits insulin-stimulated glycogen storage (49) via decreased phosphorylation of protein kinase B/Akt (54) may explain these ﬁndings. Conditions of hyperglycemia and hyperinsulinemia are also well known to increase endogenous synthesis of SFA and MUFA via sterol regulatory element-binding proteins (31, 53) and would be expected to perpetuate the increase in SFA.
In conclusion, the present studies are the first to examine the effect of acute elevations of SFA or PUFA on EGP in nondiabetic subjects. The studies clearly demonstrate that elevations of SFA are associated with increased EGP, accounted for entirely by impaired suppression of glycogenolysis. These studies suggest that increased plasma SFA, derived from either diet or endogenous synthesis, may play an important role in the regulation of hepatic glucose disposal in humans.

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