Effects of Free Fatty Acid Elevation on Postabsorptive Endogenous Glucose Production and Gluconeogenesis in Humans

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Effects of free fatty acids (FFAs) on endogenous glucose production (EGP) and gluconeogenesis (GNG) were examined in healthy subjects (n = 6) during stepwise increased Intralipid/heparin infusion (plasma FFAs 0.8 ± 0.1, 1.8 ± 0.2, and 2.8 ± 0.3 mmol/l) and during glycerol infusion (plasma FFAs ~0.5 mmol/l). Rates of EGP were determined with D-[6,6-2H2]glucose and 9.9 ± 1.2 vs. 9.0 ± 1.1 µmol · kg–1 · min–1). GNG decreased by ~12% during glycerol infusion (P < 0.01), whereas plasma insulin increased by ~47% (P = 0.02) after 9 h of lipid infusion. EGP declined from 9.3 ± 0.5 (lipid) and 9.0 ± 0.8 µmol · kg–1 · min–1 (glycerol) to 8.4 ± 0.5 and 8.2 ± 0.7 µmol · kg–1 · min–1, respectively (P < 0.01). Contribution of GNG similarly rose (P < 0.01) from 46 ± 4 and 52 ± 3% to 65 ± 8 and 78 ± 7%. To exclude interaction of FFAs with insulin secretion, the study was repeated at fasting plasma insulin (35 pmol/l) and glucagon (90 ng/ml) concentrations using somatostatin-insulin-glucagon clamps. Plasma glucose increased by ~50% (P < 0.005) during lipid but decreased by ~12% during glycerol infusion (P < 0.005). EGP remained unchanged over the 9-h period (9.9 ± 1.2 vs. 9.0 ± 1.1 µmol · kg–1 · min–1). GNG accounted for 62 ± 5 (lipid) and 60 ± 6% (glycerol) of EGP at time 0 and rose to 74 ± 3% during lipid infusion only (P < 0.05 vs. glycerol: 64 ± 4%). In conclusion, high plasma FFA concentrations increase the percent contribution of GNG to EGP and may contribute to increased rates of GNG in patients with type 2 diabetes. Diabetes 49:701-707, 2000

Plasma FFA elevation induced by lipid/heparin infusions during hyperinsulinemic clamps has repeatedly been shown to decrease insulin-dependent whole-body glucose disposal (5–8). Reports on a correlation between plasma FFAs and hepatic insulin sensitivity are more controversial. Fasting plasma FFAs correlate with the magnitude of hyperglycemia and endogenous glucose production (EGP) (9), which has been attributed to increased lipid oxidation in type 2 diabetes (10). Under hyperinsulinemic conditions, lipid/heparin infusion either increased (6,11) or had no effect on (12–14) EGP. At postabsorptive plasma insulin concentrations, plasma FFA elevation caused marked increases in EGP during somatostatin-insulin clamps (12,15), but not after an overnight fast (15,16). Similarly, inhibition of lipolysis by nicotinic acid or its derivative, acipimox, decreased basal EGP in some (17,18) but not other (19,20) studies. These apparent discrepancies could result from FFA-induced insulin secretion counterbalancing the stimulatory effect of FFAs on EGP (15) or from hepatic autoregulation preventing an increase in EGP under conditions that might favor hepatic gluconeogenesis (GNG) (16). Of note, increased GNG was documented in type 2 diabetes from a variety of precursors (21,22), whereas contradictory effects of FFAs on the contribution of GNG to EGP have been reported during lipid/heparin infusion or acipimox studies (16,18,20,23).

In most studies, glycerol was not infused during control experiments to match the lipid-induced rise in plasma glycerol concentrations. Thus, stimulation of hepatic GNG by FFAs could also be exclusively due to increased availability of glycerol (12), possibly the major gluconeogenic substrate under these conditions (24). A wide range of estimates of the contribution of GNG to EGP has been reported in humans; however, the methods used to make those estimates have several limitations (25–27). Recently, a method was introduced to overcome those limitations (28,29). Quantitative estimates of the contributions of GNG to EGP are made from 3H enrichments in the hydrogens bound to carbons 2 and 5 of glucose on deuterated water (2H2O) ingestion.
The present study was therefore designed to compare concentration-dependent effects of FFAs with those of glycerol on 1) the time-course of plasma glucose, insulin, and C-peptide concentrations; 2) the rates of EGP; and 3) the contribution of endogenous GNG to EGP. The study combined oral administration of \( ^{2}H_{2}O \) with bolus/continuous infusion of deuterated glucose (\( \delta ^{15}C_{6,6}^{2}H_{2} \) glucose) under conditions of prolonged fasting.

### RESEARCH DESIGN AND METHODS

**Subjects.** For the basal study, 6 healthy volunteers (3 men and 3 women, ages 25–37 years, BMI 22.8 ± 1.2 kg/m²) without family history of diabetes, dyslipidemia, or bleeding disorders fasted overnight for 8 h before the beginning of the experiments. For the pancreatic clamp study, 6 healthy subjects (5 men and 1 woman, ages 26–38 years, BMI 22.7 ± 0.7 kg/m²) were studied after identical overnight fasts; of them had participated in the first study. Female subjects were studied in the follicular phase of the menstrual cycle. The studies were spaced by intervals of 6–13 weeks. No changes in diet, weight, or lifestyle were recorded from the time of recruitment until completion of both studies. The protocols were reviewed and approved by the local human ethics board, and informed consent was obtained from all subjects.

**Study protocols.** For the basal study, experiments were begun at 5:30 A.M. (time –390 min) with insertion of Teflon catheters in antecubital veins of the right and left arm for blood sampling and infusions, respectively. At –375 min, a bolus (6.606 µmol/kg \times \text{body weight in kg}) infusion of D-[6,6-2H₂]glucose (99%, Cambridge Isotope Laboratories, Andover, MA) per kilogram body water divided into 4 equal doses spaced at intervals of 45 min (30). Body water was assumed to be 50% of body weight in women and 60% in men. Thereafter, subjects had free access to water containing 0.5% of \( ^{2}H_{2}O \) to maintain isotopic equilibrium in body water. To allow calculation of EGP, a bolus (6.606 µmol/kg \times \text{body weight in kg}) infusion of fasting blood glucose in \([\text{µmol/l}] / 500 \mu\text{mol/l}\)–continuous (0.067 µmol/kg \times \text{body weight in kg/min}) infusion of \( \delta ^{15}C_{6,6}^{2}H_{2} \) glucose (99%, Cambridge Isotope Laboratories) was commenced at –210 min (31). Plasma FFA concentrations were raised stepwise every 3 h by infusion of a triglyceride emulsion (0.5, 1.0, and 1.5 ml/min) (Intralipid 20%; Kabi Pharmacia, Uppsala, Sweden) combined with heparin (bolus 200 U; continuous 0.2 U–kg⁻¹·min⁻¹). During control experiments, glycerol was infused from 120 to 540 min (0.7 mg·kg⁻¹·min⁻¹ in 0.9% saline) to control for glycerol effects per se.

For the pancreatic clamp study, the protocol was identical to that in the basal study except 1) somatostatin (0.1 µg–kg⁻¹·min⁻¹) was infused from –10 min until 540 min to inhibit hormone secretion, 2) insulin (0.07 µU–kg⁻¹·min⁻¹) and glucagon (0.05 ng·kg⁻¹·min⁻¹) were infused to maintain postabsorptive plasma concentrations from 0 to 540 min, and 3) the glycerol infusion was increased stepwise to mimic the increase in plasma glycerol during lipid infusion (135–240 min: 0.23; 240–360 min: 0.47; 360–540: 1.5 mg · kg⁻¹ · min⁻¹). During both studies, blood samples were taken at timed intervals, immediately chilled, and centrifuged, and the supernatants were stored at –80°C until determination of hormones and metabolites.

**Analytical procedures.** Plasma glucose concentrations were measured using glucose oxidase (Glucose Analyzer II; Beckman, Fullerton, CA). Plasma concentrations of FFAs (intra- and interassay coefficients of variation [CVs] 4.3 and 5.7%), glycerol (CVs 2.0 and 3.4%), and lactate (CVs 3.3%) were determined by enzymatic methods (FFAs: Wako, Neuss, Germany; glycerol: Boehringer-Mannheim, Mannheim, Germany; lactate: Sigma, St. Louis, MO). Plasma insulin (CVs ≤8%), C-peptide (CVs ≤9%), glucagon (CVs ≤6%), and growth hormone (CVs ≤70%) were measured by radioimmunoassay (RIA) (insulin: Pharmacia-Upjohn, Uppsala, Sweden; C-peptide: CIS, Gif-sur-Yvette, France; glucagon: Serono Diagnostics, Freiburg, Germany; growth hormone: Sorin Biomedica, Saluggia, Italy). Plasma cortisol (CVs ≤6%) was determined after extraction and charcoal-dextran separation by RIA (32).

Enrichments of \( ^{2}H \) in hydrogens bound to carbon 2 (C₂), C₅, and C₆ of blood glucose were determined using gas chromatography-mass spectrometry by isolating the hydrogens in formaldehyde derivatized to hexamethyldiethylenetriamine (HMT) as previously detailed (28–30). Briefly, blood was diluted with an equal volume of water, deproteinized with ZnSO₄/Ba(OH)₂, and deionized, and the glucose was isolated by high-performance liquid chromatography. To estimate fractional GNG, aliquots of glucose were converted to arabinol-5-phosphate and ribitol-5-phosphate and to xylose, which were oxidized with periodate to yield formaldehyde containing C₂ or C₅ of glucose, respectively, with their hydrogens. For assessing EGP, the hydrogens bound to C₆ of glucose were isolated in formaldehyde by direct oxidation with periodate of an aliquot of glucose. Each formaldehyde was condensed with ammonia to form HMT. The HMTs were assayed for \( ^{2}H \) enrichments by mass spectrometry (28,29), mass 141 for the enrichment of the hydrogens on carbons C₂ and C₅ and mass 142 for those on C₆. Enrichments in plasma water were determined by Metabolic Solutions ( Methoxy Instruments, NH) using an isotope ratio mass spectrometer.

**Calculations and data analysis.** Rates of EGP were calculated from the tracer infusion rate of \( \delta ^{15}C_{6,6}^{2}H_{2} \) glucose and its enrichment divided by the mean percent enrichment of plasma \( \delta ^{15}C_{6,6}^{2}H_{2} \) glucose for the 3 collections minus the infusion rate. The percent contribution of GNG to EGP was set to 100 times the ratio of \( ^{2}H \) enrichment bound to C₆ to that at C₂ of blood glucose. The absolute contribution of GNG to EGP was calculated by multiplying percent GNG by EGP. All data are presented as means ± SE. Statistical comparisons between lipid and glycerol experiments were performed using the paired Student's t-test.

### RESULTS

**Basal study.** Lipid infusion at rates of 0.5, 1.0, and 1.5 ml/min resulted in a stepwise increase in plasma FFA concentrations, which did not change from baseline values during glycerol (control) infusion (Table 1). Plasma glycerol concentrations continuously increased, being higher (\( P < 0.01 \)) during...
glycerol infusion until 360 min, but were not different at 510–540 min. Plasma cortisol decreased ($P < 0.05$) in both experiments, whereas plasma lactate and growth hormone did not change.

Plasma glucose concentrations remained constant during the first 3 h of both parts of the basal study (Fig. 1). Then plasma glucose slightly declined, by ~7%, during the final period of lipid infusion ($P < 0.00001$ vs. time 0), but not during glycerol infusion. Plasma insulin concentrations continuously increased during lipid infusion and were ~48% higher than during glycerol infusion at 510–540 min ($P < 0.05$). In parallel, plasma C-peptide concentrations increased by ~50% during lipid infusion ($P < 0.05$ vs. baseline) but decreased by ~30% during glycerol infusion ($P < 0.025$ vs. baseline). At the end of the study, plasma C-peptide concentrations were ~2.4-fold higher during lipid than during glycerol infusion ($P = 0.0015$). Plasma glucagon concentrations were similar before and during both experiments (Fig. 1).

Rates of EGP were similar (time 0: $9.3 ± 0.5$ vs. $9.0 ± 0.8 \mu mol \cdot kg^{-1} \cdot min^{-1}$) and declined to $8.4 ± 0.5$ and $8.2 ± 0.7 \mu mol \cdot kg^{-1} \cdot min^{-1}$ ($P < 0.01$ and $P < 0.005$) during lipid and glycerol experiments, respectively (Fig. 3). GNG contributed $46 ± 4$ (lipid) and $52 ± 3\%$ (control) to EGP at time 0 and $55 ± 7$ (lipid) and $61 ± 9\%$ (control) at 180 min. Then GNG continuously rose ($P < 0.01$ vs. time 0) to $66 ± 8$ and $73 ± 7\%$ at 360–540 min, without a difference between lipid and glycerol experiments. **Pancreatic clamp study.** Plasma FFA concentrations increased ~5.7-fold ($P < 0.01$) during lipid infusion and...
remained constant during glycerol infusion (Table 2). Plasma glycerol concentrations rose during both protocols \( (P < 0.05) \) and were slightly but significantly higher at 360 and 540 min of the glycerol protocol \( (P < 0.05) \). Plasma growth hormone concentrations decreased during both experiments \( (P < 0.05) \). Plasma concentrations of cortisol and lactate were not different between the 2 protocols.

Plasma glucose declined by ~19\% \( (P < 0.05) \) and ~29\% \( (P < 0.005) \) during the first 90 min of lipid and glycerol infusion, respectively (Fig. 2). In contrast to the basal study, plasma glucose concentrations increased thereafter by ~50\% to 7.9 ± 0.6 mmol/l \( (540 \text{ min}; P < 0.005 \text{ vs. baseline and vs. control}) \) during lipid infusion, being ~1.8-fold higher than during glycerol infusion \( (4.4 ± 0.7 \text{ mmol/l}) \). Plasma insulin was maintained at fasting peripheral concentrations throughout the experiments, so portal insulin concentrations were likely lower than during the basal study. Plasma C-peptide concentrations were similar at baseline and rapidly declined below the detection limit of 0.3 \text{ ng/ml} \( (P < 0.0005) \) during somatostatin exposure in both protocols. Plasma glucagon concentrations were maintained at their fasting concentrations (Fig. 2).

Absolute rates of GNG and EGP at the end of the study are presented in Fig. 3. Rates of EGP were similar to those of the basal study at time 0 (lipid 9.6 ± 1.3; glycerol 9.0 ± 1.1 \text{ mmol·kg}^{-1}·\text{min}^{-1}) and were unchanged at the end of the experiments (lipid 9.9 ± 1.9; glycerol 9.0 ± 1.1 \text{ mmol·kg}^{-1}·\text{min}^{-1}). Absolute mean enrichments at C6 ranged from 0.67 to 0.79\% and percent enrichments at C6 of glucose at –10, –5, and 0 min were essentially constant, as were those at 510, 520, and 540 min (Table 3). At time 0, GNG accounted for 62 ± 5 and 60 ± 6\% of EGP during lipid and glycerol infusion, respectively. Until 180 min, GNG remained unchanged in both protocols (lipid 59 ± 3; control 63 ± 4\% NS). During high-dose lipid infusion, GNG rose to 74 ± 3\% \( (P < 0.005 \text{ vs. time 0}) \) and was higher than during glycerol infusion \( (360–540 \text{ min}: 64 ± 4\% P < 0.05 \text{ vs. lipid}) \). Using the ratio of \(^2\text{H}\) enrichments at C5 and C2, the enrichment at C2 is assumed to approximate that in body water \( (25,26) \). The enrichment at C2 was compared with that in plasma water at 540 min. The C2/water ratio was 1.00 ± 0.05 in the glycerol and 1.12 ± 0.05 in the lipid experiments \( (P \leq 0.05) \).

**DISCUSSION**

These studies demonstrate that short-term plasma FFA elevation induces insulin secretion in humans that is sufficient to prevent an increase in plasma glucose concentration. If plasma insulin is maintained at fasting peripheral concentrations by somatostatin-insulin infusion, FFA exposure results in a marked rise of plasma glucose concentrations and augments the contribution of gluconeogenesis to endogenous glucose production.

Plasma FFA elevation per se was likely responsible for the stimulation of insulin secretion, because 1) plasma glucose concentrations were not higher than during control experiments and 2) reduction of hepatic insulin clearance is unlikely to occur \( (5) \). The results are partly in line with a previous study reporting a small increase in plasma C-peptide without changes in plasma insulin \( (15) \). Insufficient duration or lower degree of plasma FFA elevation may explain the difference in results. Stimulation by FFAs of glucose-stimulated insulin secretion was demonstrated in humans \( (33,34) \) and rats \( (35) \), whereas reduction of plasma FFAs by acipimox

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**TABLE 2**

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<th>180 min</th>
<th>360 min</th>
<th>540 min</th>
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<td><strong>FFAs (mmol/l)</strong></td>
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<tr>
<td>Lipid</td>
<td>0.37 ± 0.09</td>
<td>1.04 ± 0.16*†</td>
<td>1.64 ± 0.26†§</td>
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<td><strong>Glycerol (mmol/l)</strong></td>
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<tr>
<td>Lipid</td>
<td>0.14 ± 0.01</td>
<td>0.17 ± 0.01*†</td>
<td>0.26 ± 0.04*†</td>
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<td>Control</td>
<td>0.18 ± 0.02</td>
<td>0.24 ± 0.01†</td>
<td>0.35 ± 0.03§</td>
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<td><strong>Lactate (mmol/l)</strong></td>
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<td>Lipid</td>
<td>0.75 ± 0.15</td>
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<td><strong>Growth hormone (ng/ml)</strong></td>
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<tr>
<td>Lipid</td>
<td>0.7 ± 0.1</td>
<td>&lt;0.5†</td>
<td>&lt;0.5†</td>
<td>&lt;0.5†</td>
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<tr>
<td>Control</td>
<td>0.6 ± 0.0</td>
<td>&lt;0.5†</td>
<td>&lt;0.5†</td>
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<td><strong>Cortisol (µg/dl)</strong></td>
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<tr>
<td>Lipid</td>
<td>9.9 ± 1.9</td>
<td>10.2 ± 1.9</td>
<td>4.9 ± 1.1†</td>
<td>4.6 ± 1.2†</td>
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<tr>
<td>Control</td>
<td>9.8 ± 1.6</td>
<td>11.8 ± 1.3</td>
<td>7.9 ± 1.0</td>
<td>5.9 ± 0.8</td>
</tr>
</tbody>
</table>

Data are means ± SE of 6 paired experiments. *\( P < 0.05 \); †\( P < 0.05 \); ‡\( P < 0.005 \text{ vs. control}; §\( P < 0.005 \text{ vs. basal} \).
Pancreatic clamp study

Data are means ± SE of 6 experiments per group. Absolute enrichments: *0.747 ± 0.061 at 0 min, 0.789 ± 0.060 at 540 min; †0.697 ± 0.042 at 0 min, 0.773 ± 0.062 at 540 min; ‡0.706 ± 0.044 at 0 min, 0.697 ± 0.048 at 540 min; §0.673 ± 0.045 at 0 min, 0.651 ± 0.040 at 540 min.

result from 1 or more of the following:

~3.5 mmol/l in plasma glucose concentrations. This effect may

Plasma FFA elevation was shown to increase GNG in
diabetic humans (16). Also, the authors of that study could not exclude a gluconeogenic effect of glycerol, whose concentration was markedly increased (16). In any case, they proposed that the increase in GNG is normally matched by a decrease of glycogenolysis so that EGP will remain unchanged. Recently, evidence for such hepatic autoregulation by FFAs was supported by a study reporting that a rebound of plasma FFA concentrations of up to 1.8 mmol/l after administration of nicotinic acid augmented the contribution of GNG to EGP (18). However, effects of nicotinic acid per se on hepatic metabolism could have contributed to these results. In the present study, the increased contribution of GNG regardless of unchanged EGP can be also due to the concomitant rise in glucose concentration to ~8 mmol/l, which will suppress glucose production, or to intracellular accumulation of glucose-6-phosphate, which will inhibit glycogen phosphorylase activity and stimulate glycogen synthase in the absence of counterregulatory hormones (44).

The contribution of GNG to EGP was in good agreement with previous studies (28,29,45) and was higher only at the end of lipid infusion compared with baseline or control conditions. Percent GNG from the ratio of \(^{13}C\) enrichments in C5/C2 of blood glucose might result in overestimation of the contribution of GNG to EGP to the extent that glucose-6-phosphate before its conversion to glucose undergoes triose phosphate cycling and underestimation of it to the extent that glycogen cycling occurs. Glycogen cycling is undetected in the fasted state (46), and triose phosphate cycling as the pentose phosphate cycle probably contributes no more than 2% to any overestimation of fractional GNG in the fasted state (30,45,47). Nevertheless, changes in their contributions induced by FFAs or glycerol cannot be ruled out. Estimates using the C5/C2 enrichment ratio assume complete isotopic equilibration of glucose-6-phosphate with fructose-6-phosphate. If so, at steady state, the enrichment in C2 equals that in body water (18,45,46). The reason in the lipid study for the C2/body water ratio of ~1.12 is unclear. However, using the C5/water ratio rather than C5/C2 ratio would only increase the percent GNG in the lipid compared with the glycerol study.

Finally, the increase of plasma glucose concentrations could also result indirectly from a reduction in whole-body glucose clearance (5–7,48) which is due to direct inhibition of skeletal muscle glucose transport activity with subsequent reduction in rates of glucose oxidation and glycogen syn-
thesis (7,8,48). Under pancreatic clamp conditions of the present study, the inability to counteract any decline of whole-body glucose clearance by increased secretion of insulin to inhibit EGP and stimulate glucose uptake may then lead to a net increase of plasma glucose concentrations.

In conclusion, these findings are consistent with the hypothesis that FFAs are involved in the regulation of hepatic glucose metabolism (49). However, only at high plasma FFA concentrations did the contribution of gluconeogenesis to glucose production increase, whereas endogenous glucose production remained unchanged in healthy humans.

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