FFA cause hepatic insulin resistance by inhibiting insulin suppression of glycogenolysis

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Boden, Guenther, Peter Cheung, T. Peter Stein, Karen Kresge, and Maria Mozzoli. FFA cause hepatic insulin resistance by inhibiting insulin suppression of glycogenolysis. Am J Physiol Endocrinol Metab 283: E12–E19, 2002.—Free fatty acids (FFA) have been shown to inhibit insulin suppression of endogenous glucose production (EGP). To determine whether this is the result of stimulation by FFA of gluconeogenesis (GNG) or glycogenolysis (GL) or a combination of both, we have determined rates of GNG and GL (with 2H2O) clamping performed either with or without simultaneous lipid/heparin infusion, FFA increased from 499 to 1,247 μmol/l (P < 0.001), EGP from 15.7 to 2.0 μmol·kg−1·min−1 (P < 0.01), GNG from 8.2 to 3.7 μmol·kg−1·min−1 (P < 0.05), and GL from 7.4 to −1.7 μmol·kg−1·min−1 (P < 0.02). During insulin plus lipid/heparin infusion, FFA increased from 499 to 1,247 μmol/l (P < 0.001), EGP decreased 64% less than during insulin alone (−5.1 ± 0.7 vs. −13.7 ± 3.4 μmol·kg−1·min−1). The decrease in GNG was not significantly different from the decrease of GNG during insulin alone (−2.6 vs. −4.5 μmol·kg−1·min−1, not significant). In contrast, GL decreased 66% less than during insulin alone (−3.1 vs. −9.2 μmol·kg−1·min−1, P < 0.05). We conclude that insulin suppressed EGP by inhibiting GL more than GNG and that elevated plasma FFA levels attenuated the suppression of EGP by interfering with insulin suppression of GL

gluconeogenesis; endogenous glucose production; glucagon; euglycemic-hyperinsulinemic clamping; free fatty acid

METHODS

Subjects

Sixteen healthy volunteers (11 males, 5 females) participated in three studies. Two male subjects participated in studies 1 and 2, and all other subjects participated in only one study. The subjects’ ages, weights, heights, and body compositions are shown in Table 1. None of the subjects had a family history of diabetes or any other endocrine disorder, and none was taking any medications. Their weights were stable for at least 2 mo, and their diets contained a minimum of 250 g/day of carbohydrate for at least 2 days before the studies. Informed written consent was obtained from all subjects after explanation of the nature, purpose, and potential risks of these studies. The study protocol was approved by the Institutional Review Board of Temple University Hospital.

Experimental Design

All subjects were admitted to the Temple University Hospital General Clinical Research Center the day before the studies. At 6:00 PM, the subjects ingested a meal of 14 kcal/kg body wt consisting of 53% carbohydrate, 15% protein, and 32% fat. After that, they fasted for the duration of the study but were allowed water ad libitum. At 11:00 PM, a baseline blood sample was obtained. The subjects then drank
Table 1. Study subjects

<table>
<thead>
<tr>
<th></th>
<th>Insulin</th>
<th>Insulin + Lipid</th>
<th>Insulin + Somatostatin + Glucagon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>M</td>
<td>M</td>
<td>M</td>
</tr>
<tr>
<td>Age, yr</td>
<td>32.7 ± 6.8</td>
<td>27.8 ± 3.2</td>
<td>24.4 ± 6.7</td>
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<tr>
<td>Height, cm</td>
<td>175.1 ± 3.7</td>
<td>175.3 ± 5.1</td>
<td>179.3 ± 4.9</td>
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<td>Weight, kg</td>
<td>73.1 ± 4.4</td>
<td>71.8 ± 4.3</td>
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<tr>
<td>Body fat, %</td>
<td>22.5 ± 1.3</td>
<td>24.0 ± 2.5</td>
<td>26.2 ± 1.2</td>
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<tr>
<td>BMI, kg/m²</td>
<td>23.7 ± 0.8</td>
<td>23.7 ± 1.1</td>
<td>25.8 ± 1.3</td>
</tr>
</tbody>
</table>

Values are means ± SE. M, males; F, females; BMI, body mass index.

2.5 g of $^2$H$_2$O (99.9% hydrogen 2; Isotec, Miamisburg, OH/kg body weight). They drank the same amount of $^2$H$_2$O 4 h later at 3:00 AM. Body water was assumed to be 50% of body weight in women and 60% of body weight in men. Additional water ingested during the fast was enriched to 0.5% with $^2$H$_2$O to prevent dilution of the isotopic steady state. The studies began at 8:00 AM the next day with the subjects reclined in bed. A short polyethylene catheter was inserted in an antecubital vein for infusion of isotope. Another catheter was placed in a contralateral forearm vein for blood sampling. This arm was wrapped with a heating blanket (~70°C) to arterialize venous blood. The following studies were performed. Study 1 was a 4-h euglycemic-hyperinsulinemic clamp. Plasma FFA levels decreased to very low levels because of insulin-induced inhibition of lipolysis. Study 2 was a 4-h euglycemic-hyperinsulinemic clamp with simultaneous intravenous infusion of lipid plus heparin. Plasma FFA levels rose because heparin-mediated lipolysis from the infused fat exceeded insulin-mediated antilipolysis in adipose tissue. The studies were performed in random order separated by 1–2 mo. Because plasma glucagon levels decreased during study 1, a third study was performed. Study 3 was a combined 4-h euglycemic-hyperinsulinemic-pancreatic clamp.

Procedures

Euglycemic-hyperinsulinemic clamping with and without lipid/heparin. Regular human insulin (Humulin R; Eli Lilly, Indianapolis, IN) was infused intravenously at a rate of 7 pmol·kg$^{-1}$·min$^{-1}$ for 4 h, and plasma glucose concentrations were clamped at ~5 mmol/l by a feedback-controlled variable glucose infusion (study 1). Changes in specific activity of [3H]$\text{glucose}$ were avoided by adding [3-$^3$H]glucose to the infusion of unlabeled glucose. In study 2, euglycemic-hyperinsulinemic clamping was performed as described for study 1. In addition, Liposyn II (Abbott Laboratories, North Chicago, IL), a 20% triglyceride emulsion (10% safflower, 10% soybean oil), plus heparin (0.4 U·kg$^{-1}$·min$^{-1}$) were infused at a rate of 1.5 ml/min for 4 h. Study 3 (combined euglycemic-hyperinsulinemic-pancreatic clamps) was identical to study 1 except for the coinfusion of somatostatin (500 μg/h, to block endogenous pancreatic hormone secretion) and glucagon (0.3 ng·kg$^{-1}$·min$^{-1}$) to replace basal glucagon secretion. Serial measurements of rates of GNG and GL and glucose turnover, substrate, and hormone analyses were obtained before and during the clamps.

Glucose turnover. Glucose turnover was determined with [3-$^3$H]glucose, which was infused intravenously for 6 h, starting with a bolus of 40 μCi followed by a continuous infusion of 0.4 μCi/min. This produced steady-state tracer-specific activities within 120 min. Glucose was isolated from blood for determination of [3-$^3$H]glucose specific activity, as described previously (33). Rates of total body glucose appearance ($G_{A}$) and disappearance ($G_{D}$) were calculated using Steele’s equation for non-steady-state conditions (35). Rates of EGP were obtained by subtracting rates of glucose infused to maintain euaglycemia (GIR) from $G_{A}$.

GNG. Rates of GNG were determined with the $^3$H$_2$O method of Landau et al. (19–21). In the current study, we have used the C-5-to-$^3$H$_2$O ratio, which we have shown to give the same results as those obtained with the C-5-to-C-2 ratio (5). $^3$H enrichment of C-5 was determined by gas chromatography-mass spectrometry (Hewlett-Packard 5973 MSD, HP 5890 GC) as previously described (7). Enrichment of $^3$H in plasma water was determined in all subjects with an isotope ratio-mass spectrometer (PDZ Europa, London, UK) by use of an ABCA-G module and a standard curve with known enrichments ranging from 0.25 to 1.0%. $^3$H$_2$O enrichment was stable throughout the studies (see Fig. 2). To correct for the dilution of the $^3$H on C-5 of glucose, which occurred as a result of the infusion of unlabeled glucose during the clamp, the C-5-to-$^3$H$_2$O ratio was multiplied by $G_{R_{G}}$, i.e., the sum of exogenous and endogenous glucose entering the glucose space (10, 13).

$$GNG (\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}) = ^3\text{H} \cdot \text{C-5} / ^3\text{H}_2\text{O} \times G_{R_{G}}$$

GL was calculated as the difference between EGP and GNG:

$$GL (\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}) = EGP - GNG.$$
C-5 and $^2$H$_2$O enrichment, C-5-to-$^2$H$_2$O ratio, and 
[3-3H]glucose Specific Activity

The C-5 atom percent excess (APE) and the C-5-to-$^2$H$_2$O ratio both declined during the clamps as a result of the glucose infusions, but there were no significant differences between the insulin and the insulin plus lipid groups (Fig. 2).

$^2$H$_2$O APEs were the same in both groups and did not change during the clamps.

[3-3H]glucose specific activity was stable throughout the studies and was not different when the two groups were compared.

$G_R$,$_s$, $G_R$,$_d$, and GIR

Effects of insulin and insulin plus lipid on $G_R$,$_s$, $G_R$,$_d$, and GIR are shown in Table 2. Lipid inhibited insulin-stimulated $G_R$,$_d$ and GIR by 20 and 40%, respectively, at 240 min.

EGP, GNG, and GL

EGP decreased from 15.7 ± 1.4 (at 0 min) to 2.0 ± 2.5 (at 240 min, $P < 0.001$) μmol·kg$^{-1}$·min$^{-1}$ in the insulin group and from 15.8 ± 0.7 to 10.6 ± 1.4 μmol·kg$^{-1}$·min$^{-1}$ in the insulin plus lipid group ($P < 0.001$). The difference between the two groups was statistically significant ($P < 0.02$ at 240 min; Fig. 3).

GNG decreased from 8.2 ± 0.6 (at 0 min) to 3.7 ± 1.4 ($P < 0.05$ at 240 min) μmol·kg$^{-1}$·min$^{-1}$ in the insulin group. In the insulin plus lipid group, the decrease in GNG (from 7.1 ± 0.8 to 4.6 ± 1.0 μmol·kg$^{-1}$·min$^{-1}$) was not statistically significant. The difference between the two groups also was not statistically significant.

Fig. 1. Euglycemic (~5.5 mmol/l)-hyperinsulinemic (~450 pmol/l) clamping with ($\bullet$, $n = 6$ subjects) and without ($\circ$, $n = 7$) lipid/heparin infusion. Data are means ± SE.

C-5 and $^2$H$_2$O enrichment, C-5-to-$^2$H$_2$O ratio, and 
[3-3H]glucose Specific Activity

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GNG decreased from 8.2 ± 0.6 (at 0 min) to 3.7 ± 1.4 ($P < 0.05$ at 240 min) μmol·kg$^{-1}$·min$^{-1}$ in the insulin group. In the insulin plus lipid group, the decrease in GNG (from 7.1 ± 0.8 to 4.6 ± 1.0 μmol·kg$^{-1}$·min$^{-1}$) was not statistically significant. The difference between the two groups also was not statistically significant.
GL decreased continuously (from 7.4 ± 1.2 at 0 min to -1.7 ± 1.6 at 240 min, P < 0.01) in the insulin group. In the insulin plus lipid group, GL initially (0–120 min) decreased in parallel with the insulin alone group (from 9.1 ± 0.9 to 0.7 ± 1.3 μmol·kg⁻¹·min⁻¹, P < 0.02). However, after 120 min, GL rose so that at 240 min GL was significantly higher in the lipid-infused group (6.1 ± 1.6 vs. -1.7 ± 1.6 μmol·kg⁻¹·min⁻¹, P < 0.01).

GNG Precursors, Glucagon, and Cortisol

GNG precursor concentrations (the sum of plasma alanine, glutamine, glutamate, lactate, and glycerol concentrations) did not change in either group and were not significantly different in the two groups at any time (Fig. 4). Individual precursor levels are shown in Table 3. In the insulin plus lipid study, glycerol was higher than in the insulin alone study (probably because of heparin-induced lipolysis), whereas alanine and glutamic acid concentrations were lower.

Plasma glucagon concentrations decreased in the insulin group from 52 ± 3 at 0 min to 31 ± 3 pg/ml at

**Table 2. **$G_{R_a}, G_{R_d},$ and GIR  

<table>
<thead>
<tr>
<th>Study</th>
<th>Insulin</th>
<th>Insulin + Lipid</th>
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<tbody>
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<td>Time, min</td>
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<tr>
<td></td>
<td>120</td>
<td>180</td>
</tr>
<tr>
<td>$G_{R_a}$</td>
<td>15.3 ± 1.2</td>
<td>20.9 ± 1.9</td>
</tr>
<tr>
<td>$G_{R_d}$</td>
<td>14.8 ± 0.8</td>
<td>20.3 ± 2.1</td>
</tr>
<tr>
<td>GIR</td>
<td>0.0 ± 0.0</td>
<td>12.2 ± 2.1</td>
</tr>
</tbody>
</table>

Data are means ± SE. Units are μmol·kg⁻¹·min⁻¹. $G_{R_a}$, glucose rate of disappearance; $G_{R_d}$, glucose rate of appearance; GIR, glucose infusion rate. *P < 0.05 and †P < 0.01, insulin vs. insulin + lipid.
240 min (P < 0.01). Glucagon concentrations did not change in the insulin plus lipid group [56 ± 3 vs. 51 ± 5 pg/ml, not significant (NS)]. As a result, glucagon concentrations were significantly higher in the insulin plus lipid compared with the insulin group during the last 2 h of the studies. Cortisol concentrations decreased to a similar extent in the insulin group (from 351 ± 101 to 216 ± 60 nmol/l, P < 0.05) and in the insulin plus lipid group (from 310 ± 87 to 186 ± 53 nmol/l, P < 0.05). The two groups were not different from each other at any time.

**DISCUSSION**

The results of this study confirmed previous reports showing that physiological increases in plasma FFA concentrations partially inhibited the insulin suppression of EGP (1, 4, 11, 12, 29, 32, 34). When FFA levels in the current study decreased to <100 μM under the influence of postprandial-like insulin concentrations (∼450 pM), EGP fell from 15.7 ± 1.4 to 2.0 ± 2.5 μmol·kg⁻¹·min⁻¹ in 4 h. In contrast, when FFA levels rose (to ∼1,200 μM), EGP decreased ∼60% less (from 15.8 ± 0.7 to 10.6 ± 1.4 μmol·kg⁻¹·min⁻¹).

**Methodological Considerations**

To determine whether the difference in EGP suppression between the two groups was the result of inhibition of insulin action on GNG or GL or a combination of both, we measured GNG with the ²H₂O method of Landau et al. (19–21). This method determines the part of GNG that contributes to G_R in the circulation. Under postabsorptive (low-insulin) conditions, when glycogen synthesis is negligible (19, 28, 37), this technique provides reliable estimates of GNG, i.e., of glucose derived from all non-glucose precursors. Under hyperinsulinemic conditions, as in our study, two problems arise. 1) The [²H]glucose derived from GNG that enters the circulation is diluted by nonlabeled glucose infused to maintain euglycemia. This dilution effect can be corrected by multiplying the C-5-to-²H₂O ratio by G_R (i.e., the sum of exogenous and endogenous glucose entering the glucose space; see Refs. 10 and 13). 2) Hyperinsulinemia stimulates glycogen synthesis and thus the flux of GNG-derived glucose into glycogen. To the extent that this glucose remains in glycogen, i.e., does not return to the circulation, GNG will be underestimated. Hence, under hyperinsulinemic conditions, the ²H₂O method provides a minimal estimate of all GNG-derived glucose but an accurate account of the GNG-derived glucose contributing to G_R either directly or via glycogen. GL is not measured with this technique but is calculated as the difference between EGP and GNG. Consequently, it represents GL-derived glucose entering the blood plus the amount of GNG-derived glucose remaining in glycogen.

**Effect of Insulin**

In the present study, GNG declined from 8.2 to 3.7 μmol·kg⁻¹·min⁻¹ during the 4 h of hyperinsulinaemia (P < 0.05). Similar results have been reported from two recent studies, both using the ²H₂O method. Gastaldelli et al. (13) showed in overnight-fasted normal subjects that 2.5 h of euglycemic hyperinsulinemia (of similar degree as in our study) decreased GNG from 6.7 to 4.5 μmol·kg⁻¹·min⁻¹ (P < 0.003). Edgerton et al. (10), in a study of four 18-h-fasted dogs, using a 3-h portal venous insulin infusion that resulted in an approximately fourfold lower level of peripheral hyperinsulinemia compared with our study or the study by Gastaldelli et al. (114 vs. 450 pM), reported a non-significant decrease in GNG (from 7.8 to 6.5 μmol·kg⁻¹·min⁻¹; see Ref. 10). The same investigators obtained similar results using two other techniques to measure GNG, namely the hepatic arteriovenous difference and the [¹⁴C]phosphoenolpyruvate technique (10). Considering that the ²H₂O method overestimates insulin effects on GNG (by underestimating GNG because of glycogen cycling), recent data, including this study, seem to indicate that postprandial levels of insulin have only a modest impact on GNG. This is a significant change from the previously held notion that rates of GNG are completely suppressed in response to postprandial rises in insulin (31). This concept was based on in vitro and animal studies showing insulin-induced suppression of GNG and key GNG enzymes (16, 23, 38). Unphysiologically high insulin concentrations and longer exposure to insulin may explain at least some...
of the discrepancies between the older and newer data.

Given that a reduction in GNG could account for only a small part, it follows that most of the insulin-induced suppression of EGP had to be the result of suppression of GL. Indeed, GL decreased from 7.4 to \(-1.7\) \(\mu\)mol\(\cdot\)kg\(^{-1}\)\(\cdot\)min\(^{-1}\) in our study, from 4.5 to \(-0.9\) \(\mu\)mol\(\cdot\)kg\(^{-1}\)\(\cdot\)min\(^{-1}\) in the study by Gastaldelli et al. (13), and from 5.6 to \(2.2\) \(\mu\)mol\(\cdot\)kg\(^{-1}\)\(\cdot\)min\(^{-1}\) in the study by Edgerton et al. (10). Hence the currently available data indicate that insulin suppresses EGP primarily by suppressing GL.

**Effect of Insulin Plus Lipid**

Raising plasma FFA did not change GNG beyond the changes seen with insulin alone. This was somewhat surprising since FFA has been shown to stimulate GNG in animal experiments (14, 16, 17, 39) and also in human subjects (3, 7). The human studies, however, were performed under basal insulin conditions and therefore were not comparable to the present study, where insulin levels were elevated to approximately eightfold over basal levels.

During the initial 2 h, FFA also did not seem to affect insulin-mediated suppression of GL, i.e., GL decreased rapidly and at comparable rates during both insulin and insulin plus lipid infusions. After 2 h, however, GL continued to decrease in the insulin group, whereas GL rose in the insulin plus lipid group so that, after 3 and 4 h, GL was significantly higher in the insulin plus lipid than the insulin group.

**How Did FFA Inhibit Insulin Suppression of GL?**

Insulin infusions in the periphery decrease levels of glucagon (8, 10, 13), a hormone that has been established as a potent physiological regulator of GL (22, 25, 27). In study 1, glucagon levels decreased from 52 \(\pm\) 3 to 31 \(\pm\) 3 pg/ml during insulin infusion, whereas no change in plasma glucagon concentration occurred during insulin plus lipid infusions (56 \(\pm\) 3 vs. 51 \(\pm\) 5 pg/ml, NS). This suggested the possibility that the complete inhibition of GL during insulin infusion may have reflected the combined suppressive effects of increased insulin and decreased glucagon levels, whereas the absence of a significant inhibition of GL during insulin plus lipid infusion (at 240 min) may have been the result of continued GL stimulation by glucagon. Therefore, it was necessary to perform a control experiment (study 3) in which glucagon was prevented from decreasing. The results showed similar inhibition of GL in response to hyperinsulinemia regardless of whether glucagon levels decreased (study 1) or remained unchanged (study 3) (Fig. 5). Thus they demonstrated that the insulin-induced fall in GL observed in study 1 was unrelated to the decrease in plasma glucagon and supported data by others showing that hyperinsulinemia alone is sufficient to completely suppress EGP (and thus GL; see Ref. 36). On the other hand, the delay, by at least 2 h, in onset of FFA-induced inhibition of insulin action on GL is very similar to the delay in onset of FFA-induced inhibition of insulin-stimulated glucose uptake in skeletal muscle (reviewed in Ref. 2). Thus it seems likely that FFA caused hepatic insulin resistance by interfering with the inhibitory action of insulin on GL, which has been reported to result primarily from shunting of GL-derived glucose into glycogen (28).

In summary, we have confirmed studies by others showing that, in healthy, nonobese volunteers, insulin suppresses EGP primarily by inhibiting GL. We have expanded these findings by showing that elevated plasma FFA levels attenuate insulin suppression of EGP by interfering with insulin suppression of GL.
These observations may have physiological and pathophysiological significance. In healthy subjects, FFA-mediated attenuation of insulin-induced EGP suppression may prevent hypoglycemia after a protein- and fat-rich meal when insulin levels are elevated more than glucose levels. In diabetic patients, partial unresponsiveness of EGP to hyperinsulinemia is a major problem contributing to hyperglycemia (9). These patients commonly have elevated plasma FFA levels that may contribute to this problem by inhibiting insulin-mediated suppression of GL.

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REFERENCES


