Causal Linkage between Insulin Suppression of Lipolysis and Suppression of Liver Glucose Output in Dogs

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Abstract

Suppression of hepatic glucose output (HGO) has been shown to be primarily mediated by peripheral rather than portal insulin concentrations; however, the mechanism by which peripheral insulin suppresses HGO has not yet been determined. Previous findings by our group indicated a strong correlation between free fatty acids (FFA) and HGO, suggesting that insulin suppression of HGO is mediated via suppression of lipolysis. To directly test the hypothesis that insulin suppression of HGO is causally linked to the suppression of adipose tissue lipolysis, we performed euglycemic-hyperinsulinemic glucose clamps in conscious dogs (n = 8) in which FFA were either allowed to fall or were prevented from falling with Liposyn plus heparin infusion (LI; 0.5 ml/min 20% Liposyn plus 25 U/min heparin with a 250 U prime). Endogenous insulin and glucagon were suppressed with somatostatin (1 μg/min/kg), and insulin was infused at a rate of either 0.125 or 0.5 mU/min/kg. Two additional experiments were performed at the 0.5 mU/min/kg insulin dose: a double Liposyn infusion (2×LI; 1.0 ml/min 20% Liposyn, heparin as above), and a glycerol infusion (19 mg/min). With the 0.125 mU/min/kg insulin infusion, FFA fell 40% and HGO fell 33%; preventing the fall in FFA with LI entirely prevented this decline in HGO. With 0.5 mU/min/kg insulin infusion, FFA levels fell 64% while HGO declined 62%. Preventing the fall in FFA at this higher insulin dose largely prevented the fall in HGO; however, steady state HGO still declined by 18%. Doubling the LI infusion did not further affect HGO, suggesting that the effect of FFA on HGO is saturable. Elevating plasma glycerol levels did not alter insulin’s ability to suppress HGO. These data directly support the concept that insulin suppression of HGO is not direct, but rather is mediated via insulin suppression of adipose tissue lipolysis. Thus, resistance to insulin control of hepatic glucose production in obesity and/or non—insulin-dependent diabetes mellitus may reflect resistance of the adipocyte to insulin suppression of lipolysis. (J. Clin. Invest. 1996; 98:741–749.) Key words: adipose tissue • insulin action • hepatic glucose output • free fatty acids • lipolysis

Introduction

The effect of insulin to suppress hepatic glucose output (HGO) in fasting conditions has been shown to be determined predominantly by systemic rather than portal insulin concentrations (1–4). Also, the time course of insulin suppression of HGO has been shown to be virtually identical to that for stimulation of peripheral glucose uptake (5–7). These observations have led to the suggestion that insulin control of liver glucose production is dominated by an extrapleatic effect of the hormone. We have previously shown that the slow effect of insulin on glucose uptake (Rg) is due to insulin transport across the capillary endothelial barrier of insulin-sensitive tissues (8). Given that insulin’s effect on HGO is mediated by peripheral insulin levels, and that the dynamics of the effect are identical to those of Rg, we postulated that the appearance of insulin in the interstitial fluid of extrapleatic tissues both increases Rg and activates a secondary blood-born signal that suppresses HGO. We have termed this concept the “single-gateway-hypothesis” (8, 9); the single gateway being the capillary endothelium in insulin-sensitive peripheral tissues.

While we have previously demonstrated a correlative relationship between free fatty acids (FFA) and HGO during insulin administration (1), such a correlation does not prove that insulin regulates HGO through FFA. What is needed is a study evaluating insulin suppression of HGO under conditions in which FFA reductions with insulin are prevented. Thus, in the present study, hyperinsulinemic-euglycemic clamps were performed in which FFA were either allowed to decline or were prevented from declining with Liposyn and heparin (LI). Results indicated that preventing the fall in FFA during insulin blocks the suppression of HGO. These data provide direct support for the concept that insulin suppression of FFA is a necessary link in the overall effect of insulin to suppress HGO. Further, these results support the possibility that hepatic insulin resistance could be due to a failure of insulin to lower FFA release from adipose tissue rather than a defect within the liver itself.

Methods

Animals. Experiments were conducted on eight conscious male mongrel dogs (26.8±0.5 kg, range: 23.4–31.5 kg). Dogs were housed under controlled kennel conditions (12 h light:12 h dark) in the University of Southern California (USC) Medical School Vivarium. Animals had free access to standard chow (25% protein, 9% fat, 49% carbohydrate, and 17% fiber; Wayne Dog Chow, Alfred Mills, Chicago, IL) and tap water. Food was withdrawn 15 h before experiments. Dogs were well accustomed to laboratory procedures and were used for experiments only if judged to be in good health as determined by visual

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observation, weight stability, body temperature, and hematocrit. 7–10 d before experiments, chronic catheters were surgically implanted. One catheter was inserted in the jugular vein and advanced to the right atrium for sampling of central venous blood, and a second catheter was inserted in the femoral vein and advanced to the vena cava for tracer and insulin infusion. All catheters were led subcutaneously to the neck and exteriorized. Catheters were flushed with heparinized saline (100 U/ml) twice a week and the exteriorization site was cleaned with hydrogen peroxide (4%). On the morning of each experiment, acute catheters were inserted in peripheral veins for infusion of glucose and, when necessary, Liposyn (Abbott Laboratories, North Chicago, IL) or glycerol (Sigma Immunochemicals, St. Louis, MO). Dogs received iron supplement tablets (65 mg; Rugby Laboratories, Norcross, GA) for 3 d after experiments. Experiments in individual animals were separated by at least 1 wk. The experimental protocol was approved by the USC Institutional Animal Care and Use Committee.

**Experimental protocol.** Euglycemic glucose clamps were conducted with either a “low” (0.125 mU/min/kg porcine insulin; Novo-Nordisk, Copenhagen, Denmark) or “high” (0.5 mU/min/kg) dose peripheral insulin infusion. During the low-dose infusion, FFA were either allowed to decline (control) or prevented from declining with Liposyn and heparin (LI; 0.5 ml/min 20% Liposyn plus 25 U/min heparin with a 250 U prime). Three protocols were performed at the high-dose insulin infusion. FFA were again either allowed to decline (control) or were prevented from declining with LI. In addition, a third experiment was performed in which the LI infusion was doubled (2×LI; 1.0 ml/min 20% Liposyn, heparin as above). Finally, since the Liposyn solution contains 5% glycerol as an emulsifier and heparin elicits glycerol release, a glycerol-only protocol was performed at the high-insulin dose in a subset of four animals. For these experiments, glycerol was infused at 19 mg/min so as to match the glycerol level achieved with the low-dose insulin plus LI protocol. For paired statistical comparisons missing cells were replaced with the mean of the respective groups.

**Table I. Experimental Design**

<table>
<thead>
<tr>
<th>Dog</th>
<th>Insulin 0.125 mU/min/kg</th>
<th>Insulin 0.5 mU/min/kg</th>
<th>Glycerol (mg/min)</th>
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</thead>
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<tr>
<td></td>
<td>LI (ml/min)</td>
<td>LI (ml/min)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>x</td>
<td>x</td>
<td>19</td>
</tr>
<tr>
<td>2</td>
<td>µ</td>
<td>x</td>
<td>x</td>
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<td>x</td>
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<td>x</td>
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<td>x</td>
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<tr>
<td>8</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
</tbody>
</table>

Euglycemic glucose clamps (denoted as x) performed in each animal. For paired statistical comparisons missing cells were replaced with the mean (denoted µ) of the respective groups.

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**Assays.** Immediately after sampling, plasma glucose and lactate were measured with an autoanalyzer (YSI 2700; Yellow Springs Instrument Co., Yellow Springs, OH) using glucose oxidase and lactate oxidase. For measurement of [3-H]glucose concentration, samples were deproteinized with zinc sulfate and barium hydroxide, the supernatant was evaporated in a vacuum, and the sample was redissolved in water and counted in ReadySafe scintillation fluid (Beckman liquid scintillation counter; Beckman, Fullerton, CA). Tracer infusates and plasma samples were processed and counted in an identical manner. Insulin was measured by an ELISA originally developed for human serum or plasma by Novo-Nordisk and adapted for dog plasma in our laboratory with the kind assistance of B. Dinesen (11). The method is based on two murine monoclonal antibodies that bind to different epitopes on the insulin molecule. Prolactin is not bound by the antibodies. Materials for the insulin assay, including the dog standard, were kindly provided by Novo-Nordisk. Glucagon was assayed using a kit obtained from Novo-Nordisk. The glucagon kit includes an ethanol deproteinization step and uses antiserum K5563. FFA were analyzed using a kit from Wako (NEFA C; Wako Pure Chemical Industries, Osaka, Japan) which uses the acylation of coenzyme-A. Glycerol samples were deproteinized with zinc sulfate and barium hydroxide and measured spectrophotometrically using glycerokinase and glycerophosphate dehydrogenase (12).

**Calculations.** HGO and glucose disappearance (Rd) were calculated using Steele’s model with a labeled glucose infusion as detailed previously (6). Tracer-determined whole body glucose appearance was assumed to be equal to HGO (we are aware that under some circumstances renal glucose production may be significant [13]). Basal (−60 to −30 min) and steady state (240 to 270 min) clamp values were defined as the average of four samples taken every 10 min. Michaelis-Menten parameters were calculated with MLAB (Civilized Software, Bethesda, MD) implemented on an IBM-compatible computer.

**Statistical analysis.** Repeated measure ANOVA was calculated using SAS (Cary, NC) also implemented on an IBM-compatible computer. Overall steady state comparisons to basal were performed using two-way ANOVA (time and LI) separately at the low- and high-insulin dose. A priori (planned) individual steady state comparisons to basal were evaluated by paired t test. Comparisons at steady state were performed by one-way repeated measure ANOVA using Tukey’s procedure. Data are reported as mean ± SEM with P values < 0.05 considered significant. For paired statistical comparisons the two missing experiments (one in the low-dose insulin control group and one in the double LI group) were replaced by the mean of the respective groups (denoted µ in Table I). Statistics for experiments involving the glycerol infusion, which were performed on a subset of four of the seven dogs, were calculated by ANOVA or paired t test using the corresponding matched experiments.
Results

Glucose, glucose specific activity, and glucagon. Fasting basal glucose averaged 94.2±1.1 mg/dl and was clamped with an overall coefficient of variation of 7% (Fig. 1A; all experiments). Glucose concentration could not be clamped at basal during the LI/low-dose insulin infusion; for this group steady state glucose concentration was slightly higher than basal (110.1±5.8 vs. 96.1±2.1 mg/dl; Table II basal vs. steady state P = 0.017 with significant interaction P = 0.0312, two-way ANOVA); glucose was successfully clamped at basal for all groups during high-dose insulin infusion. Because the exogenous glucose infusate was prelabeled, glucose specific activity (Fig. 1B) was also approximately constant. Glucagon-like immunoreactivity was suppressed by ~50% with somatostatin (79.0±6.72 to 39.9±23.0 ng/liter; Fig. 1C). Glucagon-like immunoreactivity was not completely suppressed during the somatostatin infusion, presumably due to cross-reactivity of glucagon-like proteins. Cross-reaction with glucagon-like peptides results in an overestimation of the glucagon concentration, suggesting that the true level may actually have been more suppressed than suggested by Fig. 1C.

Lactate. Steady state lactate concentrations during low-dose insulin infusion were higher than preclamp levels. However, there was no effect with or without LI infusion (Table II, P > 0.05 ANOVA). No significant changes were observed in lactate during the high-dose insulin infusion (basal vs. steady state not different and no effect by LI dose; P > 0.05).

Insulin. Steady state insulin concentrations (Table II) during the low-dose insulin infusion experiments were not significantly different between control and LI infusion (69±7 vs. 67±6 pm; P > 0.05, ANOVA), indicating that the LI infusion did not affect insulin clearance (endogenous insulin was suppressed by somatostatin). However, steady state insulin concentrations at this dose were significantly lower than basal (P = 0.028; ANOVA) indicating that the 0.125 mU/min/kg peripheral insulin infusion underreplaced insulin. Steady state insulin concentrations during the high-dose insulin infusions were approximately twofold higher than basal and were not different among the control, LI, and 2×LI groups (Table II, ANOVA P > 0.05).

FFA. Despite the underreplacement of insulin during the low-dose insulin infusion study, steady state FFA levels were lower than basal (343±14 vs. 575±39 μmol/liter, P = 0.0004 paired t test; Fig. 2A, Table II). Infusion of LI prevented the decline in FFA, and steady state levels were significantly higher than basal (592±27 μmol/liter, P = 0.00063 paired t test). As LI was initiated 30 min before the start of the insulin infusion, FFA levels were elevated for the entire clamp period.

During the high-dose insulin infusion, steady state FFA levels were further suppressed (Fig. 3; 181±51 vs. 343±14 μmol/liter) although this difference was not significant by ANOVA (P > 0.05). LI again elevated FFA levels for the duration of the clamp period (Fig. 2D). While steady state FFA levels tended to be above basal, this increase did not achieve statistical significance (730±85 vs. 577±51 μmol/liter; P = 0.07; paired t test). Doubling the LI infusion rate had the expected effect of further increasing FFA levels (730±85 vs. 1,251±84 μmol/liter; ANOVA; P < 0.05).

Peripheral glucose uptake. Despite significant declines in both FFA and insulin during the low-dose insulin without LI, no significant changes were observed in Rg (Fig. 2B, Table III). With LI, FFA levels were elevated but this did not significantly affect Rg (Fig. 3B; steady state Rg with LI not different than insulin alone; P = 0.09). While total glucose uptake was unaffected by LI infusion, the glucose concentration increased (96.1±2.1 to 110.1±5.8 mg/dl, P < 0.05 ANOVA) indicating

Table II. Steady State Peripheral Hormone Concentrations during Euglycemic Clamps

<table>
<thead>
<tr>
<th></th>
<th>Low-dose insulin (0.125 mU/min/kg)</th>
<th>High-dose insulin (0.5 mU/min/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control/LI</td>
<td>Control/LI</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>Basal</td>
<td>ss</td>
</tr>
<tr>
<td></td>
<td>97.1±2.3</td>
<td>96.1±2.1</td>
</tr>
<tr>
<td></td>
<td>97.7±4.0</td>
<td>110.1±5.8</td>
</tr>
<tr>
<td>Lactate (mg/liter)</td>
<td>Basal</td>
<td>ss</td>
</tr>
<tr>
<td></td>
<td>54±5</td>
<td>54±9</td>
</tr>
<tr>
<td></td>
<td>75±7</td>
<td>76±16</td>
</tr>
<tr>
<td>Insulin (pmol/liter)</td>
<td>Basal</td>
<td>ss</td>
</tr>
<tr>
<td></td>
<td>107±4</td>
<td>97±16</td>
</tr>
<tr>
<td></td>
<td>69±7</td>
<td>67±6</td>
</tr>
<tr>
<td>Glucagon (ng/liter)</td>
<td>Basal</td>
<td>ss</td>
</tr>
<tr>
<td></td>
<td>54±5</td>
<td>82±7</td>
</tr>
<tr>
<td></td>
<td>27±3</td>
<td>44±5</td>
</tr>
<tr>
<td>FFA (μmol/liter)</td>
<td>Basal</td>
<td>ss</td>
</tr>
<tr>
<td></td>
<td>575±39</td>
<td>543±37</td>
</tr>
<tr>
<td></td>
<td>343±14</td>
<td>952±71</td>
</tr>
<tr>
<td>Glycerol (μmol/liter)</td>
<td>Basal</td>
<td>ss</td>
</tr>
<tr>
<td></td>
<td>44±8</td>
<td>39±15</td>
</tr>
<tr>
<td></td>
<td>22±7</td>
<td>173±32</td>
</tr>
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</table>

Figure 1. Average glucose (A), glucose specific activity (B), and glucagon concentration pooled across all experimental groups (C) (n = 37; see Table I).

Hepatic Glucose Output Regulated by FFA
that the increase in FFA at this dose did have an effect on 
decreasing glucose clearance. The decrease in glucose clearance 
is likely explained by the Randle hypothesis (substrate compe-
tition). High-dose insulin, without LI, significantly elevated 
\( R_d \) from basal (5.41 ± 0.43 vs. 3.40 ± 0.29 mg/min/kg, 
\( P = 0.001 \)). Increases in FFA at this insulin dose, slightly, but systemati-
cally, decreased \( R_d \) (Fig. 3, A and B; ANOVA linear trend test; 
\( P = 0.04 \)), again consistent with the Randle hypothesis.

**HGO.** During low-dose insulin without LI infusion, HGO 
rapidly declined from 3.18 ± 0.25 mg/min/kg to 2.14 ± 0.11 mg/
min/kg (Fig. 2 C, Table III; basal vs. steady state, \( P = 0.003 \)). 
This fall in HGO cannot be attributed to an increase in insulin 
(insulin decreased at this dose) and is most likely due to the 
suppression of glucagon. Interestingly, when FFA were pre-
vented from decreasing, HGO initially fell but thereafter re-
covered to a level not different from basal (Fig. 2 C; 3.21 ± 0.24 
vs. 3.22 ± 0.32 mg/min/kg; \( P = 0.99 \)). This suggests that gluca-
gon’s effect to maintain basal HGO is in part dependent on the 
maintenance of basal FFA levels.

During the high-dose insulin without LI, HGO declined by 
\( 60\% \) (3.34 ± 0.27 to 1.28 ± 0.15 mg/min/kg; \( P = 0.0001 \)). 
While preventing the decline in FFA almost prevented this fall 
in HGO, the steady state production was still 18% lower than 
basal (2.68 ± 0.41 vs. 3.25 ± 0.34 mg/min/kg; \( P = 0.014 \)). Dou-
bling the LI infusion rate did not further affect steady state 
HGO (2.75 ± 0.34 vs. 2.68 ± 0.41 mg/min/kg, \( P > 0.05 \) ANOVA; 
Fig. 3 B). While doubling the LI infusion rate did not affect 
steady state HGO, there was a tendency to prevent some of 
the transient fall (see Fig. 2 F); the average HGO between 60 
and 180 min was 22% lower in the LI experiments compared 
with the double LI experiments (2.41 ± 0.33 vs. 2.95 ± 0.42 mg/
min/kg; \( P = 0.2 \); Fig. 2 F).

Steady state FFA, \( R_d \), and HGO values are summarized in 
Fig. 3. Individual basal values, which were not different among 
protocols (ANOVA, \( P > 0.05 \)), have been pooled to facilitate 
comparisons. In the absence of LI infusion (open bars), FFA 
and HGO had very similar responses; with LI both FFA and 
HGO were prevented from decreasing. However, the effect of 
the increase in FFA on HGO appeared to saturate. At the 
high-insulin dose, increasing FFA from 181 ± 51 to 730 ± 85 
\( \mu \)mol/liter increased HGO from 1.28 ± 0.15 to 2.68 ± 0.41 mg/
min/kg, whereas a further increase in FFA (to 1,251 ± 84 \( \mu \)mol/
liter) had no further effect (2.75 ± 0.34 mg/min/kg). This satura-
tion was well described by classical Michaelis-Menten analysis

![Figure 2. FFA (A and D), glucose disposal (B and E), and HGO (C and F) dynamics during low-dose (left column; 0.125 mU/
min/kg insulin) and high-dose (right column; 0.5 mU/min/kg insulin) insulin infusions. FFA concentrations were either al-
lowed to fall (filled circles), prevented from falling with an LI infusion (open circles; 0.5 
ml/min LI), or double LI infusion (open tri-
angles; high insulin infusion only).](https://doi.org/10.1172/JCI118846)

<table>
<thead>
<tr>
<th>( R_d ) (mg/min/kg)</th>
<th>Low-dose insulin (0.125 mU/min/kg)</th>
<th>High-dose insulin (0.5 mU/min/kg)</th>
</tr>
</thead>
<tbody>
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<td>Control LI</td>
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<td>3.30 ± 0.23</td>
</tr>
<tr>
<td>LI</td>
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<td>3.38 ± 0.34</td>
</tr>
<tr>
<td>ss</td>
<td>3.31 ± 0.35</td>
<td>4.76 ± 0.38</td>
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<tr>
<td>HGO (mg/min/kg)</td>
<td>3.09 ± 0.24</td>
<td>3.75 ± 0.34</td>
</tr>
<tr>
<td>Control LI</td>
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<td>3.14 ± 0.30</td>
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<tr>
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<td>3.28 ± 0.27</td>
<td>2.68 ± 0.41</td>
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Table III. Steady State Glucose Fluxes during 
Euglycemic Clamps

![Downloaded from http://www.jci.org](https://doi.org/10.1172/JCI118846)

Downloaded from http://www.jci.org on October 14, 2017. https://doi.org/10.1172/JCI118846
Hepatic Glucose Output Regulated by FFA

For both the low-dose and high-dose insulin infusions, the half-maximal effect of FFA on HGO was not altered by insulin; that is, $K_m$ was $300 \pm 175$ and $272 \pm 180$ μmol/liter low- and high-dose insulin, respectively. However, the maximal effect ($V_{max}$) may have been slightly decreased at the high-insulin dose (3.5 vs. 4.1 mg/min/kg).

Glycerol. As glycerol was used as an emulsifier in the LI solution and is released by the breakdown of triglyceride, the possibility exists that glycerol, rather than FFA, affected steady state HGO. To investigate this possibility, glycerol infusion experiments were performed on a subset of four animals at the 0.5 mU/min/kg insulin dose (Fig. 5 with corresponding steady state shown in Fig. 6). Preclamp values were again not different among groups (Fig. 6; ANOVA; $P > 0.05$) and have been pooled for comparison. As with the larger set of animals ($n = 7$), FFA and HGO were suppressed at this insulin dose and preventing the fall in FFA largely prevented the fall in HGO. However, as expected, LI infusion also elevated plasma glycerol levels with respect to both basal (180±45 vs. 153±16 μmol/liter; $P > 0.05$) and steady state control (16±10 μmol/liter; $P < 0.05$). However, infusing glycerol so as to approximately match the steady state levels obtained in the LI experiments (180±45 vs. 153±16 μmol/liter; $P > 0.05$) did not

Figure 3. Steady state ($n = 7$) FFA (A), glucose disposal (B), and HGO (C). Preclamp values represent the average basal values for the groups shown (pooled basal values not different by ANOVA; see text for details). Steady state values are shown without LI (open bars), with LI (narrow hatch), and with double LI (wide hatch).

Figure 4. Michaelis-Menten fit for the low-dose (open circles, ○) and high-dose (filled circles, ●) insulin infusion.

Figure 5. Dynamic changes in FFA (A), glycerol (B), and HGO (C) during high-dose insulin infusion (filled circles), high-dose insulin infusion + LI (open circles), and high-dose insulin infusion + glycerol (filled triangles). Data shown are for the subset ($n = 4$) of animals that received glycerol infusion experiments (see Table I).
prevented the fall in HGO; steady state HGO with and without glycerol infusion were not significantly different (1.21 ± 0.40 vs. 1.43 ± 0.24 mg/min/kg; P = 0.56). These data indicate that glycerol was not the factor which prevented the fall in HGO during the LI experiments.

**Discussion**

The disposal of glucose after meals depends upon insulin’s ability to increase peripheral glucose uptake and decrease HGO. The decrease in HGO is an important component of glucose tolerance. While the regulation of HGO after meals is affected by many signals (e.g., the increase in glucose, changes in gluconeogenic substrate supply, FFA, glucagon, etc.), the liver response to insulin per se has been shown to be impaired in non–insulin-dependent diabetes mellitus (NIDDM, hepatic insulin resistance). The underlying cause of this hepatic insulin resistance is not yet clear. Results in this study demonstrate that under fasting conditions, insulin suppression of HGO is mediated, in part, by the suppression of plasma FFA. In the absence of glucagon, insulin’s dose-dependent suppression of HGO was almost completely blocked by preventing the fall in FFA (Figs. 2 and 3). However, elevating FFA above basal did not increase HGO above basal, suggesting that the effect is saturable (Fig. 4). The suppression of HGO was not prevented by glycerol infusion (Figs. 5 and 6) suggesting that FFA, rather than glycerol, was the signal that prevented the fall in HGO.

The dependence of HGO suppression on a concomitant fall in FFA is consistent with previous studies in dogs indicating that HGO is regulated by peripheral rather than portal insulin levels (1–3). That is, studies in which insulin was infused into the peripheral circulation, or into the portal circulation at twice the peripheral infusion rate, resulted in equal systemic insulin levels but vastly different portal insulin levels. However, the higher portal insulin concentrations with portal infusion did not result in further suppression of HGO. That portal insulin delivery does not result in a more rapid or substantially greater suppression of HGO has been confirmed recently by Lewis et al. (14) in humans. In the Lewis study, euglycemic glucose clamps were performed with either portal insulin delivery (response to a tolbutamide infusion) or peripheral insulin delivery (exogenous insulin infusion). In paired experiments in which the peripheral insulin levels were matched (peripheral insulin infusion at half the calculated endogenous secretion rate), portal insulin delivery resulted in 50% suppression of HGO and peripheral insulin delivery resulted in 37% suppression. While this difference was statistically significant, it still suggests that the majority of HGO suppression was mediated by peripheral insulin (i.e., portal insulin delivery resulted in a steady state HGO that was only 16% lower than that obtained with peripheral insulin delivery at half the portal rate). Further, the rate of HGO suppression observed in the Lewis study was not increased with portal insulin delivery. That is, HGO suppression during the first 60 min of the clamp was virtually identical in the paired portal and half-peripheral insulin infusion experiments. Thus, studies in both dog and human strongly suggest that HGO is largely determined by a peripheral insulin-dependent signal. While we originally speculated that such a signal might originate in skeletal muscle, the strong correlation between FFA and HGO demonstrated in our previous study (1) suggested that adipose tissue was the “peripheral signal” site. However, while the observed correlation between FFA and HGO in our previous study was impressive, it cannot be taken as proof of causality. The present study was therefore undertaken to answer the question: Would HGO have declined if FFA levels had been prevented from falling? Results obtained here clearly indicate that the decline in FFA is required for HGO to be fully suppressed by elevations in plasma insulin (Fig. 3). This supports the hypothesis that one mechanism by which insulin controls HGO in the fasting state is by regulating plasma FFA via insulin control of lipolysis.

The results in this study, indicating that FFA concentrations must decline if insulin is to be fully effective at suppressing HGO, as well as results in the previous study, indicating that HGO responds primarily to peripheral insulin (1), were both obtained in the absence of glucagon and under conditions of euglycemia. During a meal, of course, both glucose and insulin increase while glucagon levels decrease. Thus, care must be taken in extrapolating the present results to conditions of elevated glucose or normal glucagon levels. While it has not been definitively shown that the acute removal of glucagon
completely suppresses glycogenolysis, it is likely that this component of HGO was suppressed in the present experiments and that the gluconeogenic component was dominant. Further, the relative sensitivities as well as the time courses of insulin suppression of these two components of HGO have been shown by Cherrington and colleagues to differ (15). Thus, the effect of FFA on insulin suppression of HGO may have to be evaluated under normal or elevated glucagon conditions. In addition, Pagliassotti et al. have shown recently that portal glucose delivery may affect both liver glucose uptake and HGO (16), leading to speculation that under portal hyperglycemia, there may be a greater effect of portal insulin. Thus, the direct versus indirect effects of insulin may also have to be evaluated under conditions of portal glucose delivery.

The present results provide insight into the role of glucagon in determining HGO in the postabsorptive state. This component of HGO has been estimated previously by Cherrington and colleagues to be ~ 60–70% of basal production (17). That is, the acute removal of glucagon together with portal insulin replacement resulted in a 60–70% drop in HGO (17). In the present study the fall in HGO with acute suppression of glucagon was only 33%; however, insulin was administered peripherally rather than portally and steady state plasma insulin was significantly lower than basal. In our previous study, suppression of glucagon together with replacement portal insulin infusion resulted in ~ 50% suppression of HGO (1), a value closer to that reported by Cherrington. Nevertheless, the suppression of glucagon by somatostatin results in a concomitant decline in FFA, and if this decline is prevented HGO initially falls but then slowly recovers back to preclamp levels (see Fig. 2 C). The slow recovery suggests an effect of FFA to increase gluconeogenesis with a delayed time course relative to the increase in FFA. Further, it suggests that part of the fall in HGO observed during acute suppression of glucagon may be due to a concomitant fall in FFA. Reasons for the acute fall in FFA with glucagon suppression and replacement insulin are not entirely clear. A physiologic role of glucagon per se in maintaining FFA levels is controversial. Several groups have reported a glucagon effect to increase lipolysis (18–20), whereas other studies have failed to observe any effect (21, 22). Thus, while the fall in FFA observed in this study during low-dose insulin infusion may have been due to suppression of glucagon, there are several other possibilities. For example, growth hormone is suppressed with somatostatin (18) and is known to have a lipolytic effect (23, 24). Also, lactate increased in the present study and this may have contributed to the suppression of lipolysis (25). In any case, the role of glucagon in maintaining basal HGO may need to be reevaluated in a study in which FFA levels are carefully controlled.

In the present study, there was no indication that glycerol rather than FFA was responsible for attenuating the insulin-induced inhibition of HGO (Fig. 5). This result is consistent with results obtained by Jahoor et al. (26). However, the Jahoor study was performed after an 86-h fast and under those conditions the contribution of glycerol to gluconeogenesis may have been significantly increased. Glycerol normally accounts for only 3–10% of basal HGO (27), but may be increased to up to 60% in extreme starvation (28). Results in the present study clearly indicate that after an overnight fast glycerol has little effect on HGO (Figs. 5 and 6). The glycerol infusion used in the present study (19 mg/min) resulted in an approximate threefold increase in glycerol without any increase in HGO (Fig. 6). While the data of Fig. 6 indicate that glycerol did not account for the increase in HGO seen with the LI infusion, care should be taken in concluding that glycerol had no effect; that is, the results in Fig. 6 are based on four experiments and although this allowed for sufficient power to rule out an effect on HGO equal to that observed with LI infusion, it cannot rule out a smaller effect. Nonetheless, the conclusion that glycerol does not significantly alter HGO is supported by a study by Boden et al. (29) which also did not find any alterations in HGO during clamps in which glycerol was either allowed to fall or prevented from falling, and by a study by Nurjhan et al. (30) that indicated that FFA, rather than glycerol per se, determined glycerol conversion to glucose.

The role of FFA in HGO regulation observed here is supported by a recent study by Fanelli et al. (31) demonstrating the importance of FFA for the HGO response during counterregulation. In the Fanelli study, insulin-induced hypoglycemia elicited a counterregulatory response which elevated both FFA and HGO. However, when the increase in FFA was prevented with Acipimox (a nicotinic acid derivative), the rise in HGO was markedly attenuated. This attenuation was largely due to a decrease in gluconeogenesis (with Acipimox HGO was reduced ~ 40%, and gluconeogenesis from alanine was reduced ~ 70%). Mechanisms by which FFA might increase gluconeogenesis include an increase in NADH (necessary for the conversion of 1,3-biphosphoglycerate to glyceraldehyde-3-phosphate), an increase in ATP production, and the activation of pyruvate carboxylase by acetyl-CoA (32).

While elevated FFA may increase gluconeogenesis, it is important to note that such changes may not necessarily yield equivalent changes in HGO (33). For example, Clore et al. demonstrated a fall in both FFA and HGO during sleep (34), and that preventing the fall in FFA with heparin increased gluconeogenesis without any change in HGO (35). These results led the authors to hypothesize that the relative contributions of gluconeogenesis and glycogenolysis are autoregulated; that is, decreases in gluconeogenesis are compensated for by increases in glycolysis. Interestingly, while such studies suggest that lowering gluconeogenesis increases glycogenolysis, it is unclear if the converse is also true; that is, the lowering of glycogenolysis increases gluconeogenesis. The data of Fig. 2C suggest that FFA may play an important role in such an autoregulatory process. When glucagon was suppressed and insulin was slightly underreplaced, both FFA and HGO fell; however, when FFA levels were elevated the fall in HGO was only transient. In any case, it is important to note that under the hypoglucagonemic conditions of the present study, autoregulation between glycolysis and gluconeogenesis may have been disabled. If this were true, changes in FFA would be mirrored by changes in HGO, whereas in the presence of glucagon, changes in gluconeogenesis brought on by changes in FFA could be masked by autoregulatory increases in glycolysis. Further studies in which the FFA effect on insulin suppression of HGO is evaluated under differing glucagon levels are needed to answer this question.

The effects of lowering FFA on both fasting HGO and hepatic insulin sensitivity have been investigated by several groups. Studies in which FFA are pharmacologically lowered have been, in part, motivated by the belief that fasting hyperglycemia is due to an elevation in fasting HGO (tracer methodology supporting this contention has been recently challenged by several groups [36–38]). These studies have pro-
duced conflicting results on any concomitant changes in HGO. For example, Johnston et al. (39) demonstrated that fasting HGO was unaffected during a 6-h tracer equilibration during which FFA were either elevated with Intralipid or decreased with nicotinic acid. This result is in agreement with a study by Puhakainen and Yki-Jarvinen in which overnight suppression of FFA with the nicotinic acid derivative Acipimox failed to produce a decrease in either HGO or fasting glucose in NIDDM subjects (33). However, a similar study by Fulcher et al. (again, NIDDM subjects with overnight suppression of FFA by Acipimox) did result in a 20% decrease in fasting HGO (40). The difference in the Fulcher study and the Yki-Jarvinen study might be explained by hepatic autoregulation. Subjects in the Fulcher study had undergone 14 d of Acipimox treatment and had been fasted under conditions of low FFA, conditions that may have accelerated hepatic glycogen depletion. Conversely, the subjects in the Yki-Jarvinen study had only received Acipimox for 6 h before the determination of HGO. Therefore, it is possible to speculate that in the presence of adequate glycogen stores, the liver may compensate for a decrease in gluconeogenesis by a concomitant increase in glycolysis. This hypothesis is supported by a study in overnight fasted subjects in which gluconeogenesis was decreased with ethanol without any concomitant change in fasting HGO (41). In any case, data in the present study (Fig. 4) clearly support the contention that elevations in FFA above basal do not increase HGO above basal.

While it is not yet clear if elevated FFA levels can lead to an increase in fasting glucose production, it is clear that such an elevation can lead to hepatic insulin resistance. Clamp studies in which insulin is elevated have consistently shown that HGO is less suppressed if FFA are prevented from falling. In the clamps performed by Boden et al. (29) (see above), insulin was elevated to ~70 μU/ml and HGO was ~50% less suppressed if FFA were prevented from falling with an Intralipid infusion. Similar results were achieved in a study by Bevilacqua et al. (42), in which insulin was raised to ~60 μU/ml and ~57% of the fall in HGO was eliminated when FFA were prevented from falling. Interestingly, results obtained in both of those studies (29,42) were obtained under conditions in which glucagon was allowed to change normally; nevertheless the results are quite similar to those obtained in the present study in which glucagon was suppressed with somatostatin. In the present study, insulin was elevated to ~35 μU/ml (210 pmol/liter) and this resulted in a 2.06 mg/min/kg drop in HGO; with LI infusion the fall in HGO was limited to 0.57 mg/min/kg (FFA were overreplaced during the LI infusion. However, if HGO is estimated from Fig. 4 with the correct FFA replacement, then the estimated decrease would still only have been 0.9 mg/min/kg). Thus, under the suppressed glucagon conditions of the present study, ~56% of the drop in HGO could have been prevented if FFA had been maintained at basal. Taken together, these studies all suggest that approximately half the insulin-induced fall in HGO under fasting conditions is mediated by the FFA signal.

This study has important implications for understanding hepatic insulin resistance in NIDDM. Several studies have shown NIDDM subjects to be resistant to FFA suppression by insulin (43–46), and evidence exists for both an increase in lipolysis (30) and a decrease in reesterification (47). Data also exist to suggest that increased lipid oxidation may contribute to hepatic insulin resistance in NIDDM (46), and that FFA suppression and HGO suppression are concomitantly impaired (48). Recent studies using 13C-nuclear magnetic resonance imaging have shown that increased HGO in NIDDM subjects is due to increased gluconeogenesis (49). Thus, the possibility exists that hepatic insulin resistance in NIDDM is due, at least in part, to a failure of insulin to suppress FFA which plays a preeminent role in regulating gluconeogenesis.

In conclusion, our previous study clearly established that in the absence of glucagon, insulin suppression of FFA and HGO are linearly correlated (1). The present study established that the fall in FFA is, in fact, necessary if HGO is to be fully suppressed. Thus, in the absence of glucagon, FFA plays a significant role in the regulation of HGO. Under fasting conditions, ~50% of insulin suppression of HGO can be prevented by preventing the insulin-induced fall in FFA. That a fall in FFA is required for insulin to suppress HGO supports the hypothesis that hepatic insulin resistance may be causally related to a reduced ability of insulin to suppress lipolysis.

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