Inhibition of lipolysis causes suppression of endogenous glucose production independent of changes in insulin

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Mittelman, Steven D., and Richard N. Bergman. Inhibition of lipolysis causes suppression of endogenous glucose production independent of changes in insulin. Am J Physiol Endocrinol Metab 279: E630–E637, 2000.—We have shown that insulin controls endogenous glucose production (EGP) indirectly, via suppression of adipocyte lipolysis. Free fatty acids (FFA) and EGP are suppressed proportionately, and when the decline in FFA is prevented during insulin infusion, suppression of EGP is also prevented. The present study tested the hypothesis that suppression of lipolysis under conditions of constant insulin would yield a suppression of EGP. N6-cyclohexyladenosine (CHA) was used to selectively suppress adipocyte lipolysis during euglycemic clamps in conscious male dogs. FFA suppression by CHA caused suppression of EGP. Liposyn control experiments, which maintained FFA levels above basal during CHA infusion, completely prevented the decline in EGP, whereas glyceral control experiments, which maintained glyceral levels close to basal, did not prevent a decline in EGP. These controls suggest that the EGP suppression was secondary to the suppression of FFA levels specifically. A difference in the sensitivity of FFA and EGP suppression (FFA were suppressed ~85% whereas EGP only declined ~40%) was possibly caused by confounding effects of CHA, including an increase in catecholamine and glucagon levels during CHA infusion. Thus suppression of lipolysis under constant insulin causes suppression of EGP, despite a significant rise in catecholamines.

In support of this idea, infusion of Liposyn and heparin to maintain FFA levels prevented suppression of EGP during hyperinsulinemic euglycemic clamps (26) as well as after oral glucose (15). This evidence supports the concept that plasma FFA are the second signal of insulin suppression of liver glucose production.

Numerous studies (7, 10, 11, 16, 24, 27, 32) have examined the relationship between FFA and EGP suppression, using pharmacological means to lower FFA levels. The results of these studies are conflicting. Inhibition of lipolysis has been shown to decrease (10, 16, 27, 32), not change (24), or even increase (11) EGP. Some of this inconsistency may be due to different experimental protocols; specifically, some studies did not maintain glucose-regulating hormones, such as insulin and glucagon, constant (10, 11). There may also be confounding effects of some lipid-lowering drugs to increase the levels of counterregulatory hormones, such as glucagon (21), growth hormone (11, 13, 21), cortisol (7, 11, 24), and/or catecholamines (7, 21).

The present study attempts to reexamine whether acute suppression of lipolysis will cause suppression of EGP in vivo. This study was performed under conditions of clamped insulin, glucagon, and glucose. Insulin was underreplaced to maximize the range of FFA levels achieved. N6-cyclohexyladenosine (CHA), an A1-selective adenosine agonist, was employed to suppress lipolysis. CHA is a potent antilipolytic agent that acts via the inhibition of adenylate cyclase, causing a decrease in cAMP levels in the adipocyte (31). This mechanism of CHA action is distinct from that of nicotinic acid derivatives. Liposyn control infusions were used to distinguish the effects of lipolysis suppression from other possible effects of CHA on EGP. Glycerol control experiments were also performed to test for the relationship between glycerol and EGP. Finally, saline control experiments were done to separate the effects of CHA from those of the pancreatic clamps themselves.
METHODS

Animals. Experiments were performed on male mongrel dogs (23.9 ± 1.3 kg). The maintenance of and surgery on the animals have been previously described (25). Chronic catheters were implanted at least 7 days before experiments. One catheter was inserted into the jugular vein and advanced to the right atrium for sampling of central venous blood. A second catheter was placed in the portal vein 4 cm upstream from the porta hepatis for portal replacement infusions of glucagon and insulin. We have previously shown that infusions given at this upstream site are overall equally distributed among the lobes of the liver (4). A third catheter was placed in the femoral vein and advanced to the inferior vena cava for the infusion of [3H]glucose, somatostatin, and CHA. On the morning of the experiment, acute catheters were inserted into the saphenous veins for the variable infusion of labeled glucose, and when necessary, Liposyn plus heparin or glycerol.

Euglycemic clamp protocol. Nine dogs underwent CHA dose-response experiments, performed as follows. After a fasting sample was taken at t = −150 min, infusions of somatostatin (SRIF, 1 μg·kg⁻¹·min⁻¹, Bachem California, Torrance, CA) and HPLC-purified d-[3-H]glucose (25 μCi + 0.25 μCi/min, NEN Research Products Du Pont, Boston, MA) were begun into the femoral catheter. Portal infusions of insulin (0.2 mU·kg⁻¹·min⁻¹, Novo-Nordisk, Copenhagen, Denmark) and glucagon (1.0 ng·kg⁻¹·min⁻¹, porcine glucagon, Sigma, St. Louis, MO) were also initiated at this time. This insulin dose was designed to underreplace insulin levels so that FFA concentrations would be at or above basal at the onset of CHA infusion. Growth hormone was not replaced in these studies. Glucose was clamped at basal by a variable glucose infusion, which was labeled with d-[3-H]glucose (1.8 μCi/g) to stabilize specific activity (12). At t = −60 min, samples were taken every 20 min until t = 0, at which time CHA infusion was initiated. CHA was infused for 60 min at consecutive increasing doses (0.167, 0.500, and 1.667 μg·kg⁻¹·min⁻¹, CHA, Sigma). Samples were taken at 5-min intervals for the first 20 min of each CHA dose and then at 30, 45, and 60 min into each CHA infusion. All samples were assayed for glucose, insulin, glucagon, FFA, and glycerol (see Assays).

All nine dogs underwent the CHA dose-response experiment (CHA). Additionally, in five dogs an infusion of Liposyn [20% Liposyn (LI), 0.5 ml/min, Abbot Laboratories, North Chicago, IL] and heparin (250 U prime + 25 U/min) was started at t = 0 to maintain FFA levels above their basal value (CHA + LI). It should be noted that Liposyn + heparin infusions also elevate glycerol levels, as Liposyn contains 2.5 g of glycerol per 100 ml, and heparin mobilizes glycerol from triglycerides. Five dogs underwent glycerol controls (CHA + GLY) in which glycerol levels were clamped during CHA infusion using online measurements and a variable infusion of glycerol (Sigma). Finally, three dogs were used for saline controls (SAL) in which CHA was not given, and the pancreatic clamp was maintained for 180 min. Three animals in a previous study from this laboratory had undergone similar saline controls and were also included in the present study (2).

Assays. Samples for insulin, glucagon, and tracer assay were collected in tubes coated with lithium fluoride and heparin. Trasylol (aprotinin; 75 μl/ml blood; FBA Pharmaceuticals, New York, NY) was added to the samples for glucagon assay. Samples for assay of FFA and glycerol were collected in EDTA and paraoxon (Sigma) to suppress lipoprotein lipase activity (35). Samples for FFA, glycerol, glucose, and tracer were immediately centrifuged, and the plasma was separated and kept on ice for processing that day. Plasma for insulin assay was stored at −20°C, and plasma for glucagon assay was stored at −70°C. Glucose was measured immediately after sampling with a YSI 2700 autoanalyzer (Yellow Springs Instrument, Yellow Springs, OH). Samples for tracer assay were deproteinized using barium hydroxide and zinc sulfate. The supernatants were then evaporated in a vacuum, reconstituted in water, and counted in Ready Safe scintillation fluid (Beckman liquid scintillation counter; Beckman, Fullerton, CA). Tracer infusates were processed identically to plasma samples. Insulin was measured by an ELISA originally developed for human serum or plasma by Novo-Nordisk and adapted for dog plasma. The method is based on two murine monoclonal antibodies that bind to different epitopes on insulin but not to proinsulin. Materials for the insulin assay, including the dog standard, were kindly provided by Novo-Nordisk. Glucagon was measured using a Linco RIA kit (Linco Research, St. Charles, MO) with glucagon standards from Novo-Nordisk. FFA were measured using a kit from Wako (NEFA C; Wako Pure Chemical Industries, Richmond, VA), which utilizes a colorometric assay based on the acylation of coenzyme-A. Glycerol was measured using a colorometric triglyceride kit from Sigma (GPO-Trinder), which uses glycerol kinase and glycerol phosphate oxidase. This assay was modified to enhance sensitivity (100, rather than 10 μl, was used for each sample, and the assay mixture was centrifuged at 3,000 g for 5 min to remove visible precipitates before reading at 540 nm).

Additionally, basal and “steady-state” samples were assayed for catecholamines for seven of the nine CHA experiments. This assay utilizes catecholamine O-methyltransferase to attach labeled S-adenosyl methionine to both epinephrine and norepinephrine (22).

Calculations. Basal values were defined as the average of the t = −20 and t = 0 time points, and steady state was defined as the average of the final two samples taken during each CHA dose period (t = 45 and t = 60 min into each protocol). EGP and glucose uptake were calculated using the equations of Finegood et al. (12). To control for effects of CHA infusion on EGP other than suppression of lipolysis, the change in EGP during CHA infusion was subtracted from the average change in EGP during CHA + LI infusion. This calculation was also performed on the CHA + GLY experiments.

Statistics. All outcome variables were measured by general linear model ANOVA, which tested for an effect of animal, protocol, CHA dose, and an interaction between protocol and CHA dose. When a significant effect of CHA dose was identified, the ANOVA was repeated on each protocol individually, and when significance was reached, Dunnett’s t-tests were used to compare individual doses to basal while correcting for multiple comparisons. When a significant effect of protocol was identified, ANOVA was repeated on each dose individually, and Tukey’s t-test was used to make multiple comparisons between protocols when significance was reached. Linear trend tests were performed to examine dose dependency of outcome variables by CHA. Linear regression analysis between EGP and FFA was performed for both protocols. Paired t-tests were performed to compare replacement levels of insulin and glucagon to fasting levels. All statistics were performed using Minitab for Windows, release 12.22 (Minitab, State College, PA), except for linear trend tests and paired t-tests, which were performed using Microsoft Excel 2000; all calculations and statistics were done on an IBM-compatible computer.
RESULTS

Euglycemic clamp values. Glucose was clamped at its basal value throughout, with an average coefficient of variation (CV) of 4.2%, and was not affected by protocol or CHA dose (P = nonsignificant (NS); Table 1; Fig. 1). Likewise, glucose specific activity did not change significantly from its basal value (P = NS, average CV = 6%). Insulin was underreplaced by portal infusion of 0.2 mU kg⁻¹ min⁻¹ (31 ± 6 vs. 99 ± 3 pM, basal vs. fasting, P < 0.001, paired t-test, Fig. 2) but remained constant over the experimental protocol (P = NS). Basal glucagon levels were underreplaced from fasting levels (53 ± 6 vs. 73 ± 5 pg/ml, P = 0.001, paired t-test); however, glucagon levels increased over the course of CHA infusion (P < 0.001). This increase in glucagon tended to occur in the SAL control experiments as well (Table 2, P = 0.052).

FFA and glycerol. FFA were suppressed by CHA infusion in a dose-dependent manner (P < 0.001 ANOVA, P < 0.005 linear trend test; Table 1; Fig. 3). This suppression was completely prevented by Liposyn and heparin infusion (no effect of CHA dose on FFA levels in LI experiments, P = NS). Glycerol levels were also suppressed in a dose-dependent manner during CHA infusion, from 0.80 ± 0.12 to 0.58 ± 0.09, 0.26 ± 0.10, and 0.19 ± 0.07 mg/dl (P = 0.001 ANOVA, P = 0.05 linear trend test) but declined only slightly and not significantly from 0.76 ± 0.16 to 0.60 ± 0.15, 0.69 ± 0.24, and 0.53 ± 0.11 mg/dl in the CHA + GLY protocol (no effect of CHA dose, P = 0.31). Glycerol levels were not measured in the CHA + LI protocol due to interference with the glycerol assay; however, they were likely elevated above their basal levels due to the high glycerol content of Liposyn and the lipolytic effect of heparin. Glycerol declined slightly during SAL control experiments (P < 0.05).

Catecholamines. CHA infusion alone caused an increase in mean epinephrine (P = 0.07) and norepinephrine (P = 0.07).

Table 1. Hormone and metabolite levels during the CHA only protocol

<table>
<thead>
<tr>
<th></th>
<th>Fasting</th>
<th>Basal</th>
<th>SS1</th>
<th>SS2</th>
<th>SS3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, mg/dl</td>
<td>103.1 ± 3.5</td>
<td>104.8 ± 4.6</td>
<td>102.2 ± 3.8</td>
<td>101.2 ± 2.8</td>
<td>99.6 ± 3.4</td>
</tr>
<tr>
<td>SA, dpm/µg</td>
<td>—</td>
<td>6.7 ± 0.5</td>
<td>6.8 ± 0.4</td>
<td>7.0 ± 0.3</td>
<td>6.9 ± 0.3</td>
</tr>
<tr>
<td>Glucagon, ng/l</td>
<td>72 ± 9</td>
<td>58 ± 10</td>
<td>66 ± 9</td>
<td>69 ± 7</td>
<td>86 ± 13†</td>
</tr>
<tr>
<td>Insulin, µM</td>
<td>96 ± 8*</td>
<td>34 ± 3</td>
<td>28 ± 4</td>
<td>29 ± 3</td>
<td>34 ± 3</td>
</tr>
<tr>
<td>FFA, µM</td>
<td>627 ± 48</td>
<td>748 ± 95</td>
<td>489 ± 70†</td>
<td>218 ± 42‡</td>
<td>108 ± 26‡</td>
</tr>
<tr>
<td>Glycerol, mg/dl</td>
<td>0.70 ± 0.10</td>
<td>0.80 ± 0.12</td>
<td>0.58 ± 0.09</td>
<td>0.26 ± 0.10‡</td>
<td>0.19 ± 0.07‡</td>
</tr>
<tr>
<td>EPI, pg/ml</td>
<td>77 ± 24</td>
<td>68 ± 28</td>
<td>84 ± 21</td>
<td>100 ± 45</td>
<td>144 ± 61</td>
</tr>
<tr>
<td>NOR EPI, pg/ml</td>
<td>190 ± 38</td>
<td>208 ± 35</td>
<td>234 ± 50</td>
<td>258 ± 52</td>
<td>360 ± 56‡</td>
</tr>
</tbody>
</table>

Values are means ± SE. SA, specific activity; FFA, free fatty acids; EPI, epinephrine; NOR EPI, norepinephrine; CHA, N⁶-cyclohexyladenosine. *P < 0.05; †P < 0.005; ‡P < 0.001 vs. basal period.

Fig. 1. Glucose (top) and glucose specific activity (bottom) during N⁶-cyclohexyladenosine (CHA) dose-response, euglycemic clamp experiments. CHA was infused in 3 sequential doses of 0.167 (1), 0.500 (2), and 1.667 ng kg⁻¹ min⁻¹ (3).

Fig. 2. Insulin (top) and glucagon (bottom), pooled from all 3 CHA dose-response protocols.
Fig. 3. Suppression of free fatty acid (FFA) levels, expressed as a change from their basal values. CHA only (●), CHA + LI (○), and CHA + GLY (△). LI, liposyn; GLY, glycerol.

Fig. 5. Glucose uptake during CHA only (●), CHA + LI (○), and CHA + GLY (△) protocols.

Table 2. Saline control experiments

<table>
<thead>
<tr>
<th></th>
<th>Basal</th>
<th>SS1</th>
<th>SS2</th>
<th>SS3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, mg/dl</td>
<td>99.0 ± 7.5</td>
<td>93.5 ± 9.9</td>
<td>90.7 ± 9.7</td>
<td>88.1 ± 9.3</td>
</tr>
<tr>
<td>Glucagon, ng/l</td>
<td>48.2 ± 8.6</td>
<td>65.7 ± 4.9</td>
<td>66.1 ± 2.6</td>
<td>68.5 ± 3.5</td>
</tr>
<tr>
<td>Insulin, pM</td>
<td>33.3 ± 5.0</td>
<td>28.5 ± 4.5</td>
<td>32.1 ± 3.9</td>
<td>31.3 ± 2.5</td>
</tr>
<tr>
<td>FFA, µM</td>
<td>896 ± 173</td>
<td>662 ± 129</td>
<td>519 ± 127*</td>
<td>541 ± 126</td>
</tr>
<tr>
<td>Glycerol, mg/dl</td>
<td>1.08 ± 0.18</td>
<td>0.60 ± 0.07</td>
<td>0.55 ± 0.09*</td>
<td>0.39 ± 0.04*</td>
</tr>
<tr>
<td>EGP, mg·kg·min⁻¹</td>
<td>2.4 ± 0.3</td>
<td>2.3 ± 0.2</td>
<td>2.3 ± 0.2</td>
<td>2.1 ± 0.2</td>
</tr>
<tr>
<td>R₄, mg·kg⁻¹·min⁻¹</td>
<td>2.6 ± 0.2</td>
<td>2.5 ± 0.2</td>
<td>2.7 ± 0.2</td>
<td>2.5 ± 0.1</td>
</tr>
</tbody>
</table>

Values are means ± SE. *P < 0.05 vs. CHA only protocol.

Glucose fluxes did not change significantly during SAL control experiments (Table 2). As a percentage of their basal values, both catecholamines increased significantly with CHA infusion (P < 0.01 for both comparisons, Fig. 4).

Glucose influx. Glucose fluxes did not change significantly during SAL control experiments (Table 2). Glucose uptake increased significantly with CHA dose, from 3.4 ± 0.2 to 3.8 ± 0.3, 3.9 ± 0.3, and 4.3 ± 0.4 mg·kg⁻¹·min⁻¹ with successive CHA doses (26% overall increase, P < 0.01 ANOVA, P < 0.05 linear trend test, Fig. 5). Glucose uptake was not different among the CHA only, CHA + LI, and CHA + GLY protocols. EGP was significantly suppressed in a dose-dependent manner by CHA infusion alone (P < 0.001 ANOVA, P < 0.005, linear trend test; Fig. 6). Liposyn infusion prevented the suppression of EGP (P < 0.05 vs. CHA protocol periods 2 and 3) and, in fact, was associated with a tendency for EGP to increase (P = 0.08). EGP suppression was not changed by maintaining glycerol levels near basal (P = NS vs. CHA only). To separate the effects of lipolysis suppression from those of CHA itself, the difference in EGP between the CHA + LI and the CHA only and CHA + GLY protocols was calculated (Fig. 7, see METHODS). This net suppression of EGP increased from 0.6 ± 0.2, 1.2 ± 0.2, and 1.6 ± 0.2 mg·kg⁻¹·min⁻¹ over the three consecutive CHA doses and was not different with glycerol infusion.

There was a strong correlation between FFA and EGP in the CHA only protocol (P < 0.01, r² = 0.19), which was improved when both variables were calculated as a percentage of basal (P < 0.001, r² = 0.48); the latter is described by the equation: EGP%Basal = 0.335 × FFA%Basal + 0.646 (Fig. 8). The y-intercept implies that complete suppression of FFA would yield only ~35% suppression of EGP. A similar correlation was seen during the CHA + GLY control experiments (Equation: EGP%Basal = 0.280 × FFA%Basal + 0.747, r² = 0.52, not shown). However, the y-intercept was slightly higher, implying that glycerol may have had a minor effect to spare EGP in the face of suppressed FFA levels.

DISCUSSION

Historically, insulin was believed to be an exclusively direct regulator of liver glucose production. However, recent evidence from this laboratory (1, 20, 25, 26, 29) and others (14, 17, 23) has suggested that at least some of insulin’s effect may be indirect and may be mediated by the lowering of plasma FFA. To further examine this indirect (Single Gateway) mechanism, the present study was designed to test whether pharmacological suppression of FFA with constant insulinemia will reduce EGP. The adenosine agonist CHA...
was used to suppress lipolysis. When FFA was suppressed in a dose-response manner, EGP was suppressed as well. In fact, the relative suppression of EGP was strongly correlated with the suppression of FFA. Suppression of EGP occurred during constant insulin and glucose, slightly increasing glucagon, implying that EGP suppression was causally related to the suppression of lipolysis. This conclusion was supported by Liposyn plus heparin infusions, which maintained FFA and glycerol levels during CHA infusion and completely prevented CHA suppression of EGP. It appears that the majority of the effect of CHA to suppress EGP was due to a decrease in FFA levels, as maintenance of glycerol levels alone did not prevent CHA’s effect.

Despite the correlation between FFA and EGP, the suppression of EGP was only ~40% complete with ~85% suppression of FFA (Fig. 8). This implies that either EGP is under only partial control of FFA or that CHA infusion had a separate confounding effect to bolster EGP in the face of FFA suppression. In previous studies from this laboratory (25, 26), we have found a more proportional relationship between FFA and EGP. Thus it seems likely that CHA must cause an increase in EGP separate from its effect on lipolysis. This is supported by the fact that EGP actually increased in the present Liposyn control experiments.

The effect of CHA to increase EGP could represent a direct effect of this drug on the liver or an indirect effect mediated by counterregulatory hormones. CHA has previously been demonstrated to directly stimulate glycogenolysis in the perfused rat liver (6). This likely is an effect of CHA to stimulate adenylate cyclase, which occurs with a 50% effective dose (ED50) of 580 nM (28). Although CHA concentrations were not measured in the present study, they can be estimated. We are unaware of published data on the clearance of CHA in the dog; however, the effect of bolus injections of CHA (1–1,000 μg/kg) on vascular conductance in anesthetized dogs completely subsided within 20 min (A. S. Clanachan, personal communication). Although clearance cannot be assessed from biological effectiveness alone, these data suggest that CHA is cleared rapidly [half-time (t1/2) < 20 min]. Assuming a conservative estimate of t1/2 = 20 min, the expected CHA concentrations in the dose-response experiments are ~80, 240, and 800 nM for each successive dose. Thus, based on the ED50 of CHA stimulation of adenylate cyclase in vitro, it is quite possible that a direct effect of CHA to stimulate glycogenolysis was present to some degree, especially during the highest dose of CHA.

Supporting the possibility that there is an indirect effect of CHA to stimulate EGP, both epinephrine and norepinephrine were elevated with CHA infusion. The dose-response infusion of CHA caused an ~75 pg/ml increase in epinephrine levels and ~150 pg/ml increase in norepinephrine. Stevenson et al. (30) have shown that an increase in epinephrine levels of 300 pg/ml can cause a transient 1 mg·kg⁻¹·min⁻¹ increase in EGP. A similar rise in norepinephrine levels did not significantly increase EGP (8); however, local norepinephrine release by hepatic sympathetic nerves may have a greater effect on EGP than suggested by the systemic levels. Glucagon levels also increased slightly, from 58 ± 10 to 86 ± 13 ng/l. Although only modest changes in systemic catecholamine and glucagon levels were seen in the present study, these levels do not necessar-
ily reflect the local hepatic levels of these and other unmeasured counterregulatory hormones. Additionally, growth hormone was suppressed and not replaced or measured in this study. Therefore, it is not possible to rule out a permissive or confounding effect of growth hormone in these experiments. However, growth hormone should have differed between the SAL control experiment and the CHA experiments only to the extent that CHA affected its secretion or clearance.

These two effects of CHA to potentially increase EGP, direct stimulation of adenylate cyclase and stimulation of catecholamine levels, could have masked any effects of suppressed FFA levels to lower EGP. Therefore, the effect of CHA to lower EGP is even more impressive, given that it occurred in the face of elevated catecholamine levels and despite having a demonstrated direct effect to stimulate glycogenolysis in vitro.

In the present study, both FFA and glycerol levels were suppressed from their basal values by CHA infusion. Since Liposyn plus heparin increases both FFA and glycerol, control experiments were performed in which glycerol levels were clamped during CHA infusion (CHA + GLY). In these experiments, FFA were suppressed slightly but not significantly further than the CHA only experiments. EGP suppression was nearly identical in these two protocols, implying that the suppression of glycerol by CHA had little if any effect on EGP. This agrees with our previous finding that insulin suppression of EGP is prevented by Liposyn plus heparin infusion but is unaffected by glycerol infusion (26). Thus it appears that FFA play the primary role in modulating both insulin and CHA suppression of EGP.

Although we and others have shown that insulin suppression of EGP and FFA is well correlated and that preventing the fall in FFA will also prevent the fall in EGP (15, 16A, 25, 26), the Single Gateway Hypothesis remains to be proven unequivocally. To prove causality, it is also necessary to show that suppression of FFA levels, independent of changes in insulin, will cause a suppression of EGP. Many studies have addressed this question, yet a consensus remains elusive. The suppression of FFA levels has been shown to enhance hepatic suppression by insulin in normal rats (16) and in human subjects with non-insulin-dependent diabetes mellitus (27, 32) or cirrhosis (10); however, none of these studies showed a decline in EGP brought about by the lowering of FFA themselves. Fery et al. (11) found that the suppression of lipolysis with acipimox in normal humans raised whole body glucose production; however, this was likely due to declining insulin and increasing glucagon levels during this study (11). The inconsistency of previous studies may be related to lack of precise hormonal control, differences in species or population, and different time periods examined. Several studies have also shown that antilipolytic drugs such as nicotinic acid and acipimox can cause increases in glucagon, growth hormone, cortisol, and possibly catecholamines (11, 13, 21, 24).

The results of the present study agree in part with those from a recent study by Chen et al. (7) in which nicotinic acid administration to humans caused a suppression of FFA levels and gluconeogenesis, with no change in EGP due to a compensatory increase in glycogenolysis (7). The decline in gluconeogenesis was prevented by infusion of Liposyn and heparin. Like the present study, suppression of lipolysis resulted in an increase in plasma epinephrine levels, which likely contributed to the increase in glycogenolysis. There are several possible mechanisms whereby FFA might control EGP. FFA have long been known to stimulate gluconeogenesis in vitro (9, 33). The precise mechanism of this stimulation is unknown, but several possibilities have been proposed. One involves the production of reducing equivalents by FFA oxidation in the hepatocyte (33, 34). In fact, several key enzymes in the gluconeogenesis pathway are stimulated by an increase in the NADH-to-NAD ratio (33). The oxidation of FFA also yields energy in the form of ATP, which can act to stimulate gluconeogenesis and inhibit glycolysis (34). Acetyl-CoA, the end product of FFA oxidation, has been shown to stimulate pyruvate carboxylase, a key regulatory enzyme for the activation of gluconeogenesis (34). Acetyl-CoA also reacts with oxaloacetate to form citrate, which inhibits phosphofructokinase (PFK-1), a key step in glycolysis. FFA might also raise cAMP levels (9), which would stimulate gluconeogenesis by well-defined mechanisms.

However, it is not likely that FFA control EGP solely by modulating gluconeogenesis. Puhakainen and Yki-Järvinen (24) demonstrated that suppression of gluconeogenesis alone does not decrease total hepatic glucose output, implying some sort of autoregulation that maintains EGP constant. If this is the case, then FFA must either regulate both gluconeogenesis and glycolysis separately or control flux through the glucose-6-phosphatase enzyme. In fact, glucose-6-phosphatase gene expression is stimulated by FFA both in vivo and in vitro (18, 19). Evidence for or against the acute regulation of glucose-6-phosphatase by FFA is elusive, as the measurement of this enzyme’s activity in vivo remains problematic.

An important caveat in this study is that insulin was underreplaced during these experiments, although levels were maintained constant before and during CHA infusion. Thus hepatic underinsulization may have had a permissive effect that allowed FFA to control EGP. Because we have previously shown that insulin has both direct and indirect effects on the liver to control EGP (20), minimizing hepatic insulin should minimize the direct effect, thereby increasing the power to observe an effect of FFA on EGP. Indeed, this may in part explain why some studies did not observe an effect of FFA suppression on EGP. Further studies are needed to fully elucidate the interaction between insulin and FFA on hepatic glucose metabolism.

Glucose uptake was increased by CHA infusion by ~25%. This was not changed by Liposyn or glycerol control infusion and therefore was not likely due to the effect of CHA to lower FFA or glycerol. It is possible
that CHA had some direct effect on peripheral tissues to increase glucose uptake, perhaps by increasing regional blood flow (3).

In summary, we have shown that the suppression of FFA, independent of changes in glucose, insulin, or glucagon levels, can elicit suppression of EGP. With FFA suppression, EGP was suppressed ∼40%, despite an approximate doubling of catecholamine levels. This relationship between lipolysis and glucose production can have important implications for the treatment of insulin resistance and diabetes. Perhaps treatment of these conditions should focus on the resistance of the adipocyte rather than that of the liver.

The stimulation of counterregulatory hormones (glucagon, growth hormone, and catecholamines) confounds direct testing of the Single Gateway Hypothesis using antilipolytic drugs such as CHA and nicotinic acid analogs. Therefore, additional studies linking FFA as a precise moment-to-moment regulator of EGP are justified.

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