Effects of free fatty acids on hepatic glycogenolysis and gluconeogenesis in conscious dogs

CHANG AN CHU, STEPHANIE M. SHERCK, KAYANO IGAWA, DANA K. SINDELAR, DOSS W. NEAL, MAYA EMSHWILLER, AND ALAN D. CHERRINGTON

Department of Molecular Physiology and Biophysics, Vanderbilt University School of Medicine, Nashville, Tennessee 37232-0615

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An increase in free fatty acids (FFA) stimulates hepatic gluconeogenesis (10, 14, 37). In vitro studies have shown that perfusion of rat liver with lipid increases gluconeogenesis (36, 37) and that an increase in FFA oxidation stimulates the activities of key enzymes in the gluconeogenic pathway (3, 26). In accord with this, Boden and Jadali (5) reported that a rise in plasma FFA increased hepatic glucose production (HGP) in normal human subjects. In addition, Saloranta et al. (30) showed that an increase in FFA and glycerol availability, brought about by Intralipid infusion, increased gluconeogenesis and glucose production in type 2 diabetic patients. In contrast, in a recent study, Roden et al. (27) did not detect a change in HGP in response to an increase in FFA in normal humans. Likewise, Johnston et al. (18) reported that an acute increase in the plasma FFA level did not change HGP in type 2 diabetic subjects. In agreement with the latter, Puhakainen and colleagues (24, 25) showed that a decrease in plasma FFA, although it reduced gluconeogenesis, did not change HGP in patients with type 2 diabetes. Most recently, Boden et al. (4) showed in both normal and type 2 diabetic subjects that increasing and decreasing plasma FFA stimulated and inhibited gluconeogenesis, respectively, but did not alter glucose production. In another recent study, Stingl et al. (35) showed that in normal humans HGP and glycogenolysis both decreased in response to an increase in plasma FFA level. It is obvious from the above discordance that the effect of an increase in FFA availability on HGP in vivo, as well as its gluconeogenic and glycogenolytic components, requires further investigation.

Previous studies by Shulman et al. (32) and Chu et al. (7) showed that hyperglycemia significantly inhibited HGP in the dog, even when basal levels of insulin and glucagon were maintained. In accord with this, Sacca et al. (29) reported that hyperglycemia had an inhibitory effect on glucose production in the presence of fixed basal pancreatic hormone concentrations, bringing about glucose availability, brought about by Intralipid infusion, in type 2 diabetic subjects. In agreement with the latter, Puhakainen and colleagues (24, 25) showed that a decrease in plasma FFA, although it reduced gluconeogenesis, did not change HGP in patients with type 2 diabetes. Most recently, Boden et al. (4) showed in both normal and type 2 diabetic subjects that increasing and decreasing plasma FFA stimulated and inhibited gluconeogenesis, respectively, but did not alter glucose production. In another recent study, Stingl et al. (35) showed that in normal humans HGP and glycogenolysis both decreased in response to an increase in plasma FFA level. It is obvious from the above discordance that the effect of an increase in FFA availability on HGP in vivo, as well as its gluconeogenic and glycogenolytic components, requires further investigation.

Using the rat and dog, respectively, Rossetti et al. (28) and Sindelar et al. (34) showed that hyperglycemia inhibits glucose production through an effect on hepatic gluconeogenesis. In line with this, Petersen et al. (23) reported that hyperglycemia significantly inhibited net hepatic gluconeogenesis in the presence of a pancreatic clamp in humans. The available data, therefore, indicate clearly that hyperglycemia per se can inhibit hepatic gluconeogenesis. An earlier study by

Address for reprint requests and other correspondence: A. D. Cherrington, Dept. of Molecular Physiology and Biophysics, 702 Light Hall, Vanderbilt Univ. School of Medicine, Nashville, TN 37232-0615 (E-mail: alan.cherrington@mcmail.vanderbilt.edu.)

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Shulman et al. (32) in the dog and a recent study by Hellerstein et al. (17) in the rat indicated that hyperglycemia can also decrease hepatic gluconeogenesis. On the other hand, recent studies by Rossetti et al. in the rat and Sindelar et al. in the dog could not confirm this conclusion. A study in isolated rat hepatocytes by Sanchez-Gutierrez et al. (31) indicated that hyperglycemia significantly inhibited gluconeogenesis. A study by Davidson (12), on the other hand, suggested that hyperglycemia did not do so. It is also of interest, therefore, to investigate the effect of hyperglycemia on hepatic gluconeogenesis.

It is known that chronic hyperlipidemia increases insulin resistance in various animal models and humans. An increase in plasma FFA decreases the effect of insulin on the liver and peripheral tissues (muscle and adipose tissues), which in turn results in hyperglycemia in these subjects. In fact, patients with type 2 diabetes have long been known to exhibit hyperglycemia and hyperlipidemia concurrently. Because hyperlipidemia stimulates HGP while hyperglycemia inhibits it, it is of interest to examine the interaction between high plasma FFA levels and hyperglycemia in controlling hepatic glycogenolysis and gluconeogenesis in vivo.

The aims of the present study, therefore, were to determine the effects of hyperlipidemia alone, hyperglycemia alone, and hyperlipidemia plus hyperglycemia on hepatic glycogenolysis and gluconeogenesis in vivo in the presence of fixed basal levels of insulin and glucagon.

**METHODS AND MATERIALS**

Experiments were carried out on 24 18-h-fasted conscious mongrel dogs (20–30 kg) of either sex that had been fed a standard diet of meat and chow described elsewhere (7–9). The animals were housed in a facility that met American Association for the Accreditation of Laboratory Animal Care guidelines, and the protocols were approved by the Vanderbilt University Medical Center Animal Care Committee.

A laparotomy was performed 16–18 days before each experiment to implant catheters and Doppler flow probes into or around appropriate blood vessels, as described elsewhere (7–9). Each dog was used for only one experiment. All dogs studied had 1) a leukocyte count <18,000/mm³, 2) a hematocrit >35%, 3) a good appetite, and 4) normal stools.

Each experiment consisted of a 100-min tracer equilibration and hormone adjustment period (−140 to −40 min), a 40-min basal period (−40 to 0 min), and a 180-min test period (0–180 min). In all studies, a priming dose of purified [3-3H]glucose (42 μCi) was given at −140 min, followed by a constant infusion of [3-3H]glucose (0.35 μCi/min), [U-14C]alanine (0.35 μCi/min), and indocyanine green (ICG, 0.1 mg·m⁻²·min⁻¹). An infusion of somatostatin (0.8 μg·kg⁻¹·min⁻¹) was started at −130 min to inhibit endogenous insulin and glucagon secretion. Concurrently, intraportal replacement infusions of insulin (300 U·kg⁻¹·min⁻¹) and glucagon (0.5 ng·kg⁻¹·min⁻¹) were started. The plasma glucose level was monitored every 5 min, and euglycemia was maintained by adjusting the rate of insulin infusion. The final alteration in the insulin infusion rate was made at least 30 min before the start of the basal period, and the rate of insulin infusion (mean of 235 U·kg⁻¹·min⁻¹) remained unchanged thereafter. The study included four groups: EuG,
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FFA + EuG, hyperglycemia (HG), and FFA + HG (Fig. 1). Intralipid (0.02 ml·kg⁻¹·min⁻¹, 20% fat emulsion; Pharma-
cia & Upjohn) and heparin (0.5 U·kg⁻¹·min⁻¹) were infused
during the test period via the right saphenous vein in the
FFA + EuG and FFA + HG groups. Dextrose (20%) was
infused to clamp the arterial blood glucose level at 120–130
mg/dl (equal to 165–178 mg/dl plasma glucose) via the right
cephalic vein in the HG and FFA + HG groups. In the EuG
group, saline was infused peripherally during the test period.
Data from five of the six dogs in each of the EuG and HG
groups were published previously in the context of another
study (7, 33). The data are reproduced here to facilitate
comparison of the groups.

Plasma and blood glucose, plasma [³H]- and [¹⁴C]glucose,
blood lactate, glyceral, β-hydroxybutyrate (β-OHB), alanine,
glutamine, glutamate, glycine, serine, threonine, and plasma
FFA were determined using previously described methods (7–
9). The levels of insulin, glucagon, cortisol, epinephrine, and
norepinephrine were also determined as described elsewhere
(7–9). Transonic flow probes and ICG were used to measure
total hepatic blood flow (7–9). Because a high level of Intralipid
interferes with the measurement of ICG in plasma, the net
hepatic balance and fractional extraction of metabolites were
calculated using Transonic-determined flow. The net hepatic
balance and fractional extraction of blood glucose, lactate, glyc-
erol, β-OHB, alanine, other gluconeogenic amino acids, and
plasma FFA in the present study were calculated using arte-
riovenous difference (a-v) methods described elsewhere (7–9).

Total glucose production and utilization were determined
using both one- and two-compartment models, as previously
described (7–9). The results were similar regardless of which
approach was employed because the deviations from steady
state were minimal. The glucose production and utilization data
shown in Figs. 1–9 and Tables 1–3 are those calculated using
the two-compartment model. It should also be noted, since the
kidneys produce a small amount of glucose, that the rate of
endogenous glucose production determined by the tracer
method slightly (0.2 mg·kg⁻¹·min⁻¹) overestimates total hep-
atic glucose release (20). Gluconeogenic efficiency was as-
essed using a double-isotope technique described elsewhere
(7–9). Because the conversion of [¹⁴C]alanine to [¹⁴C]glucose by
the kidney is minimal (21), [¹⁴C]glucose production in our study
was almost exclusively attributable to the liver. The hepatic
gluconeogenic flux from circulating gluconeogenic precursors
was calculated using the methods described previously (7–9).
Briefly, the net hepatic balances of the gluconeogenic prec-
ursors alanine, glycine, serine, threonine, glutamine, glutamate,
lactate, and glyceral were measured in the present study. The
net hepatic balance of pyruvate was assumed to be 10% of
lactate balance. The hepatic gluconeogenic flux rate was calcu-
lated by dividing the above uptake rates by two to account for
the incorporation of the C-3 precursors into the C-6 glucose mole-
cule. Net hepatic glycogenolysis was calculated as follows (7–9):

\[
\text{net hepatic glycogenolysis} = \text{NHGO} + \text{NHLO}
\]

\[+ \text{Glc}_{\text{oxid}} - \text{GNG flux}\]

where NHGO is net hepatic glucose output and NHLO is net
hepatic lactate output. When net hepatic lactate uptake
occurred, its net output was considered to be zero and it was
considered as a gluconeogenic precursor; Glc_{oxid} is hepatic
glucose oxidation. The latter was assumed to be 10% of basal
NHGO (16). Therefore, in the present study, it was assumed
to be 0.2 mg·kg⁻¹·min⁻¹ in all of the groups (7, 16). We have
measured glucose oxidation in a number of studies, and it
ranges from 0.1 to 0.2 mg·kg⁻¹·min⁻¹ after an overnight
fast. Importantly, it does not change significantly regardless
of the physiological changes being brought about. GNG is the
hepatic gluconeogenic flux rate measured by the a-v differ-
ence technique, as described elsewhere (7–9). All parameters
were expressed in glucose equivalents.

Statistical analysis. All statistical comparisons were made
using repeated-measures ANOVA with post hoc analysis by univari-
ate F-tests or the paired Student’s t-test where appropriate.
Statistical significance was accepted at \( P < 0.05 \). Data
are expressed as means ± SE.

RESULTS

Hormone levels and hepatic blood flow. The arterial
plasma levels of insulin and glucagon remained unchanged
and at basal levels in all groups (Fig. 2) as did the arterial plasma
levels of epinephrine, norepinephrine, and cortisol (Fig. 2). Hepatic
blood flow also remained unchanged and equivalent in all
groups (Table 1). The arterial blood levels, net hepatic balances, and frac-
tional extractions of FFA, β-OHB, and glycerol. The arterial plasma
level of FFA increased from 746 ± 69 to 1,850 ± 245 (\( P < 0.05 \)) and from 746 ± 131 to 1,817 ±
189 (\( P < 0.05 \)) μmol/l in the FFA + EuG and

Fig. 2. Arterial plasma levels of insulin, glucagon, epinephrine,
norepinephrine, and cortisol during the basal and the experimental
periods in the presence of a pancreatic clamp in conscious 18-h-fasted
dogs.

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Fig. 3. Arterial plasma levels and net hepatic uptake and fractional extraction of FFA during the basal and the experimental periods in the presence of a pancreatic clamp in conscious 18-h-fasted dogs. $P < 0.05$, FFA + HG vs. HG (**), FFA + HG vs. EuG (#), FFA + EuG vs. HG ($), and FFA + EuG vs. EuG (+).

FFA + HG groups, respectively, during Intralipid infusion (Fig. 3). FFA levels remained unchanged in the EuG and HG groups. The net hepatic uptake of FFA increased from 2.5 ± 0.3 to 4.5 ± 0.5 (P < 0.05) and from 2.6 ± 0.5 to 4.5 ± 0.9 (P < 0.05) μmol·kg⁻¹·min⁻¹ in the FFA + EuG and FFA + HG groups, respectively (Fig. 3). It did not change significantly in the EuG and HG groups. Net hepatic fractional extraction of FFA did not change significantly in any group (Fig. 3).

The arterial blood glycerol level increased from 84 ± 8 to 209 ± 14 (P < 0.05) and from 80 ± 7 to 176 ± 12 (P < 0.05) μmol/l in the FFA + EuG and FFA + HG groups, respectively, during Intralipid infusion (Fig. 4). Glycerol levels remained unchanged in the EuG and HG groups. Net hepatic uptake of glycerol increased from 1.5 ± 0.2 to 3.5 ± 0.3 (P < 0.05) and from 1.5 ± 0.3 to 3.2 ± 0.3 (P < 0.05) μmol·kg⁻¹·min⁻¹ in the FFA + EuG and FFA + HG groups, respectively (Fig. 4). It did not change significantly in the EuG and HG groups.

The arterial blood level of β-OHB increased from 26 ± 3 to 40 ± 8 (P < 0.05) and from 27 ± 5 to 39 ± 11 (P < 0.05) μmol/l in the FFA + EuG and FFA + HG groups, respectively, during Intralipid infusion (Fig. 4). It did not change significantly in the EuG and HG groups. Net hepatic output of β-OHB increased from 0.8 ± 0.1 to 1.6 ± 0.2 (P < 0.05) and from 0.9 ± 0.3 to 1.3 ± 0.3 (P < 0.05) μmol·kg⁻¹·min⁻¹ in the FFA + EuG and FFA + HG groups, respectively (Fig. 4). In the latter group, β-OHB output fell back to baseline by the end of the study. It did not change significantly in the EuG and HG groups.

Glucose kinetics. The arterial blood glucose level increased from 79 ± 4 to 121 ± 2 and 78 ± 3 to 131 ± 1 mg/dl in the HG and FFA + HG groups, respectively (Fig. 5). It remained unchanged in the EuG and FFA + EuG groups. Net hepatic glucose output did not change significantly in the EuG and FFA + EuG groups (Fig. 5). In response to hyperglycemia alone, net hepatic glucose output decreased by 1.5 ± 0.4 mg·kg⁻¹·min⁻¹ (from 2.2 ± 0.3 to 0.7 ± 0.5 mg·kg⁻¹·min⁻¹, P < 0.05) by 90 min and remained reduced thereafter. In response to infusion of Intralipid and glucose, net hepatic glucose output decreased by 1.7 ± 0.3 mg·kg⁻¹·min⁻¹ (P < 0.05; Fig. 5). The changes in tracer-determined glucose production paralleled those in net hepatic glucose balance (Fig. 5). Glucose utilization in the EuG and FFA + EuG groups did not change significantly (Table 2). On the other hand, glucose utilization increased moderately from...
The hepatic glucose output and tracer-determined endogenous glucose production during basal and experimental periods in the presence of a pancreatic clamp in conscious 18-h-fasted dogs. *P < 0.05, FFA + HG vs. EuG (#), FFA + EuG vs. HG ($), FFA + HG vs. FFA + EuG (!), and HG vs. EuG (@).

Arterial blood glucose levels and changes in net hepatic lactate balance significantly in EuG and HG groups.

Glucocereogenisis and glycogenolysis. The hepatic glucocereogenisis flux rate did not change significantly (0.6 ± 0.1 to 1.3 ± 0.2 (P < 0.05) and from 0.6 ± 0.1 to 1.2 ± 0.2 (P < 0.05) mg·kg⁻¹·min⁻¹ in the FFA + EuG and FFA + HG groups, respectively (Fig. 7A). The overall amount of glucose produced by hepatic glucocereogenisis during the 3-h test period was 128 ± 22 mg/kg in the EuG group (Fig. 7A). In response to hyperglycemia, the overall amount of glucose produced by hepatic glucocereogenisis was reduced by 30 ± 9 mg/kg (the area between HG and EuG curves in Fig. 7B; not significant) or 23% inhibition (Fig. 8). Elevated plasma FFA increased overall glucocereogenisis flux by 59 ± 12 mg/kg (P < 0.05) or 46% stimulation (Fig. 8). In response to combined hyperglycemia and hyperlipidemia, overall glucocereogenisis flux was increased by 56 ± 10 mg/kg (P < 0.05) or 44% (Fig. 8).
Net hepatic glycogenolysis decreased slightly from 1.4 ± 0.3 to 1.0 ± 0.2 mg·kg⁻¹·min⁻¹ in the EuG group by the end of the study (Fig. 9). In response to hyperglycemia alone, it decreased from 1.6 ± 0.2 to 0.7 ± 0.2 (P < 0.05) mg·kg⁻¹·min⁻¹ in 60 min and was stable thereafter (Fig. 9). In response to the high level of plasma FFA alone, it decreased from 2.0 ± 0.2 to 1.3 ± 0.2 (P < 0.05) mg·kg⁻¹·min⁻¹ in 90 min. In response to hyperglycemia and the high level of FFA together, it decreased from 1.7 ± 0.2 to −0.1 ± 0.2 (P < 0.05). Net hepatic glycogenolysis over the 3-h test period was 196 ± 26 mg/kg in the EuG group. Hyperglycemia decreased it by 96 ± 20 mg/kg (the area between HG and EuG curves in Fig. 9; P < 0.05), a 49% inhibition (Fig. 8). Elevated plasma FFA and glycerol decreased it by 82 ± 16 mg/kg (P < 0.05) or 42% (Fig. 8). Hyperlipidemia and hyperglycemia together decreased it by 177 ± 22 mg/kg (P < 0.05) or a 90% inhibition (Fig. 8).

**DISCUSSION**

In the present study, we were able to directly examine the effect of an increase in plasma FFAs and glycerol alone or in the presence of hyperglycemia on hepatic glycogenolysis and gluconeogenesis in vivo. The arterial levels of insulin, glucagon, epinephrine, norepinephrine, and cortisol remained at basal values in all groups, thereby simplifying data interpretation. The increments in plasma FFAs and glycerol were indistinguishable in the FFA + EuG and FFA + HG groups, as were the increments in arterial glucose concentrations in the HG and FFA + HG groups.

Under euglycemic conditions in the control group, net hepatic glucose output and net hepatic glycogenolysis decreased slightly over the course of the study. The overall contribution of net hepatic glycogenolysis to HGP was 196 ± 26 mg/kg over the 3-h test period. Gluconeogenesis did not change significantly during the study, and overall hepatic gluconeogenic flux was 128 ± 22 mg/kg (−40% of HGP). Hyperglycemia alone significantly decreased net hepatic glucose output from 2.2 ± 0.3 to 0.7 ± 0.5 mg·kg⁻¹·min⁻¹ (P < 0.05) as the result of a 49% reduction in net hepatic glycogenolysis and a 23% reduction in hepatic gluconeogenic flux. Our data therefore demonstrate that moderate hyperglycemia per se can significantly inhibit hepatic glycogenol-

![Fig. 7. Hepatic gluconeogenic flux and change in hepatic gluconeogenic flux during the basal and experimental periods in the presence of a pancreatic clamp in conscious 18-h-fasted dogs. P < 0.05, FFA + HG vs. HG (*), FFA + HG vs. EuG (#), FFA + EuG vs. HG ($), and FFA + EuG vs. EuG (+).](image)

![Fig. 8. Inhibition of net hepatic glycogenolysis and gluconeogenic flux during the experimental period in the presence of a pancreatic clamp in conscious 18-h-fasted dogs. The glycogenolytic and gluconeogenic areas under the curve in the EuG study during the experimental period were 196 ± 26 and 128 ± 22 mg/kg, respectively.](image)
hyperglycemic or hyperlipidemic means of the rates at the Table 1. Hepatic blood flow during the basal and test periods of euglycemic or hyperlipidemic + euglycemic or hyperglycemic or hyperlipidemic + hyperglycemic groups of conscious 18-h-fasted dogs maintained on a pancreatic clamp.

<table>
<thead>
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<th>Group</th>
<th>Basal Period</th>
<th>Test Period, min</th>
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<tr>
<td>FFA + HG</td>
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</table>

Data are means ± SE; units are ml·kg⁻¹·min⁻¹. EuG, euglycemia; FFA, free fatty acid; HG, hypoglycemia. The basal period data are means of the rates at the −40 and 0-min time points.

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Fig. 9. Net hepatic glycogenolysis and change in net hepatic glycogenolysis during the basal and experimental periods in the presence of a pancreatic clamp in conscious 18-h-fasted dogs. P < 0.05, FFA + HG vs. HG (*), FFA + HG vs. EuG (#), FFA + EuG (!), and HG vs. EuG (@).

The effect of hyperglycemia on gluconeogenesis has been more controversial. An earlier study by Shulman et al. showed that hyperglycemia can inhibit glucose production while having only a modest effect on glycogenolysis. In previous studies, Rossetti et al. (28), Chu and colleagues (7, 9), and Sindelar et al. (34), among others, showed that hyperglycemia can inhibit glucose production by means of a decrease in hepatic glycogenolysis. The effect of hyperglycemia on gluconeogenesis has been more controversial. An earlier study by Shulman et al. (32) showed that, in the 36-h fasted dog with fixed basal insulin and glucagon, hyperglycemia significantly decreased the conversion of [14C]alanine and [14C]lactate to [14C]glucose. Net hepatic lactate uptake was significantly inhibited (>80%) by the hyperglycemia, whereas net hepatic alanine uptake was not changed significantly. Because the net hepatic lactate uptake accounted for ~55% of net hepatic gluconeogenic flux in the study by Shulman et al., it is obvious that hyperglycemia decreased net hepatic gluconeogenic flux ~45%. In a recent study, Hellerstein et al. (17) reported that, in 20-h fasted rats (no hormone data shown), hyperglycemia (glucose infusion of 35 mg·kg⁻¹·min⁻¹ iv) significantly inhibited HGP (10.3 ± 0.6 vs. 3.6 ± 0.5 mg·kg⁻¹·min⁻¹; control group vs. iv glucose infusion) and gluconeogenesis (8.0 ± 0.6 vs. 3.4 ± 0.5 mg·kg⁻¹·min⁻¹; control group vs. iv glucose infusion). Using isolated rat hepatocytes, Sanchez-Gutierrez et al. (31) demonstrated a significant positive correlation between the glucose level in the medium and the inhibition of gluconeogenesis.

Rossetti et al. (28), on the other hand, reported that, in 6-h fasted rats maintained with basal levels of insulin and glucagon, hyperglycemia (plasma glucose ~240 mg/dl) significantly decreased HGP and gluconeogenesis but had no effect on gluconeogenesis. Sindelar et al. (34) showed that, in overnight-fasted dogs, modest hyperglycemia (blood glucose ~140 mg/dl) abolished net hepatic glucose output but did not inhibit the gluconeogenic rate. In the study by Rossetti et al., gluconeogenesis was estimated only at the last time point (120 min) using the PEP specific activity/uridine diphasophoglucone specific activity method. In the study by Sindelar et al., the net hepatic balances of gluconeogenic amino acids were only measured in the last 30 min of test period, thereby preventing the contribution of gluconeogenesis to HGP over the whole test period from being calculated. It is possible, therefore, that an earlier inhibitory effect of hyperglycemia on the overall contribution of hepatic gluconeogenesis to HGP could have been overlooked in those two studies.

In response to an increase in plasma FFA and glyceral, net hepatic glucose output did not change significantly, but net hepatic glycolysis decreased from 2.0 ± 0.2 to 1.3 ± 0.2 mg·kg⁻¹·min⁻¹ (P < 0.05, a 35% inhibition). The overall contribution of net hepatic glycogenolysis to HGP over 3 h decreased significantly by 82 ± 16 mg/kg (P < 0.05; a 42% inhibition). Gluconeogenic flux, on the other hand, increased significantly...
from 0.6 ± 0.1 to 1.3 ± 0.2 mg·kg⁻¹·min⁻¹ (P < 0.05; a 2-fold stimulation). The overall contribution of hepatic gluconeogenic flux increased by 59 mg/kg (P < 0.05; a 46% stimulation). The combination of hyperglycemia and an increase in plasma FFA and glycerol completely abolished net hepatic glycogenolysis (1.7 ± 0.2 to −0.1 ± 0.2 mg·kg⁻¹·min⁻¹, P < 0.05) such that it decreased by 177 ± 22 mg/kg (P < 0.05; 90% inhibition). Gluconeogenic flux, on the other hand, increased from 0.6 ± 0.1 to 1.2 ± 0.2 mg·kg⁻¹·min⁻¹ (P < 0.05; 2-fold stimulation), and the overall contribution of hepatic gluconeogenic flux increased by 56 ± 10 mg/kg (P < 0.05; a 44% stimulation). It should be noted that the increased FFA levels may have decreased glucose oxidation in the liver. To the extent they did so we would have overestimated net hepatic glycogenolysis since we assumed a fixed glucose oxidation rate. Given the low basal rate of glucose oxidation, this effect would have been very small (<0.2 mg·kg⁻¹·min⁻¹). If this occurred, the inhibition of gluconeogenesis that occurred in the FFA and FFA + hyperglycemia protocols may in reality have been slightly greater than estimated. In both Intralipid protocols, the blood glycerol level rose by more than twofold. This gave rise to increases in net hepatic glycerol uptake of ~1.8 μmol·kg⁻¹·min⁻¹, which could have accounted for an increase in gluconeogenesis of 0.16 mg·kg⁻¹·min⁻¹. Assuming that all of the glycerol taken up by the liver was converted to glucose, the rise in glycerol uptake could account for no more than 40% of the increase in gluconeogenesis. It seems most likely, therefore, that the rise in gluconeogenesis was primarily attributable to the rise in net hepatic FFA uptake. A similar conclusion was reached in the study by Stingl et al. (35). Taken together, the above findings demonstrate that an acute simulated rise in lipolysis that increased plasma FFA and glycerol significantly inhibited hepatic glycogenolysis but markedly stimulated gluconeogenesis. High FFA, glycerol, and hyperglycemia have an additive effect in inhibiting hepatic glycogenolysis. Interestingly, the stimulatory effects of FFA and glycerol on gluconeogenesis appeared to overcome the small inhibitory effect of hyperglycemia on the process.

In the present study, net hepatic lactate balance decreased slowly over time under euglycemic conditions. Hyperglycemia, on the other hand, caused an increase in net hepatic lactate output. The likely explanation for this finding is that, in response to hyperglycemia, hepatic glucose uptake increased, and as a result the intracellular glucose and glucose 6-phosphate levels rose, which in turn increased glycolytic flux. On the other hand, in the presence of euglycemia, high plasma FFA levels resulted in an increase in net hepatic lactate uptake consistent with a stimulation of gluconeogenesis. Elevation of plasma FFA levels completely eliminated the ability of hyperglycemia to cause net hepatic lactate output, again suggesting that an increase in plasma FFA levels stimulated hepatic gluconeogenesis and inhibited glycolysis.

Earlier studies in vitro (14, 36, 37) showed that FFA could stimulate gluconeogenesis in the perfused rat liver. The increase in FFA availability elevated FFA oxidation by the liver. This in turn stimulated gluconeogenesis via increases in the NADH-to-NAD⁺ ratio, in acetyl-CoA production, and in ATP production. NADPH generates reducing equivalents for the gluconeogenesis process. Acetyl-CoA stimulates hepatic gluconeogenesis through an activation of pyruvate carboxylase and an increase in citrate concentration. The latter can inhibit phosphofructokinase and thereby inhibit glycolysis (11, 22). Several earlier in vivo studies (5, 10, 13, 30) have shown that an elevated FFA level resulting from Intralipid infusion can increase gluconeogenesis in the human.

Although it has been generally accepted that an increase in FFA stimulates gluconeogenesis, the direct effects of plasma FFA on hepatic glycogenolysis have not been determined. As a result, the effects of plasma FFAs on overall HGP have been controversial. Clére et al. (10) and Johnston et al. (18) reported that an increase in plasma FFA level resulting from the infusion of Intralipid did not change HGP in normal humans or

Table 2. Tracer-determined Rd and Cl during the basal and test periods of euglycemic or hyperlipidemic + euglycemic or hyperglycemic or hyperlipidemic + hyperglycemic groups of conscious 18-h-fasted dogs maintained on a pancreatic clamp

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</tr>
<tr>
<td>FFA + HG</td>
<td>2.8 ± 0.3</td>
<td>3.0 ± 0.2</td>
<td>3.5 ± 0.3</td>
<td>3.4 ± 0.3</td>
</tr>
</tbody>
</table>

Data are means ± SE. Rd, glucose utilization; Cl, clearance. Basal period values are means of those at −40 and 0 min. *P < 0.05 vs. corresponding basal period.
Table 3. Arterial blood levels and net hepatic balances of alanine, glutamate, glutamine, glycine, serine, and threonine during the basal and test periods of euglycemic or hyperlipidemic + euglycemic or hyperglycemic or hyperlipidemic + hyperglycemic groups of conscious 18-h-fasted dogs maintained on a pancreatic clamp

<table>
<thead>
<tr>
<th>Group and Parameter</th>
<th>Basal period</th>
<th>Test period</th>
<th>Net Hepatic Balance, μmol·kg⁻¹·min⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Arterial Blood Level, μmol/l</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EuG</td>
<td>357 ± 73</td>
<td>348 ± 94</td>
<td>-3.1 ± 0.5</td>
</tr>
<tr>
<td>FFA + EuG</td>
<td>423 ± 21</td>
<td>363 ± 30</td>
<td>-2.7 ± 0.6</td>
</tr>
<tr>
<td>HG</td>
<td>304 ± 76</td>
<td>286 ± 61</td>
<td>-2.9 ± 0.5</td>
</tr>
<tr>
<td>FFA + HG</td>
<td>330 ± 80</td>
<td>312 ± 59</td>
<td>-2.4 ± 0.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><strong>Net Hepatic Balance, μmol·kg⁻¹·min⁻¹</strong></td>
</tr>
<tr>
<td>Alanine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EuG</td>
<td>76 ± 12</td>
<td>73 ± 12</td>
<td>-0.1 ± 0.1</td>
</tr>
<tr>
<td>FFA + EuG</td>
<td>79 ± 14</td>
<td>74 ± 15</td>
<td>-0.0 ± 0.0</td>
</tr>
<tr>
<td>HG</td>
<td>196 ± 54</td>
<td>188 ± 53</td>
<td>0.0 ± 0.1</td>
</tr>
<tr>
<td>FFA + HG</td>
<td>85 ± 3</td>
<td>79 ± 4</td>
<td>-0.2 ± 0.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><strong>Glutamine</strong></td>
</tr>
<tr>
<td>EuG</td>
<td>887 ± 58</td>
<td>911 ± 68</td>
<td>0.2 ± 0.6</td>
</tr>
<tr>
<td>FFA + EuG</td>
<td>919 ± 49</td>
<td>897 ± 52</td>
<td>0.9 ± 1.0</td>
</tr>
<tr>
<td>HG</td>
<td>897 ± 87</td>
<td>898 ± 93</td>
<td>0.2 ± 0.4</td>
</tr>
<tr>
<td>FFA + HG</td>
<td>951 ± 84</td>
<td>931 ± 78</td>
<td>1.1 ± 1.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><strong>Glycine</strong></td>
</tr>
<tr>
<td>EuG</td>
<td>217 ± 26</td>
<td>202 ± 34</td>
<td>-1.3 ± 0.3</td>
</tr>
<tr>
<td>FFA + EuG</td>
<td>262 ± 31</td>
<td>239 ± 30</td>
<td>-1.2 ± 0.2</td>
</tr>
<tr>
<td>HG</td>
<td>170 ± 19</td>
<td>162 ± 15</td>
<td>-0.8 ± 0.2</td>
</tr>
<tr>
<td>FFA + HG</td>
<td>260 ± 41</td>
<td>217 ± 36</td>
<td>1.5 ± 0.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><strong>Serine</strong></td>
</tr>
<tr>
<td>EuG</td>
<td>138 ± 13</td>
<td>128 ± 18</td>
<td>-0.8 ± 0.2</td>
</tr>
<tr>
<td>FFA + EuG</td>
<td>151 ± 13</td>
<td>143 ± 14</td>
<td>-0.3 ± 0.1</td>
</tr>
<tr>
<td>HG</td>
<td>124 ± 18</td>
<td>118 ± 13</td>
<td>-0.6 ± 0.2</td>
</tr>
<tr>
<td>FFA + HG</td>
<td>139 ± 18</td>
<td>122 ± 15</td>
<td>-0.7 ± 0.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><strong>Threonine</strong></td>
</tr>
<tr>
<td>EuG</td>
<td>229 ± 25</td>
<td>213 ± 26</td>
<td>-0.3 ± 0.2</td>
</tr>
<tr>
<td>FFA + EuG</td>
<td>211 ± 19</td>
<td>202 ± 17</td>
<td>-0.3 ± 0.1</td>
</tr>
<tr>
<td>HG</td>
<td>220 ± 37</td>
<td>202 ± 21</td>
<td>-0.4 ± 0.4</td>
</tr>
<tr>
<td>FFA + HG</td>
<td>218 ± 16</td>
<td>202 ± 14</td>
<td>0.5 ± 0.4</td>
</tr>
</tbody>
</table>

Data are means ± SE. Basal period is the mean of samples taken at ~40 and 0 min. Test period values are means of the values at 30, 60, 90, 120, 150, and 180 min. Negative and positive balance data mean net hepatic uptake and output, respectively. There were no significant differences among groups.

type 2 diabetic subjects. Puhakainen and colleagues (24, 25) and Lee et al. (19) reported that, in the absence of pancreatic clamp, a decrease in the FFA level by infusion of acipimox reduced hepatic gluconeogenesis but did not change HGP. These authors attributed their observations to a hepatic autoregulatory mechanism, which resulted in an increase in glycolysis that offset the fall in gluconeogenesis. An alternative explanation for the above results would be that FFAs have a direct inhibitory effect on hepatic glycolysis.

Ferrannini et al. (13) reported that, in normal humans in the presence of a hyperglycemic hyperglucagonemic hypoinsulinemic condition, HGP was higher in the presence of elevated FFA levels than in the presence of normal FFA levels. These authors suggested that FFAs stimulate gluconeogenesis and increase HGP. It should be noted that, since no insulin was infused and the glucagon level was clamped, the combination of these changes in the pancreatic hormones would have significantly stimulated hepatic gluconeogenesis, which would itself have caused an increase in HGP. Therefore, the inhibitory effect of a high plasma FFA concentration on hepatic gluconeogenesis could have been masked by the effects of changes in the insulin and glucagon levels. A recent study by Roden et al. (27) showed that, in normal humans infused with somatostatin, basal insulin, and glucagon, HGP did not change significantly in the presence or absence of high plasma FFA levels, but the gluconeogenic contribution to glucose production increased. In another recent study, Stingl et al. (35) reported that an increase in plasma FFA decreased gluconeogenesis and glucose production in healthy men in the absence of a pancreatic clamp. Because in this study the plasma insulin level in the high FFA group was twofold greater than in the saline group, the decrease in glycolysis and glucose production observed in the high FFA group could have been attributable to the inhibitory effect of elevated insulin rather than the increase to FFA per se.

The present study showed that net hepatic gluconeogenesis was inhibited by 48, 43, and 94% in response to hyperglycemia, high levels of FFA and glycerol, and hyperglycemia + high levels of FFA and glycerol, respectively. These data indicate that there is an additive effect of hyperglycemia, high FFA, and glycerol concentrations on the inhibition of hepatic gluconeogenesis. They also indicate that the inhibition caused by hyperglycemia and increased lipolysis might occur via different mechanisms. Studies in vitro (1, 2) and in vivo (6) showed that hyperglycemia per se induces a translocation of hepatic glucokinase to the cytosol, thereby increasing the activity of glucokinase, which in turn increases HGP levels and glycogen synthase activity (15) and increases glycogen synthesis. Hyperglycemia can also work through a mass action mechanism to push more glucose into the hepatocytes. The inhibitory effects of high FFA and glycerol on net hepatic gluconeogenesis appear to be mediated via their stimulatory effects on gluconeogenesis and/or an inhibitory effect on glycolysis.

In conclusion, 1) an increase in plasma FFA and glycerol significantly inhibits net hepatic gluconeogenesis and markedly stimulates gluconeogenic flux, without changing HGP, 2) hyperglycemia significantly inhibits net hepatic gluconeogenesis and stimulates hepatic glycogenolysis, but modestly inhibits gluconeogenesis, 3) simultaneous increases in plasma FFA, glycerol, and glucose have an additive effect on the inhibition of net hepatic gluconeogenesis, and 4) an increase in plasma FFA and glycerol can overcome the inhibitory effect of hyperglycemia on hepatic gluconeogenic flux.

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25. Puhakainen I and Yki-Jarvinen H. Inhibition of lipolysis decreases lipid oxidation and gluconeogenesis from lactate but not fasting hyperglycemia or total hepatic glucose production in NIDDM. Diabetes 42: 1694–1699, 1993.