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


Received 20 March 2001; accepted in final form 28 September 2001

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 in the overnight-fasted human.

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
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. Data from in vitro studies are similarly conflicting. A study 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,

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>-140</th>
<th>Equilibration</th>
<th>-40</th>
<th>Control</th>
<th>0</th>
<th>Experimental Period</th>
<th>180</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Indocyanine Green + ^3H Glucose + ^14C Alanine</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Somatostatin + Basal Portal Glucagon and Insulin (0.8 μg/kg-min) (0.5 ng/kg-min) (235 μU/kg-min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>EuG</strong> (N = 6)</td>
<td></td>
<td></td>
<td></td>
<td>Euglycemic clamp (80 mg/dl)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>FFA+EuG</strong> (N = 6)</td>
<td>Clamp blood glucose to 80 mg/dl + 20% Intralipid 0.02 ml/kg-min + Heparin 0.5 unit/kg-min</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>HG</strong> (N = 6)</td>
<td>Clamp blood glucose to 120 mg/dl</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>FFA+HG</strong> (N = 6)</td>
<td>Clamp blood glucose to 120 mg/dl + 20% Intralipid 0.02 ml/kg-min + Heparin 0.5 unit/kg-min</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Fig. 1.** Protocol. EuG, euglycemia; FFA, free fatty acid; HG, hyperglycemia.

Note: All dogs were conscious and 18 h fasted.
EFFECTS OF FFA ON HEPATIC GLYCOGENOLYSIS

FFA + EuG, hyperglycemia (HG), and FFA + HG (Fig. 1). Intralipid (0.02 ml·kg\(^{-1}\)·min\(^{-1}\)), 20% fat emulsion; Pharmacia & 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 \(^{[3]}\)H]- and \(^{[14]}\)C-glucose, blood lactate, glycerol, \(\beta\)-hydroxybutyrate (\(\beta\)-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, glycerol, \(\beta\)-OHB, alanine, other gluconeogenic amino acids, and plasma FFA in the present study were calculated using arterial-venous 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 hepatic glucose release (20). Gluconeogenic efficiency was assessed using a double-isotope technique described elsewhere (7–9). Because the conversion of \(^{[14]}\)C]alanine to \(^{[14]}\)C-glucose by the kidney is minimal (21), \(^{[14]}\)C-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 precursors alanine, glycine, serine, threonine, glutamine, glutamate, lactate, and glycerol 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 calculated by dividing the above uptake rates by two to account for the incorporation of the C-3 precursors into the C-6 glucose molecule. Net hepatic gluconeogenesis 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\(_{\text{oxid}}\) 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 difference 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 univariate \(F\)-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).

Arterial blood levels, net hepatic balances, and fractional extractions of FFA, \(\beta\)-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 (\(P < 0.05\)) \(\mu\)mol/l in the FFA + EuG and
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

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 ($\dagger$), and FFA + EuG vs. EuG (+).

Fig. 4. Arterial blood levels and net hepatic balances of glycerol and β-hydroxybutyrate (β-OHB) 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 ($\dagger$), and FFA + EuG vs. EuG (+).
No significant changes were seen in arterial blood levels or net hepatic balances of alanine, glutamate, glutamine, glycine, serine, and threonine in response to any treatment (Table 3).

**Gluconeogenesis and glycogenolysis.** The hepatic gluconeogenic flux rate did not change significantly (0.6 ± 0.1 to 0.8 ± 0.2 and 0.8 ± 0.1 to 0.7 ± 0.2 in the EuG and HG groups, respectively; Fig. 7A) in the absence of increased FFA. In response to Intralipid infusion, the gluconeogenic flux rate increased from 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 gluconeogenic flux 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 gluconeogenic flux 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 gluconeogenic flux by 59 ± 12 mg/kg (P < 0.05) or 46% stimulation (Fig. 8). In response to combined hyperglycemia and hyperlipidemia, overall gluconeogenic flux was increased by 56 ± 10 mg/kg (P < 0.05) or 44% (Fig. 8).

2.4 ± 0.3 to 3.4 ± 0.4 (P < 0.05) and from 2.8 ± 0.3 to 3.7 ± 0.4 (P < 0.05) mg·kg⁻¹·min⁻¹ by 120 min in the HG and FFA + HG groups, respectively (Table 2). Glucose clearance decreased slightly from 2.5 ± 0.2 to 2.0 ± 0.1 (P < 0.05) and from 2.7 ± 0.2 to 2.2 ± 0.2 (P < 0.05) ml·kg⁻¹·min⁻¹, respectively, by 90 min in the FFA + EuG and FFA + HG groups (Table 2), but it did not change significantly in EuG and HG groups.

**Arterial blood levels and net hepatic balances of lactate, alanine, glutamate, glutamine, glycine, serine, and threonine.** The arterial blood lactate level did not change significantly in any group (Fig. 6). Net hepatic lactate balance slowly decreased by 2.4 ± 1.3 μmol·kg⁻¹·min⁻¹ in the EuG group (Fig. 6). In response to Intralipid infusion in the absence of hyperglycemia, net hepatic lactate balance decreased significantly (7.1 ± 2.3 μmol·kg⁻¹·min⁻¹, P < 0.05). In the presence of hyperglycemia alone, net hepatic lactate balance increased (5.4 ± 1.6 μmol·kg⁻¹·min⁻¹). When plasma FFA levels rose in the presence of hyperglycemia, net hepatic lactate balance decreased significantly (5.9 ± 1.6 μmol·kg⁻¹·min⁻¹).

---

Fig. 5. Arterial blood glucose levels and changes in net 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 (@).

Fig. 6. Arterial blood level and change in net hepatic lactate balance 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 ($), FFA + EuG vs. EuG (!), and HG vs. EuG (@).
Net hepatic glycogenolysis decreased slightly from $1.4 \pm 0.3$ to $1.0 \pm 0.2$ 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 $1.6 \pm 0.2$ to $0.7 \pm 0.2$ (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 $2.0 \pm 0.2$ to $1.3 \pm 0.2$ (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 $1.7 \pm 0.2$ to $0.1 \pm 0.2$ (P < 0.05). Net hepatic glycogenolysis over the 3-h test period was $196 \pm 26$ mg/kg in the EuG group. Hyperglycemia alone significantly decreased net hepatic glucose output from $2.2 \pm 0.3$ to $0.7 \pm 0.5$ (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 glycogenolysis.

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 $196 \pm 26$ mg/kg over the 3-h test period. Gluconeogenesis did not change significantly during the study, and overall hepatic gluconeogenic flux was $128 \pm 22$ mg/kg (~40% of HGP). Hyperglycemia alone significantly decreased net hepatic glucose output from $2.2 \pm 0.3$ to $0.7 \pm 0.5$ 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 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.

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.

**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 $196 \pm 26$ mg/kg over the 3-h test period. Gluconeogenesis did not change significantly during the study, and overall hepatic gluconeogenic flux was $128 \pm 22$ mg/kg (~40% of HGP). Hyperglycemia alone significantly decreased net hepatic glucose output from $2.2 \pm 0.3$ to $0.7 \pm 0.5$ 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 glycogenolysis.

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 $196 \pm 26$ mg/kg over the 3-h test period. Gluconeogenesis did not change significantly during the study, and overall hepatic gluconeogenic flux was $128 \pm 22$ mg/kg (~40% of HGP). Hyperglycemia alone significantly decreased net hepatic glucose output from $2.2 \pm 0.3$ to $0.7 \pm 0.5$ 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 glycogenolysis.
E408 EFFECTS OF FFA ON HEPATIC GLYCOGENOLYSIS

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 (@).

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>
<tr>
<th>Group</th>
<th>Basal Period</th>
<th>Test Period, min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>30</td>
</tr>
<tr>
<td>EuG</td>
<td>27 ± 4</td>
<td>29 ± 4</td>
</tr>
<tr>
<td>FFA + EuG</td>
<td>27 ± 2</td>
<td>26 ± 2</td>
</tr>
<tr>
<td>HG</td>
<td>27 ± 2</td>
<td>29 ± 3</td>
</tr>
<tr>
<td>FFA + HG</td>
<td>29 ± 2</td>
<td>29 ± 2</td>
</tr>
</tbody>
</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.
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 glycolysis. 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.
E410 EFFECTS OF FFA ON HEPATIC GLYCOGENOLYSIS

Table 3. Arterial blood levels and net hepatic balances of alanine, glutamate, glutamine, 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>Arterial Blood Level, μmol/l</th>
<th>Net Hepatic Balance, μmol·kg⁻¹·min⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal period</td>
<td>Test period</td>
</tr>
<tr>
<td>EuG</td>
<td>357 ± 73</td>
<td>348 ± 94</td>
</tr>
<tr>
<td>FFA + EuG</td>
<td>423 ± 21</td>
<td>363 ± 30</td>
</tr>
<tr>
<td>HG</td>
<td>304 ± 76</td>
<td>286 ± 61</td>
</tr>
<tr>
<td>FFA + HG</td>
<td>330 ± 80</td>
<td>312 ± 59</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Group and Parameter</th>
<th>Arterial Blood Level, μmol/l</th>
<th>Net Hepatic Balance, μmol·kg⁻¹·min⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal period</td>
<td>Test period</td>
</tr>
<tr>
<td>EuG</td>
<td>357 ± 73</td>
<td>348 ± 94</td>
</tr>
<tr>
<td>FFA + EuG</td>
<td>423 ± 21</td>
<td>363 ± 30</td>
</tr>
<tr>
<td>HG</td>
<td>304 ± 76</td>
<td>286 ± 61</td>
</tr>
<tr>
<td>FFA + HG</td>
<td>330 ± 80</td>
<td>312 ± 59</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 glycogenolysis that offset the fall in gluconeogenesis. An alternative explanation for the above results would be that FFAs have a direct inhibitory effect on hepatic glycogenolysis.

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 glycogenolysis, which would itself have caused an increase in HGP. Therefore, the inhibitory effect of a high plasma FFA concentration on hepatic glycogenolysis 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 glycogenolysis 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 glycogenolysis was inhibited by 48, 43, and 94% in response to hyperglycemia, high levels of FFA and glycerol, and hyperglyceremia + 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 glycogenolysis. 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 glycogenolysis 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 glycogenolysis and markedly stimulates gluconeogenic flux, without changing HGP, 2) hyperglycemia significantly inhibits net hepatic glycogenolysis and stimulates hepatic glycolysis, but modestly inhibits gluconeogenesis, 3) simultaneous increases in plasma FFA, glycerol, and glucose have an additive effect on the inhibition of net hepatic glycogenolysis, and 4) an increase in plasma FFA and glycerol can overcome the inhibitory effect of hyperglycemia on hepatic gluconeogenic flux.

We appreciate assistance from Jon Hastings, Melanie Scott, Wanda Snead, and Paul Flakoll.
Part of this work was presented at the 57th Annual Meeting of the American Diabetes Association, San Antonio, TX, in June 2000.

This work was supported in part by National Institute of Diabetes and Digestive and Kidney Diseases Grants 2RO1 DK-18243 and 5P60 DK-20593 (Diabetes Research and Training Center).

REFERENCES


