Effects of Free Fatty Acids Per Se on Glucose Production, Gluconeogenesis, and Glycogenolysis

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Insulin-independent effects of a physiological increase in free fatty acid (FFA) levels on fasting glucose production, gluconeogenesis, and glycogenolysis were assessed by administering [6,6-^{2}H$_2$]-glucose and deuteriated water (^{2}H$_2$O) in 12 type 1 diabetic patients, during 6-h infusions of either saline or a lipid emulsion. Insulin was either fully replaced (euglycemic group, $n = 6$), or underreplaced (hyperglycemic group, $n = 6$). During saline infusions, plasma FFA levels remained unchanged. Glucose concentrations decreased from 6.7 ± 0.4 to 5.3 ± 0.4 mmol/l and 11.9 ± 1.0 to 10.5 ± 1.0 mmol/l in the euglycemic and hyperglycemic group, respectively. Accordingly, glucose production declined from 84 ± 5 to 63 ± 5 mg · m^{-2} · min^{-1} and from 84 ± 5 to 68 ± 4 mg · m^{-2} · min^{-1}, due to declining rates of glycogenolysis but unaltered rates of gluconeogenesis. During lipid infusions, plasma FFA levels increased twofold. In the euglycemic group, plasma glucose increased from 6.8 ± 0.3 to 7.8 ± 0.8 mmol/l. Glucose production declined less in the lipid study than in the saline study due to a stimulation of gluconeogenesis by 6 ± 1 mg · m^{-2} · min^{-1} and a decline in glycogenolysis that was 6 ± 2 mg · m^{-2} · min^{-1} less in the lipid study than in the saline study. In contrast, in the hyperglycemic group, there were no significant effects of elevated FFA on glucose production, gluconeogenesis, or glycogenolysis. In conclusion, a physiological elevation of plasma FFA levels stimulates glycogenolysis as well as gluconeogenesis and causes mild fasting hyperglycemia. These effects of FFA appear attenuated in the presence of hyperglycemia. *Diabetes* 52:260–267, 2003

Free fatty acids (FFAs) are believed to play an important role in the regulation of glucose production (1). Supporting this view, in vitro studies suggest that FFAs increase gluconeogenesis through stimulation of key-gluconeogenic enzymes (2–4). In vivo, elevation of FFA levels in hyperinsulinemic clamp studies (by lipid/heparin infusions) impair the insulin-mediated suppression of glucose production (1,5). Fur-

thermore, in clamp studies of type 2 diabetic patients, impaired suppression of glucose production by insulin appears related to impaired suppression of plasma FFA levels (6). However, in vivo effects of FFA on glucose production, gluconeogenesis, and glycogenolysis in the fasted state are less clear.

The in vivo studies are complicated by the fact that FFAs are potent insulin secretagogues (1). Since glycogenolysis, in particular, is very sensitive to insulin (7,8), even small changes in its levels may bias studies on the effects of FFAs. To assess the direct effects of FFA per se on these pathways, endogenous insulin secretion has therefore been inhibited in a number of studies by administering somatostatin (SRIF) (9–14), but conclusions still differ (Table 1).

In some studies, an insulin-independent direct relationship between plasma FFA levels and fasting glucose production rates was found, i.e., glucose production increased when FFA levels were elevated by lipid/heparin infusions (9–11) and decreased when FFA levels were lowered by N^6-cyclohexyladenosine (12). In other studies, there was no significant effect of elevated FFA levels on glucose production (13,14). In two studies, individual contributions of gluconeogenesis and glycogenolysis were determined, and a stimulating effect of FFA on gluconeogenesis was reported in both. In one of the studies (13) glycogenolysis was unaltered by elevating FFA levels, while in the other (14) glycogenolysis was inhibited.

An important question is: does an increase in glucose production induced by elevated FFA levels result in increased glucose levels? The answer is of particular interest with regard to type 2 diabetic patients, in whom elevated fasting FFA levels are suggested to lead to hyperglycemia by increasing glucose production. In several previous studies, glucose levels were clamped (9,11,12). Plasma glucose levels were allowed to change freely in response to changes in FFA levels in only two studies in humans. Boden et al. (10) found that production, as well as plasma glucose levels, increased upon elevating FFA levels. Roden et al. (13) also observed an increase in plasma glucose levels in response to elevated FFA levels. However, there was no change in glucose production rates, suggesting that FFA affected glucose levels via another mechanism (13).

A methodological issue of potential significance is in the use of SRIF. SRIF may reduce splanchnic blood flow (15) and stimulate peripheral glucose clearance (16). More importantly, SRIF acutely reduces portal insulin levels. That is because insulin is replaced by peripheral infusion in most in vivo studies (and in all human studies). There-

TABLE 1
Studies that have assessed the effects of FFA per se on glucose production (GP), gluconeogenesis (GNG), and glycogenolysis (GGL)

	Species	Change in FFA	Change in plasma glucose	Change in GP	Change in GNG	Change in GGL	Ref. no.
Ferrannini 1983	Human	Increase	Clamped	→	—	—	9
Boden 1991	Human	Increase	Increase	→	—	—	10
Rebrin 1996	Dogs	Increase	Clamped	→	—	—	11
Mittelman 2000	Dogs	Decrease	Clamped	↓	—	—	12
Roden 2000	Human	Increase	Increase	No change	→	No change	13
Chu 2002	Dogs	Increase	No change	No change	→	↓	14

fore, because insulin is normally secreted into the portal vein, peripheral insulin levels remain essentially unaltered while portal insulin levels are lower than normal. Since glycogenolysis is sensitive to very small changes in insulin levels (7,8), acute reduction in portal insulin during SRIF infusion could then alter the sensitivity of the liver to FFA. In any case, the stimulating effect of FFA on glucose production in humans appears primarily to have been detected under acutely reduced portal insulin levels.

We therefore assessed the effects of a physiological elevation of FFA levels on glucose production, gluconeogenesis, and glycogenolysis in otherwise healthy type 1 diabetic patients to determine whether elevated FFA levels increase glucose production in the absence of acute reductions in portal insulin levels brought about by SRIF. If so, we asked whether changes in both gluconeogenesis and glycogenolysis occur. Studies were performed both after overnight replacement and underreplacement of insulin to further explore the importance of prevailing insulin levels on the effects of FFA.

RESEARCH DESIGN AND METHODS

Subjects. A total of 12 lean male type 1 diabetic patients were recruited from the outpatient clinic. They were in good glycemic control (Table 2) and without signs of diabetic retinopathy, nephropathy, neuropathy, or macrovascular complications. All were treated with short-acting insulin (Actrapid; Novo, Bagsvaerd, Denmark) three times a day and long-acting insulin (Insulatard; Novo) at bedtime. The patients were distributed into two groups, designated euglycemic (EU) group and hyperglycemic (HY) group. The groups were comparable in age, HbA$_{1c}$, BMI, lipid profile, and daily insulin requirements (Table 2). Patients were considered eligible for the study even if they had detectable low levels of C-peptide, provided that they lacked a C-peptide response to intravenous glucagon.

The purpose and the risks of the study were carefully explained before informed consent to participate was obtained. The protocol was reviewed and approved by the regional scientific ethical committee.

Study design. Each patient was studied on two occasions, in a lipid and a saline (control) study, separated by an 8-week interval. Subjects were instructed to eat their regular diet and abstain from exercise 3 days before each study. They injected the prescribed dose of rapid-acting insulin at noon on the day before the study, but thereafter no subcutaneous insulin was injected. Consequently, in the morning of the study day the subjects had not received short-acting insulin for 20 h and long-acting insulin for 36 h.

Subjects were admitted to the department at 7:00 P.M., after having eaten their regular dinner. Two intravenous catheters were inserted in hand veins for blood sampling and infusions. Then an insulin infusion (Actrapid; Novo), was begun to lower plasma glucose levels. In the six subjects of the euglycemic group, replacement insulin infusion was given, sufficient to maintain a plasma glucose level of ~6 mmol/l overnight, based on frequent bedside plasma glucose measurements. In the remaining six subjects (the hyperglycemic group), insulin was underreplaced to maintain a plasma glucose level of ~12 mmol/l. The rate of insulin infusion was unaltered after administration of ^{2}H$_2$O at 3:00 A.M., even if there was a small drift in plasma glucose levels in some of the subjects. Thus, changes in insulin levels could have altered incorporation of deuterium labeled glucose into glycogen. Subjects drank ^{2}H$_2$O (99% enriched; New England Nuclear, Boston, MA); 2.5 ml/kg body water (estimated as 60% of body weight) at 3:00 A.M., 1.25 ml/kg at

5:30 A.M., and 1.25 ml/kg at 6:15 A.M. (17,18). Otherwise, they rested undisturbed throughout the night.

At 6:30 A.M., a primed-continous infusion of [6,6-^{2}H$_2$]glucose was begun. The priming dose equaled the amount infused in 100 min times the fasting plasma glucose concentration (mmol/l) divided by 5 mmol/l (adjusted priming [19,20]). The infusion rate was constant at 75 mg/h. A baseline period of 120 min was allowed for tracer equlibration. At 8:30 A.M., an infusion of either saline at 45 ml/h or a 20% lipid emulsion (Intralipid; Kabi Pharmacia) at 45 ml/h was started and continued for 300 min.

Blood was collected every 15–60 min for determination of the enrichments of the two hydrogens bound to carbon 6 of blood glucose and the plasma concentrations of glucose, FFA, triglyceride, insulin, C-peptide, and glucagon. Blood for determining the enrichments of the hydrogens bound to carbons 2 and 5 of blood glucose were collected at −30, 0, 270, and 300 min. Blood samples were immediately centrifuged and plasma stored at −20°C until assay.

Assays. Plasma glucose concentrations were determined using a glucose oxidase method (Beckman Glucose Analyzer II, Fullerton, CA). Enrichments of the hydrogens bound to carbons 2, 5, and 6 of blood glucose were determined as previously described (17,18). Briefly, the supernatant, obtained after deproteinizing a blood sample with ZnSO$_4$-Ba(OH)$_2$, was deionized by passage through cation- and anion-exchange resins. Glucose in the effluent was isolated by high-performance liquid chromatography. To determine the percentage of glucose molecules with two ^{2}H atoms at carbon 6, a sample of the glucose was oxidized with periodate. The formaldehyde formed, which contains carbon 6 with its two hydrogens, was converted to hexamethylene-tetramine (HMT) by the addition of ammonia. The HMT was analyzed by gas chromatography–mass spectrometry for mass $m + 2$.

To determine the enrichment of the hydrogen bound to carbon 2, carbon 1 of a portion of the glucose was removed to yield ribulose-5-P. The ribulose-5-P was reduced to a mixture of polyol phosphates. These were oxidized with periodate, yielding formaldehyde, which contains carbon 2 with its hydrogen. HMT was made from the formaldehyde and analyzed for mass $m + 1$. To determine the enrichment of the hydrogen bound to carbon 5, another portion of the glucose was oxidized to remove its carbon 6. The resulting xylose was also oxidized with periodate, yielding carbon 5 with its hydrogen in formaldehyde. Again, HMT was formed and analyzed for mass $m + 1$. HMTs formed from formaldehyde from [6,6-^{2}H$_2$]glucose and [1-^{2}H]sorbitol of known enrichments served as standards in the assays.

TABLE 2
Clinical characteristics of the subjects

	EU group	HY group
n	6	6
Age (years)	32 ± 4	31 ± 3
BMI (kg/m^2)	23 ± 0.3	23 ± 0.8
HbA$_{1c}$ (%)*	7.7 ± 0.4	7.5 ± 0.4
Diabetes duration (years)	13 ± 2	10 ± 3
Body surface area (m^2)	1.9 ± 0.01	2.0 ± 0.05
Body weight (kg)	75 ± 2	78 ± 3
Total cholesterol (mmol)	4.4 ± 0.4	4.2 ± 0.3
LDL cholesterol (mmol)	2.7 ± 0.3	2.6 ± 0.3
HDL cholesterol (mmol)	1.4 ± 0.2	1.3 ± 0.1
Triglyceride (mmol)	0.9 ± 0.2	0.7 ± 0.1
Actrapid dose (IU/day)	32 ± 1.5	30 ± 1.2
Insulatard dose (IU/day)	22 ± 3	23 ± 4

Data are mean ± SE. *Reference interval for HbA$_{1c}$ is 4.7–7.0%. EU, euglycemic; HY, hyperglycemic.
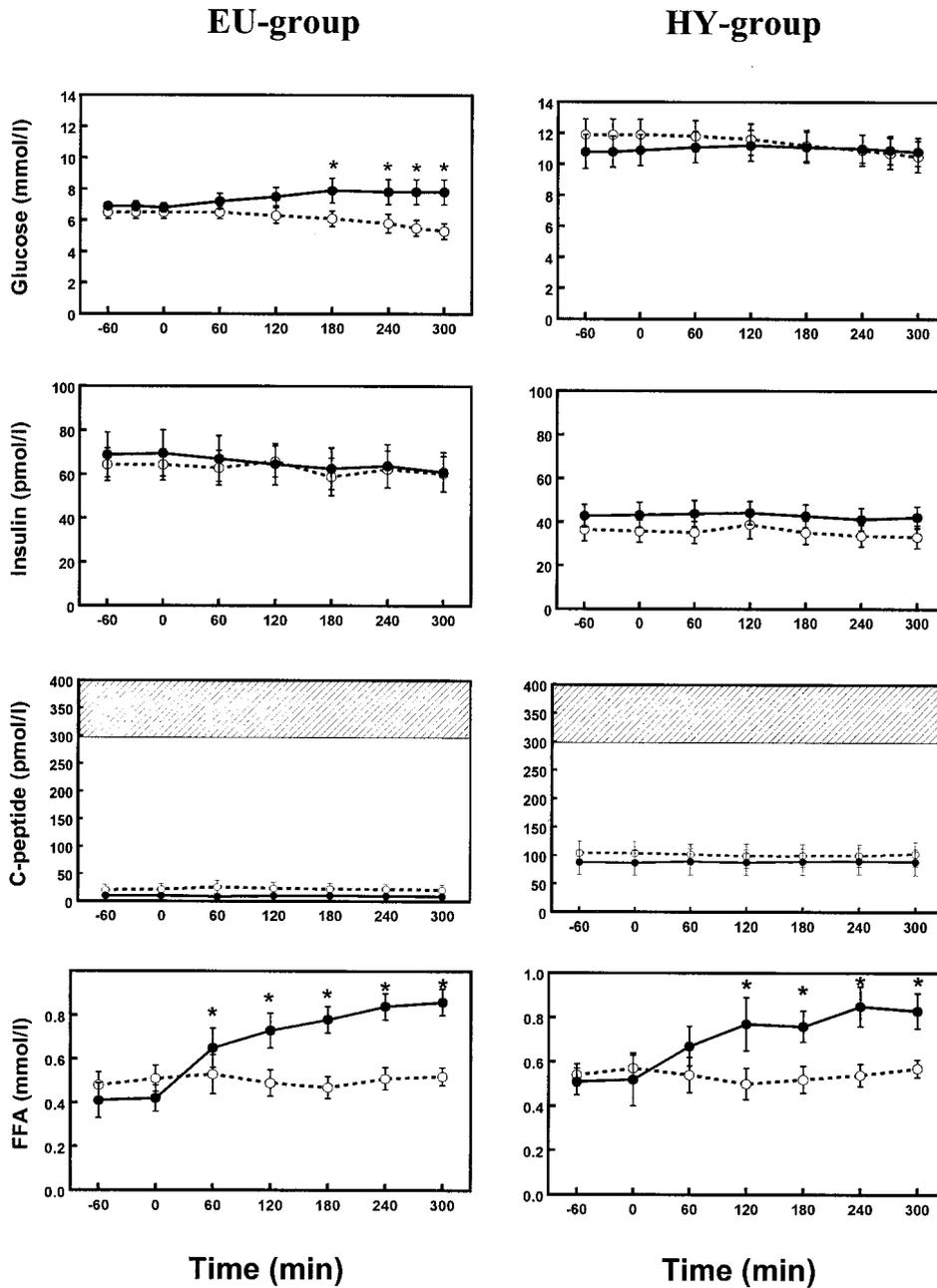
EU-group

HY-group

FIG. 1. Plasma glucose, insulin, C-peptide, and FFA concentrations during saline (----) and lipid (—) infusions. Left panels: euglycemic (EU) group; right panels: hyperglycemic (HY) group. The shaded areas in the C-peptide figures represents the lower part of the normal range. Mean ± SE. *P < 0.05, saline vs. lipid.

Plasma insulin and C-peptide concentrations were measured using a two-site time-resolved immunofluorometric assay (Wallac Oy, Turku, Finland). Plasma FFA was measured by an enzymatic colorimetric method (Wako Chemicals, Neuss, Germany) and plasma glucagon was measured by radioimmunoassay as described by Holst (21).

Calculations. Rates of glucose production and glucose disposal were calculated from the changes in the concentrations of plasma glucose and in the enrichments of the hydrogens bound to carbon 6, using Steele's non–steady-state equations (19,20). The ratio of the enrichments at carbons 5 and 2, the C5-to-C2 ratio, was equated to the fractional contribution of gluconeogensis to glucose production, so that 100 times that fraction equals the percentage of gluconeogenesis (17,18). The ratios used were the means of the ratio at −30 and 0 min (baseline) and at 270 and 300 min (infusion period). Fractional glycogenolysis is then 1 − C5-to-C2 × 100. The absolute contributions of gluconeogenesis and glycogenolysis were calculated by multiplying their fractional contributions by the rates of glucose production. Glucose metabolic clearance rate (MCR) was calculated as the rate of glucose disposal divided by the plasma glucose level.

Statistical analysis. Data are presented as mean ± SE. Differences were evaluated using the Wilcoxon's signed-rank test for paired data and the Mann-Whitney U test for unpaired data. Statistical tests and least-squares regression analysis were performed using Stata 6 software (Stata, Chicago, IL). A significance level of 5% was chosen.

RESULTS

Glucose concentrations. In the euglycemic group, baseline plasma glucose concentrations were 6.7 ± 0.4 mmol/l and declined to 5.3 ± 0.4 mmol/l (P < 0.01) on saline infusion. In contrast, in response to lipid infusion, plasma glucose increased from 6.8 ± 0.3 to 7.8 ± 0.8 mmol/l (P < 0.05) (Fig. 1).

In the hyperglycemic group, baseline plasma glucose was 11.9 ± 1.0 mmol/l and decreased to 10.5 ± 1.0 mmol/l (P < 0.01) on saline infusion. Infused with lipid, plasma glucose remained unchanged, 10.9 ± 1.0 mmol/l at baseline and 10.8 ± 0.9 mmol/l after 300 min (Fig. 1).

FFAs and triglycerides. In the euglycemic group, the baseline FFA level of 0.51 ± 0.06 mmol/l remained unal-

TABLE 3
Rates of glucose production (GP), gluconeogenesis (GNG), and glycogenolysis (GGL) in the EU group, at baseline and after 300 min of either saline or lipid infusion

EU group	Baseline $(mg \cdot m^{-2} \cdot min^{-2})$	300 min $(mg \cdot m^{-2} \cdot min^{-2})$	Difference $(mg \cdot m^{-2} \cdot min^{-2})$	P (basal vs 300 min)
GP				
Saline	84 ± 5	63 ± 5	22 ± 3	<0.05
Lipid	86 ± 4	75 ± 4	$11 \pm 2*$	<0.05
GNG				
Saline	45 ± 3	46 ± 4	1 ± 2	NS
Lipid	44 ± 3	50 ± 3	$6 \pm 1*$	<0.05
GGL				
Saline	40 ± 4	17 ± 3	-23 ± 3	<0.01
Lipid	43 ± 4	26 ± 3	$-17 \pm 2*$	<0.01

Data are mean $\pm$ SE. *$P < 0.05$, lipid vs. saline. EU, euglycemic.

tered on saline infusion. On lipid infusion, plasma FFA increased from 0.40 ± 0.06 to 0.88 ± 0.06 mmol/l ($P < 0.01$) (Fig. 1). In the hyperglycemic group, FFA levels also remained unaltered during saline infusion ($0.57–0.56$ mmol/l) but increased during lipid infusion from 0.56 ± 0.12 to 0.83 ± 0.08 mmol/l ($P < 0.01$) (Fig. 1).

Plasma triglyceride levels in the euglycemic group remained unaltered during saline infusion, 0.80 ± 0.10 vs. 0.72 ± 0.09 mmol/l, but increased from 0.91 ± 0.20 to 4.23 ± 0.90 mmol/l during lipid infusion ($P < 0.01$). In the hyperglycemic group, triglyceride levels also remained unaltered during saline infusion (0.60 ± 0.12 vs. 0.61 ± 0.08 mmol/l) and increased during lipid infusion from 0.63 ± 0.10 to 3.05 ± 0.20 mmol/l ($P < 0.01$) (data not shown).

Insulin, glucagon, and C-peptide concentrations. Baseline plasma insulin concentrations in the saline and lipid study in the euglycemic group were 65 ± 7 and 70 ± 10 pmol/l, respectively, and remained virtually unchanged (Fig. 1). The corresponding baseline insulin levels in the hyperglycemic group were lower (35 ± 5 and 45 ± 5 pmol/l) ($P < 0.05$ vs. euglycemic group) but also remained unchanged during saline as well as lipid infusion (Fig. 1).

Low levels of C-peptide were detected in a subset of the subjects. However, these levels were markedly below the lower limit of the reference interval of $300–700$ pmol/l and remained unaltered in all subjects (Fig. 1).

Baseline plasma glucagon concentrations in the saline and lipid study were 5.1 ± 0.8 and 6.5 ± 0.5 pmol/l, respectively, in the euglycemic group and 8.2 ± 0.4 and 7.7 ± 0.7 pmol/l, respectively, in the hyperglycemic group. No significant changes in glucagon levels occured in any of the studies (data not shown).

Glucose production in the euglycemic group. In the euglycemic group, glucose production decreased by 22 ± 3 mg $\cdot$ m^{-2} $\cdot$ min^{-1} in the saline study group (Table 3 and Fig. 2). Rates of gluconeogenesis did not change significantly, whereas rates of glycogenolysis were reduced by 23 ± 3 mg $\cdot$ m^{-2} $\cdot$ min^{-1}, accounting for the observed reduction in glucose production.

As in the saline study, glucose production decreased over time in the lipid study. However, the reduction of 11 ± 2 mg $\cdot$ m^{-2} $\cdot$ min^{-1} was only half that observed with saline ($P < 0.05$). Rates of gluconeogenesis increased by 6 ± 1 mg $\cdot$ m^{-2} $\cdot$ min^{-1}. Rates of glycogenolysis declined by 17 ± 2 mg $\cdot$ m^{-2} $\cdot$ min^{-1}, which was 6 ± 2 mg $\cdot$ m^{-2} $\cdot$ min^{-1}

less than the reduction in glycogenolysis observed with saline ($P < 0.05$).

In neither the saline nor the lipid study were there any relationships between fasting plasma glucose levels and rates of glucose production or the relative contribution of gluconeogenesis (Fig. 3).

Glucose production in the hyperglycemic group. Similar to the euglycemic group, in the hyperglycemic group glucose production decreased over time by 16 ± 3 mg $\cdot$ m^{-2} $\cdot$ min^{-1} with saline infusion (Table 4 and Fig. 2). Rates of gluconeogenesis remained unchanged, while rates of glycogenolysis decreased by 17 ± 2 mg $\cdot$ m^{-2} $\cdot$ min^{-1} and therefore, similar to the euglycemic group, accounted for the time-dependent decline in glucose production.

In contrast to the euglycemic group, lipid infusion in the hyperglycemic group did not affect the decline in glucose production over the 5-h study period, since the decline was similar in the saline and lipid experiments. Rates of gluconeogenesis remained unaltered. Rates of glycogenol-
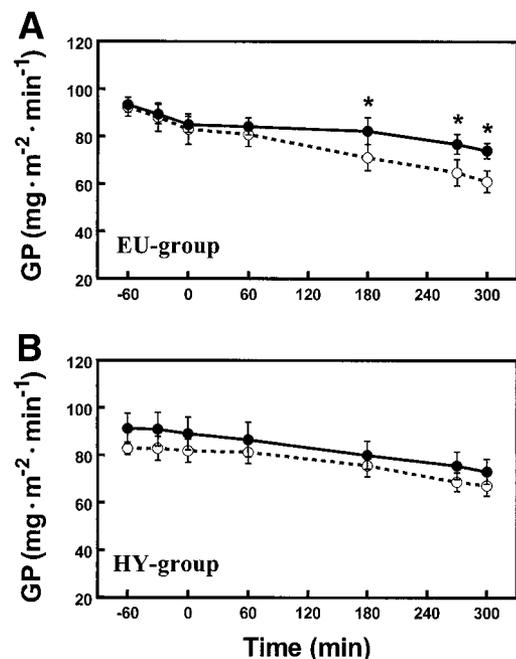

FIG. 2. Time course of glucose production (GP) during saline (----) and lipid (—) infusions in the euglycemic (EU) group (*A*) and hyperglycemic (HY) group (*B*). Mean $\pm$ SE. *$P < 0.05$ saline vs. lipid.
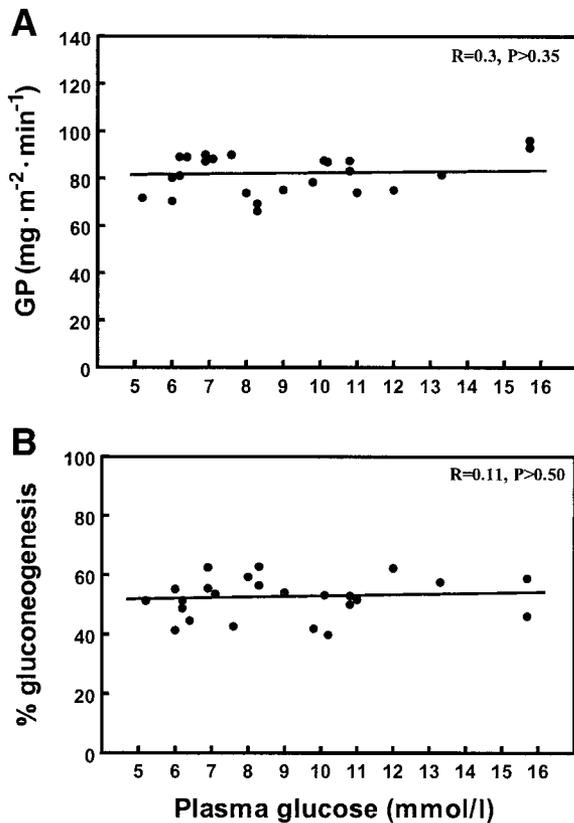
FIG. 3. Relationship between fasting plasma glucose levels and glucose production (*A*) and % gluconeogenesis (*B*). Since no correlations between these parameters were found in the saline and lipid studies in either the euglycemic or the hyperglycemic group, the data have been combined.

ysis decreased by $18 \pm 3 \text{ mg} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$, not significantly different from the reduction during saline infusion.

Similar to the euglycemic group, there were no relationships between baseline plasma glucose levels and rates of glucose production or the relative contribution of gluconeogenesis in the saline or the lipid study (Fig. 3).

Glucose disposal and glucosuria. There was a decline in glucose disposal during the saline infusion of $14 \pm 4 \text{ mg} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$ in the euglycemic group and $10 \pm 2 \text{ mg} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$ in the hyperglycemic group (both $P < 0.05$ vs. baseline). Since plasma glucose levels also declined, glucose MCR remained constant in both groups, from 69 ± 5 to $71 \pm 3 \text{ ml} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$ in the euglycemic group and

from 39 ± 2 to $42 \pm 3 \text{ ml} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$ in the hyperglycemic group.

During lipid infusion in both the euglycemic and hyperglycemic groups, glucose disposal declined by $10 \pm 2 \text{ mg} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$ (both $P < 0.01$ vs. baseline) and was not significantly different from the decline on saline infusion. However, glucose MCR decreased during lipid infusions in both the euglycemic group (71 ± 4 to $57 \pm 5 \text{ ml} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$) ($P < 0.02$) and hyperglycemic group (46 ± 2 to $40 \pm 1 \text{ ml} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$) ($P < 0.05$) (data not shown).

Urinary glucose excretion was only detected in one subject. He was in the hyperglycemic group and had the highest plasma glucose level of any subject. His glucose excretion was $12 \text{ mg} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$ during saline and $20 \text{ mg} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$ during lipid infusion.

DISCUSSION

We assessed the insulin-independent effects of a physiological increase in plasma FFA levels on glucose production in type 1 diabetic patients, in whom acute changes in portal insulin levels and the administration of SRIF are obviated. Stimulation of glucose production by FFA was confirmed in the absence of acute changes in insulin, as evidenced by an attenuation of a time-dependent decline in glucose production during lipid infusion in the euglycemic group. The increase in the rate of gluconeogenesis by FFA is in line with previous in vitro (2–4) and in vivo (13,14) observations. However, contrasting with previous studies in which FFA was found to have no effect (13) or inhibit (14) glycogenolysis, a stimulating effect of FFA on rates of glycogenolysis was evidenced by an attenuation of a time-dependent decline in glycogenolysis, in response to lipid infusion.

Few studies have addressed these effects of FFA in humans (9,10,13). Ferrannini et al. (9) found a marked stimulation of glucose production by FFA. However, insulin was not replaced during SRIF administration presumably rendering the liver particularly sensitive to stimulating factors. Boden et al. (10) studied healthy subjects in whom insulin, as well as glucagon, was replaced, and plasma glucose levels were allowed to change freely. Elevated FFA levels increased both glucose production rates and plasma glucose levels. Roden et al. (13) used the same design as Boden et al., in combination with the ^2H_2O method to assess individual contributions of gluconeogenesis and glycogenolysis. A small increase in

TABLE 4
Rates of glucose production (GP), gluconeogenesis (GNG), and glycogenolysis (GGL) in the HY group, at baseline and after 300 min of either saline or lipid infusion

HY group	Baseline ($\text{mg} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$)	300 min ($\text{mg} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$)	Difference ($\text{mg} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$)	P (basal vs. 300 min.)
GP				
Saline	84 ± 5	68 ± 4	16 ± 3	<0.05
Lipid	90 ± 7	74 ± 6	15 ± 4	<0.05
GNG				
Saline	48 ± 3	49 ± 3	1 ± 1	NS
Lipid	43 ± 4	45 ± 3	2 ± 2	NS
GGL				
Saline	36 ± 3	19 ± 2	-17 ± 2	<0.01
Lipid	47 ± 5	29 ± 5	-18 ± 3	<0.01

Data are mean $\pm$ SE. HY, hyperglycemic.

gluconeogenesis in response to elevated FFA levels was detected, but glycogenolysis and glucose production rates were unchanged. Nevertheless, marked hyperglycemia developed during lipidinfusion. We observed a stimulating effect of FFA on both glucose production, glycogenolysis and gluconeogenesis in the presence of a small increase in plasma glucose levels.

Importantly, changes in glucose production may not be independent of changes in glycemia in response to elevated FFA levels. That is because glucose production is sensitive to the inhibitory effect of glucose per se (14,22). In particular, glycogenolysis is very sensitive to changes in glucose levels, while the effect on gluconeogenesis is more controversial (14). Furthermore, glucose levels can increase during lipid infusion, not only through a stimulation of glucose production, but also by an inhibition of peripheral glucose uptake (1). Thus, elevated FFA levels have been shown to inhibit glucose oxidation and glycogen synthesis either directly (1,23) or through inhibition of skeletal muscle glucose transport and phosphorylation (24). In accord with that, glucose clearance has been reduced in response to elevation of FFA levels in studies in humans (9,10,13). Consequently, peripheral effects of FFA on plasma glucose levels may modify the response of the liver to elevated FFA levels.

Roden et al. (13) observed a marked increase in plasma glucose levels despite no change in glucose production rates during lipid infusion. FFA levels were initially elevated from ~0.5 to ~0.8 mmol/l. However, plasma glucose levels decreased. Therefore, glucose production presumably declined. FFA levels were then further elevated to ~2–3 mmol/l, and in response, plasma glucose levels increased by ~3 mmol/l. The hyperglycemia may have prevented an increase in glycogenolysis and glucose production while gluconeogenesis, being less sensitive to the inhibitory effects of glucose, increased slightly. Boden et al. (10) also initially elevated FFA levels to ~0.8 mmol/l. In contrast to the study of Roden et al. (13), glucose production increased (individual contributions of gluconeogenesis and glycogenolysis were not determined) and plasma glucose levels increased by ~1 mmol/l. FFA levels were then elevated to ~2 mmol/l, and plasma glucose levels increased by an additional ~1.7 mmol/l. However, glucose production rates remained virtually unchanged in response to the high FFA levels. Presumably, the high FFA levels primarily increased plasma glucose levels by interference with peripheral glucose utilization, while a further increase in glucose production was prevented by hyperglycemia. We observed a stimulation of glucose production and an increase in plasma glucose levels of ~1 mmol/l in response to a similar elevation of FFA levels from ~0.4 to ~0.8 mmol/l, in accord with the results of Boden et al. (10). However, these observations are extended by the demonstration that a stimulatory effect of FFA on both gluconeogenesis and glycogenolysis occurs. Taken together, an increase in plasma FFA levels appears able to stimulate both gluconeogenesis and glycogenolysis, and thereby glucose production. The likelihood of detecting this effect, however, may depend on the magnitude of changes in plasma glucose levels which, in turn, depend on the rate of lipid infusion.

Interestingly, the view that FFAs stimulate glycogenol-

ysis, is supported by a recent study by Boden et al. (25). Healthy subjects underwent a control and a lipid infusion study, using the euglycemic-hyperinsulinemic clamp technique. It was demonstrated that elevated FFA levels impair insulin-mediated suppression of glucose production, through a stimulatory effect on glycogenolysis in the face of unaltered gluconeogenesis. FFA may therefore play a more important role in the regulation of glycogenolysis than previously assumed.

Lipid infusion also affected glucose metabolism in the hyperglycemic group. Thus, although plasma glucose levels were not increased by elevated FFA levels, the time-dependent decline was prevented (Fig. 1). However, in contrast to the findings in the euglycemic group, there was no significant effect on rates of glucose production, gluconeogenesis, or glycogenolysis. We anticipated that, due to underreplacement of insulin, glucose production would be more sensitive to elevated FFA levels in the hyperglycemic than in the euglycemic group. Presumably, the lack of effect of FFA in the hyperglycemic group was due to the suppressive action of hyperglycemia on glycogenolysis and gluconeogenesis (14,22). As in the euglycemic group, glucose clearance was lower during lipid infusion. The effect, however, was subtle. Interestingly, a similar response to elevated FFA levels as in the present study was observed by Rigalleau et al. (26) in hyperglycemic type 2 diabetic patients. SRIF was not infused, but insulin levels did not change, presumably because insulin secretion was already markedly stimulated by hyperglycemia and because of the inherent defects in insulin secretion in these patients. Similar to our study, lipid infusion prevented a time-dependent decline in glucose concentrations in the patients with fasting plasma glucose of ~11 mmol/l in the presence of unaltered glucose production and disposal rates, but lower glucose clearance. Of note, hyperglycemia per se has been shown to attenuate the inhibitory effect of elevated FFA levels on glucose oxidation (27). Whether hyperglycemia also interferes with an effect of FFA on glucose transport and/or phosphorylation has, to our knowledge, not been determined. Such a mechanism could explain the more subtle effect of increased FFA levels on glucose clearance and plasma glucose levels in the presence of hyperglycemia.

The present data may bear on the importance of FFA in the pathophysiology of type 2 diabetes. Fasting FFA levels, as well as glucose production rates, are frequently elevated in type 2 diabetic patients, although the latter is debated (28). Elevated FFA levels in these patients are therefore assumed to increase gluconeogenesis, leading to increased glucose production. We raised FFAs to a level frequently encountered in type 2 diabetic patients (29). There was a small effect on glucose production in the complete absence of changes in insulin levels. In type 2 diabetic patients, fasting glucose and insulin levels are usually elevated. The small increase in glycogenolysis in response to elevated FFA levels observed in the present study would likely be obscured in type 2 diabetic patients. Since gluconeogenesis is less sensitive to the suppressive effects of insulin and glucose, increased FFA levels may contribute to the slightly increased rates of gluconeogenesis in these patients (30). It seems less likely from the present data that the often marked fasting hyperglycemia

seen in many of the patients is attributable to elevated FFA levels. Nonetheless, reducing FFA levels by Acipimox for several days effectively reduces plasma glucose levels in type 2 diabetic patients (31). This effect, however, is more likely due to the role of FFA in determining insulin action in the liver as well as the periphery during hyperinsulinemia (1,6) and hence in the postprandial state.

With regard to the pathophysiology of type 1 diabetes, previous studies indicate that fasting glucose production is increased in hyperglycemic type 1 diabetic patients (32,33). However, in our subjects (with neglible urinary glucose excretion) neither glucose production rates nor gluconeogenesis were related to plasma glucose levels across a wide range of glucose values (Fig. 3). This apparent discrepancy may be explained, at least in part, by the fact that in many previous studies, glucose production was quantified using a fixed tracer priming dose. The priming dose must be increased in proportion to the level of fasting hyperglycemia, or rates of glucose production may be overestimated (19,20). The contribution of gluconeogenesis to glucose production in the overnight fasted state of ~50–55%, is similar to the 48–55% observed in lean nondiabetic subjects using the ^2H_2O method (17) or the nuclear magnetic resonance-technique (34). In healthy subjects, absolute rates of gluconeogenesis are similar after 14 and 22 h of fasting, while rates of glycogenolysis (and therefore glucose production) decrease over time (18). Similar quantitative changes in short-term fasting were observed on saline infusion in our type 1 diabetic subjects, suggesting that a normal response of glucose production to fasting is not dependent on an intact β-cell function, but rather may be determined by intrahepatic factors.

In conclusion, in the absence of a compensatory rise in insulin secretion, a physiological elevation of plasma FFA levels stimulates glycogenolysis as well as gluconeogenesis and causes mild fasting hyperglycemia. However, these effects appear attenuated in the presence of hyperglycemia.

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