Effects of Free Fatty Acids and Glycerol on Splanchnic Glucose Metabolism and Insulin Extraction in Nondiabetic Humans

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The present study sought to determine whether elevated plasma free fatty acids (FFAs) alter the ability of insulin and glucose to regulate splanchnic as well as muscle glucose metabolism. To do so, FFAs were increased in 10 subjects to ~1 mmol/l by an 8-h Intralipid/heparin (IL/Hep) infusion, whereas they fell to levels near the detection limit of the assay (<0.05 mmol/l) in 13 other subjects who were infused with glycerol alone at rates sufficient to either match (n = 5, low glycerol) or double (n = 8, high glycerol) the plasma glycerol concentrations observed during the IL/Hep infusion. Glucose was clamped at ~8.3 mmol/l, and insulin was increased to ~300 pmol/l to stimulate both muscle and hepatic glucose uptake. Insulin secretion was inhibited with somatostatin. Leg and splanchnic glucose metabolism were assessed using a combined catheter and tracer dilution approach. Leg glucose uptake (21.7 ± 3.5 vs. 48.3 ± 9.3 and 57.8 ± 11.7 $\mu mol \cdot kg^{-1}$ leg $\cdot min^{-1}$) was lower (P < 0.001) during IL/Hep than the low- or high-glycerol infusions, confirming that elevated FFAs caused insulin resistance in muscle. IL/Hep did not alter splanchnic glucose uptake or the contribution of the extracellular direct pathway to UDP-glucose flux. On the other hand, total UDP-glucose flux (13.2 ± 1.7 and 12.5 ± 1.0 vs. 8.1 ± 0.5 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) and flux via the indirect intracellular pathway (8.4 ± 1.2 and 8.1 ± 0.6 vs. 4.8 ± 0.05 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) were greater ($P$ < 0.05) during both the IL/Hep and high-glycerol infusions than the low-glycerol infusion. In contrast, only IL/Hep increased (P < 0.05) splanchnic glucose production, indicating that elevated FFAs impaired the ability of the liver to autoregulate. Splanchnic insulin extraction, directly measured using the arterial and hepatic vein catheters, did not differ (67 ± 3 vs. 71 ± 5 vs. 69 ± 1%) during IL/Hep and high- and low-glycerol infusions. We conclude that elevated FFAs exert multiple effects on glucose metabolism. They inhibit insulin- and glucose-induced stimulation of muscle glucose uptake and suppression of splanchnic glucose production. They increase the contribution of the indirect pathway to glycogen synthesis and impair hepatic autoregulation. On the other hand, they do not alter either splanchnic glucose uptake or splanchnic insulin extraction in nondiabetic humans. *Diabetes* 51:301–310, 2002

Insulin-induced stimulation of splanchnic glucose uptake and suppression of hepatic (and perhaps renal) glucose production are impaired in people with type 2 diabetes (1–5). The contribution of abnormal fat metabolism to these defects is an area of active investigation. People with type 2 diabetes commonly have elevated FFA and glycerol concentrations (1,5,6). Although it is well established that FFAs can blunt the response of muscle to insulin (7–19), elevated FFAs have been reported to increase (20), decrease (21), or have no effect (22,23) on initial splanchnic glucose extraction. The lack of concordance between these studies may be due in part to the fact that none of these studies directly measured splanchnic glucose uptake. All measured the proportion of ingested glucose that reached the systemic circulation, which is a function of both the rapidity and completeness of intestinal glucose absorption as well as the rate of initial (i.e., first pass) splanchnic glucose extraction. In addition, glycerol as well as FFA concentrations differed between the control and experimental groups, further complicating interpretation of the data.

Because the regulation of glucose uptake differs substantially in muscle and liver, it would not be surprising if the effects of FFAs on glucose metabolism also differed in these two tissues. In muscle, glucose is transported by GLUT4 (24,25), phosphorylated by hexokinase (26), and incorporated into glycogen by muscle glycogen synthase (27,28). All of these steps are stimulated by insulin and inhibited by FFAs (14,29,30). In contrast, in liver glucose is transported by GLUT2 (24,25), phosphorylated by glucokinase (31), and incorporated into glycogen by hepatic glycogen synthase (28,32). Transport via GLUT2 is not rate-limiting (33), and glucokinase activity is primarily regulated by glucose rather than insulin (34). Studies in animals have shown that high levels of FFAs can decrease glucokinase (35) and increase glucose-6-phosphatase activity (36). However, it is not known whether elevated FFAs exert the same effects in humans and, if so, whether this results in a decrease in hepatic glucose uptake and/or hepatic glycogen synthesis. If this is indeed the case, then elevated FFAs may inhibit both hepatic as well as muscle glucose uptake and hepatic as well as muscle glycogen synthesis.

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Received for publication 4 June 2001 and accepted in revised form 7 November 2001.

FFA, free fatty acids; IL/Hep, Intralipid/heparin.

TABLE 1
Subject characteristics

Group	Number and sex distribution (F/M)	Age (years)	BMI (kg/m^2)	Percent fat
Low glycerol	5 (4/1)	32 ± 5	24.5 ± 2.4	38.5 ± 4.0
IL/Hep	10 (7/3)	34 ± 4	27.0 ± 1.7	37.6 ± 3.1
High glycerol	8 (5/3)	36 ± 4	26.3 ± 1.4	37.7 ± 6.3

Data are n or means ± SD.

FFAs can potentially influence hepatic glucose metabolism by several other mechanisms. The ability of FFAs to stimulate gluconeogenesis is well established (37–39). However, the effects of FFAs on hepatic glucose release are less certain. Most (10,15,16,22,40), but not all (39), studies indicate that elevated FFAs impair insulin-induced suppression of endogenous glucose production. Although the liver is generally assumed to be the source of the increased glucose release, this has yet to be established in humans because only the effects of FFAs on total-body (commonly referred to as endogenous) glucose production has been measured. Furthermore, although other substrates (e.g., glycerol) also increase gluconeogenesis, they do not increase glucose production because of so-called hepatic autoregulation (41–43). If elevated FFAs increase splanchnic glucose production, this implies that FFAs not only increase gluconeogenesis but also cause a greater proportion of the resultant glucose-6-phosphate to be dephosphorlyated and released into the systemic circulation. It has also been suggested that elevated FFAs may cause or exacerbate systemic hyperinsulinemia by decreasing hepatic insulin degradation and, by inference, hepatic insulin extraction (44–47). However, to date, the effects of FFAs on hepatic insulin clearance has not been directly measured in humans.

The current experiments were undertaken to answer these questions. We report that elevated FFAs impair insulin-induced stimulation of muscle but not splanchnic glucose uptake. We show that although UDP-glucose flux and the contribution of the indirect pathway to UDP-glucose flux are stimulated by an increase in either plasma FFA or glycerol concentrations, only FFAs increase splanchnic glucose production. Because FFAs did not alter the contribution of extracellular glucose to hepatic glycogen synthesis (and by implication, hepatic glucokinase activity), these data argue strongly that FFAs impair hepatic autoregulation by directly stimulating glucose-6-phosphatase activity. On the other hand, in contrast to prior predictions from in vitro and animal studies, elevated FFAs had no effect on splanchnic insulin clearance in nondiabetic humans.

RESEARCH DESIGN AND METHODS

After approval from the Mayo Institutional Review Board, 23 nondiabetic subjects gave written consent to participate in the study. All subjects were in good health and were at a stable weight. None of the first-degree relatives of the volunteers had a history of diabetes. None of the volunteers regularly engaged in vigorous exercise. Subjects were on no medications other than oral contraceptive pills, estrogen, or thyroxin replacement. All subjects were instructed to follow a weight maintenance diet containing ~55% carbohydrate, 30% fat, and 15% protein for at least 3 days before the study. Subject characteristics are given in Table 1. Age, BMI, percent body fat, sex as well as fasting plasma glucose concentrations (see below) did not differ among the three groups.

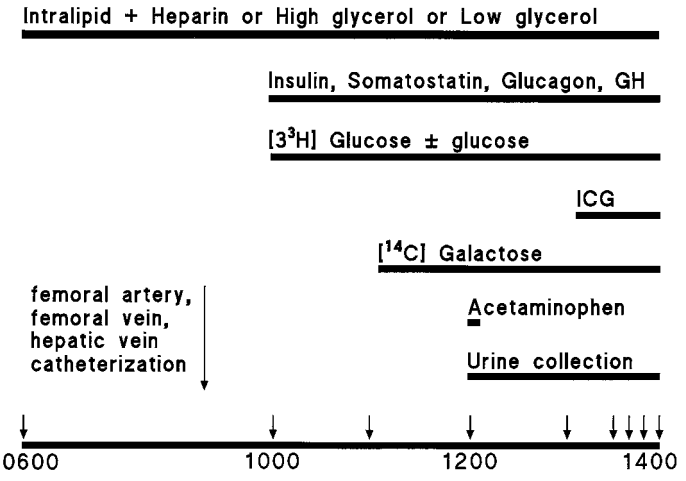

FIG. 1. Experimental design. For additional details see the text. GH, human growth hormone; ICG, indocyanine green.

Experimental design. Subjects were admitted to the Mayo Clinic General Clinical Research Center at ~1700 on the evening before each study. Subjects ingested a standard 10-kcal/kg meal (55% carbohydrate, 30% fat, and 15% protein) between 1730 and 1800. They then fasted (with the exception of an occasional sip of water) until the following morning. An outline of the experimental design is shown in Fig. 1. At 0600 on the morning of study, an 18-gauge catheter was inserted into the left forearm vein. A catheter was inserted into the bladder in 20 of the 23 volunteers. Three subjects elected not to have a bladder catheter placed; all were able to void upon request at appropriate times. An infusion of Intralipid (20%, 0.013 ml · kg^{-1} · min^{-1}; Baxter Healthcare, Deerfield, IL) and heparin (200 units prime, 0.2 unit · kg^{-1} · min^{-1} continuous) was then started in 10 subjects to increase plasma FFA concentrations (Intralipid/heparin [IL/Hep] group). Five subjects were infused with glycerol at a rate of 5 μmol · kg^{-1} · min^{-1} to match the amount of free glycerol added as an emulsifier in the Intralipid infusate (low-glycerol group). Eight subjects were infused with glycerol at a rate of 20 mol · kg^{-1} · min^{-1} to match the total amount of glycerol (i.e., free and that present in the triglyceride) contained in the Intralipid infusion (high-glycerol group).

Volunteers were moved to an intervention radiology suite at ~0800, where femoral arterial, femoral venous and hepatic venous catheters were placed as previously described (1,2). Subjects were then returned to the General Clinical Research Center for the remainder of the study. At 1000 (time 0), infusions of [3-^{3}H]glucose (12 μCi prime, 0.12 μCi/min continuous; New England Nuclear, Boston, MA), insulin (in 1.25% albumin, 1.0 mU · kg^{-1} · min^{-1}), somatostatin (72 ng · kg^{-1} · min^{-1}; Bachem California, Torrance, CA), glucagon (0.65 ng · kg^{-1} · min^{-1}; Eli Lilly, Indianapolis, IN), and growth hormone (3.0 ng · kg^{-1} · min^{-1}; Genentech, South San Francisco, CA) were started and continued until study end at 1400. A glucose infusion was also begun at 1000 and the rate adjusted to maintain plasma glucose concentrations ~8.3 mmol/l over the next 4 h. To minimize the change in plasma glucose specific activity, all infused glucose contained [3-^{3}H]glucose. In addition, the "basal" [3-^{3}H]glucose infusion was reduced in a pattern (50% from 0 to 30 min, 43% from 31 to 60 min, 31% from 61 to 90 min, 23% from 91 to 120 min, 19% from 121 to 150 min, 15% from 151 to 180 min, and 12% from 211 to 240 min) designed to mimic the anticipated pattern of change in glucose production (1,2).

An infusion of [1-^{14}C]galactose (15 μCi prime, 0.15 μCi/min continuous; New England Nuclear, Boston, MA) was initiated at 1100 and indocyanine green (0.25 mg/min into the femoral artery) at 1300. Subjects ingested 2 g of acetaminophen (2 g/20 ml acetaminophen pediatric suspension) at 1200. As part of a separate experiment, [U ^{13}C]linoleate (0.3 μmol/min), [^{13}C ^{15}N]leucine (1 mg/kg prime, 1 mg · kg^{-1} · h^{-1}), [^{15}N]phenylalanine (0.75 mg/kg prime, 0.75 mg · kg^{-1} · h^{-1}), [^{2}H$_4$]tyrosine (0.6 mg/kg prime, 0.6 mg · kg^{-1} · h^{-1}), and [^{15}N]tyrosine (0.3 mg/kg prime) were also infused from 1000 to 1400 to assess the effects of elevated FFAs on fatty acid and amino acid metabolism. Blood was collected at 0600, 1000, 1100, 1200, 1300, 1330, 1340, 1350, and 1400 and urine at 1000, 1200, and 1400. Samples for FFAs were placed in tubes containing 50 μl Paraoxon (diethyl-p-nitrophenyl-phosphate) (Sigma Chemicals, St. Louis, MO) diluted to 0.04% in diethyl ether to prevent ex vivo lipolysis (48). All blood samples were collected in prechilled syringes and dispensed into prechilled tubes. Samples were centrifuged immediately at 4°C, and the plasma obtained from these tubes was stored at −20°C until analysis.

Analytical techniques. Plasma glucose during the clamp was measured every 10 min using a Beckman Glucose Analyzer II (Beckman, Chaska, MN). Plasma C-peptide, insulin, and glucagon concentrations were measured by radioimmunoassay using reagents purchased from Linco Research (St. Louis, MO). Plasma growth hormone concentrations were measured using a double-antibody chemiluminescence method with the Access immunoassay system (Beckman). Plasma [^{3}H]glucose specific activity was measured using a liquid scintillation counter. Plasma glucose concentrations were measured using a Yellow Springs glucose and lactate analyzer (Yellow Springs Instruments, Yellow Springs, OH). Urinary [^{3}H] and [^{14}C]glucuronide specific activities were measured using high-performance liquid chromatography as previously described (49). Plasma indocyanine green concentration was determined by high-performance liquid chromatography using the method of Awni and Bakker (50).

Plasma glycerol and FFA concentrations were measured by a microfluorometric enzymatic method (51). Body composition (including fat-free mass and total fat mass) was measured by dual-energy X-ray absorptiometry (DPX-IQ scanner; Hologic, Waltham, MA) using SmartScan version 4.6 (52).

Calculations. Total-body glucose appearance and disappearance were calculated using the non–steady-state equations of Steele et al. (53) and [3-^{3}H]glucose as the tracer. Endogenous glucose production was determined by subtracting the glucose infusion rate from the tracer-determined rate of glucose appearance.

Splanchnic plasma flow was calculated by dividing the indocyanine green infusion rate by the arterial-to-hepatic venous concentration gradient. Likewise, leg plasma flow was calculated by dividing the indocyanine green infusion rate by the femoral artery–to–femoral venous concentration gradient. The corresponding blood flows were derived by dividing the respective plasma flows by (1 − hematocrit). Blood glucose concentrations were calculated by multiplying the plasma glucose concentrations by 0.85.

Splanchnic glucose extraction ratio (SER) was calculated as:

$$\text{SER} = \frac{\text{FA [3-}^3\text{H]glucose} - \text{HV [3-}^3\text{H]glucose}}{\text{FA [3-}^3\text{H]glucose}}$$

FA [3-^{3}H]glucose and HV [3-^{3}H]glucose are the concentrations of [3-^{3}H]glucose in the femoral arterial and hepatic venous plasma, respectively.

Splanchnic glucose uptake (SGU) was calculated as:

$$\text{SGU} = \text{SER} \times (\text{FA glucose} \times \text{SBF})$$

where FA glucose is the glucose concentrations in the femoral artery, and SBF is the median of quadruple determinations of splanchnic blood flow.

Splanchnic glucose production (SGP) was calculated as:

$$\text{SGP} = (\text{FA glucose} - \text{HV glucose}) \times \text{SBF} - \text{SGU}$$

where HV glucose is the glucose concentrations in the hepatic vein. Although SGP is technically a negative number, for clarity of presentation SGP is presented in the figures and text as a positive value.

Flux through UDP-glucose pool was calculated as:

$$\text{UDP glucose flux} = \left[\frac{F_{GAL}}{\text{SA of UDP} - [^{14}\text{C]glucose}}\right]$$

where F_{GAL} is the infusion rate of [^{14}C]galactose, and SA of UDP-[^{14}C]glucose is the specific activity of acetaminophen [^{14}C]glucuronide in urine (49,54).

The fractional contribution of plasma glucose to UDP glucose flux (F_{Direct}) was calculated as:

$$F_{Direct} = \left[\frac{\text{SA of urinary UDP [}^3\text{H]glucose}}{\text{SA of plasma [}^3\text{H]glucose}}\right]$$

where SA of urinary UDP [^{3}H]glucose is the specific activity of acetaminophen [^{3}H]glucuronide in urine, and SA of plasma [^{3}H]glucose is the specific activity of [^{3}H]glucose in plasma (49,54).

The fractional contribution of the indirect pathway to UDP-glucose flux ($F_{Indirect}$) was calculated as:

$$F_{Indirect} = 1 - F_{Direct}$$

Flux through the direct and indirect pathways (in $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) was calculated by multiplying F_{Direct} and $F_{Indirect}$, respectively, by UDP glucose flux.

Leg glucose extraction ratio was calculated as:

$$\text{LER} = \frac{\text{FA [3-}^3\text{H]glucose} - \text{FV [3-}^3\text{H]glucose}}{\text{FA [3-}^3\text{H]glucose}}$$

where FA [3-^{3}H]glucose and FV [3-^{3}H]glucose are the concentrations of [3-^{3}H]glucose in femoral arterial and femoral venous plasma, respectively.

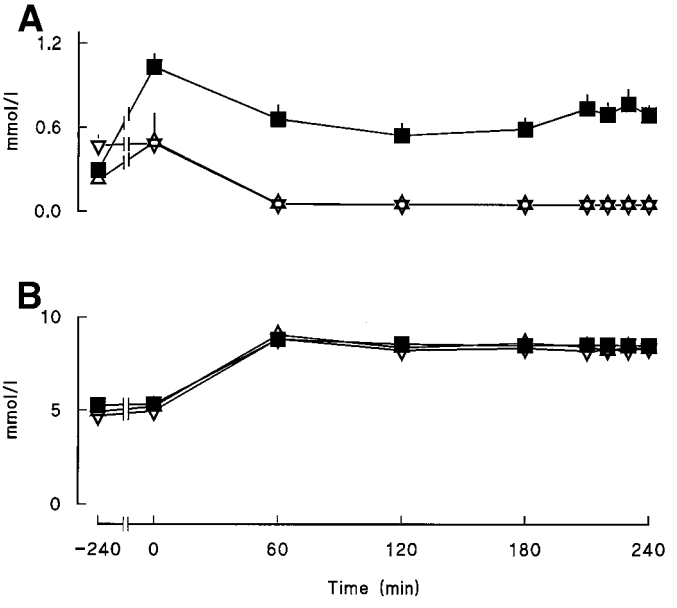

FIG. 2. FFA (*A*) and glucose (*B*) concentrations during IL/Hep and low- and high-glycerol infusions. The IL/Hep and glycerol infusions were started at −240 min, and exogenous glucose, insulin, and somatostatin infusions were started at time 0. ▽, Low glycerol; ■, IL/Hep; △, high glycerol.

Leg glucose uptake (LGU) was calculated as:

$$\text{LGU} = (\text{FA glucose} - \text{FV glucose}) \times \text{leg blood flow}$$

where LBF equals the median of quadruple determinations of leg blood flow.

Statistical analysis. Data in the figures and text are expressed as mean ± SE. Rates are expressed as micromoles per kilogram fat-free mass per minute, with the exception of leg uptake/balance, which is expressed as micromoles per kilogram leg fat-free mass per minute. Mean responses during the final 30 min of the study were determined and used for statistical analysis. ANOVA was used to determine whether the responses among groups differed. Student's nonpaired *t* test was used to test the hypothesis that splanchnic and leg glucose uptake were lower and splanchnic glucose production higher in the IL/Hep than the low- and high-glycerol groups. All other tests were two-tailed. A P value of <0.05 was considered statistically significant.

RESULTS

Plasma FFA, glucose, and glycerol concentrations. Plasma FFA concentrations did not differ before the IL/Hep and glycerol infusions among the three groups (Fig. 2*A*). IL/Hep infusion increased FFAs to 1.03 ± 0.10 mmol/l at time 0 and then decreased to 0.72 ± 0.09 mmol/l during the exogenous insulin infusion. FFAs increased slightly but not significantly during both the low- and high-glycerol infusions and then fell to almost undetectable levels during the insulin infusion in both the groups. This resulted in higher ($P < 0.02$) FFA concentrations in the IL/Hep than in the low- and high-glycerol groups both before (1.03 ± 0.10 vs. 0.49 ± 0.13 and 0.49 ± 0.05mmol/l) and during insulin infusion (0.72 ± 0.09 vs. 0.05 ± 0.00 and 0.05 ± 0.00 mmol/l).

Plasma glucose concentrations did not differ among the IL/Hep, low-glycerol, and high-glycerol groups (Fig. 2, lower panel) either before (5.3 ± 0.2 vs. 4.7 ± 0.1 vs. 4.9 ± 0.1 mmol/l) or during the IL/Hep and glycerol infusions (5.3 ± 0.2 vs. 5.0 ± 0.1 vs. 5.2 ± 0.1 mmol/l). They also did not differ during the hyperinsulinemic clamp (8.5 ± 0.2 vs. 8.3 ± 0.1 vs. 8.3 ± 0.1 mmol/l).

Plasma glycerol concentrations did not differ among the

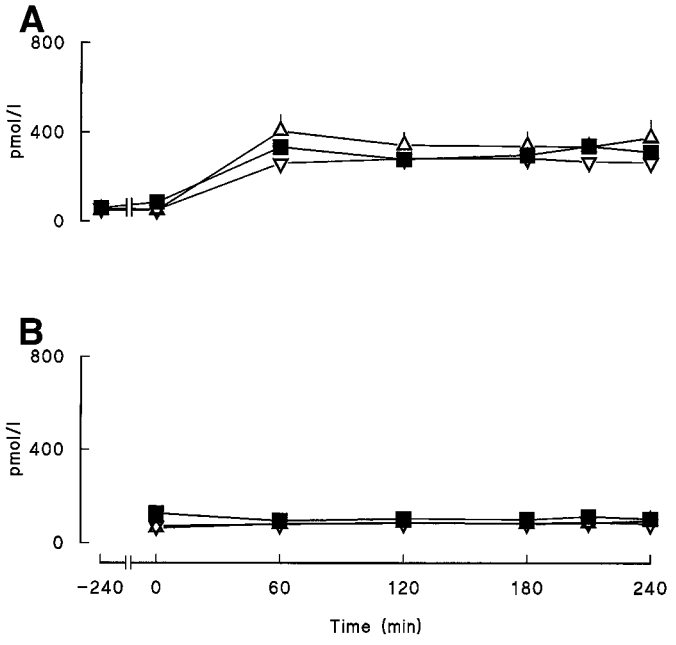

FIG. 3. Femoral artery (*A*) and hepatic venous (*B*) insulin concentrations were measured from time 0 onward during IL/Hep and low- and high-glycerol infusions. Insulin concentrations measured in forearm venous plasma at −240 min are provided as a reference. The IL/Hep and glycerol infusions were started at −240 min, and exogenous glucose, insulin, and somatostatin infusions were started at time 0. ▽, low glycerol; ■, IL/Hep; △, high glycerol.

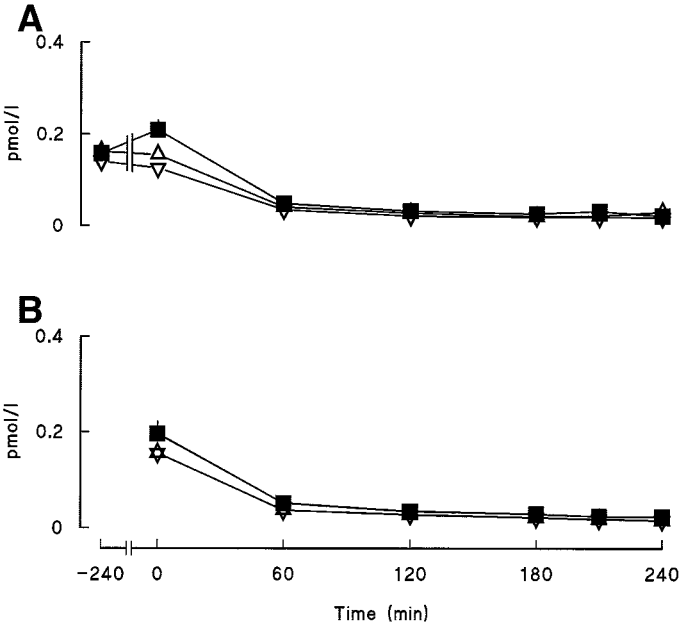

FIG. 4. Femoral artery (*A*) and hepatic venous (*B*) C-peptide concentration were measured from time 0 onward during IL/Hep and low- and high-glycerol infusions. C-peptide concentrations measured in forearm venous plasma at −240 min are provided as a reference. The IL/Hep and glycerol infusions were started at −240 min, and exogenous glucose, insulin, and somatostatin infusions were started at time 0. ▽, low glycerol; ■, IL/Hep; △, high glycerol.

three groups before the IL/Hep or the low- or high-glycerol infusions (155 ± 19 vs. 107 ± 17 vs. 121 ± 24 μmol/l). During the IL/Hep and glycerol infusions, plasma glycerol concentrations in the high-glycerol group were greater ($P < 0.05$) than those present in either the IL/Hep or low-glycerol groups both before (729 ± 95 vs. 373 ± 119 vs. 278 ± 24 μmol/l) and during (710 ± 91 vs. 313 ± 72 vs. 187 ± 30 μmol/l) the hyperinsulinemic clamp. On the other hand, plasma glycerol concentrations did not differ between the IL/Hep and low-glycerol groups at any time during the study.

Plasma insulin and C-peptide concentrations. Plasma insulin and C-peptide concentrations did not differ among the three groups before the IL/Hep and glycerol infusions. Arterial insulin and C-peptide concentrations increased ($P < 0.01$) during IL/Hep but did not change during either of the glycerol infusions (Figs. 3 and 4). The exogenous insulin and somatostatin infusions (begun at time 0) resulted in a prompt decrease in arterial (0.03 ± 0.00 vs. 0.02 ± 0.00 vs. 0.02 ± 0.01 pmol/l, respectively) and hepatic venous (0.02 ± 0.00 vs. 0.03 ± 0.01 vs. 0.02 ± 0.00 pmol/l, respectively) C-peptide concentrations to levels that approached the limit of detection in the IL/Hep and low- and high-glycerol groups.

Hepatic venous insulin concentrations did not change during the exogenous insulin and somatostatin infusions. Femoral artery and hepatic venous insulin concentrations did not differ among the three groups from 60 min onward. Femoral artery insulin concentrations averaged 338 ± 37 vs. 360 ± 63 vs. 272 ± 18 pmol/l, respectively, and hepatic venous insulin concentrations averaged 110 ± 12 vs. 90 ± 9 vs. 85 ± 6 pmol/l, respectively, during the final thirty minutes of the IL/Hep and low- and high-glycerol studies. Plasma glucagon and growth hormone concentrations also did not differ either before or during the IL/Hep and glycerol infusions (data not shown).

Splanchnic insulin extraction. Because the somatostatin infusion resulted in essentially complete suppression of insulin secretion and because arterial and hepatic venous insulin concentrations were at steady state for several hours, splanchnic insulin extraction could be accurately measured. Splanchnic insulin extraction (Fig. 5) did not differ over the final 30 min of the experiment among the IL/Hep and low- and high-glycerol groups (67 ± 3 vs. 69 ± 1 vs. 71 ± 5%).

Glucose infusion rate and [3-^{3}H]glucose specific activity. The glucose infusion rate required to maintain target plasma glucose concentrations (Fig. 6, upper panel)

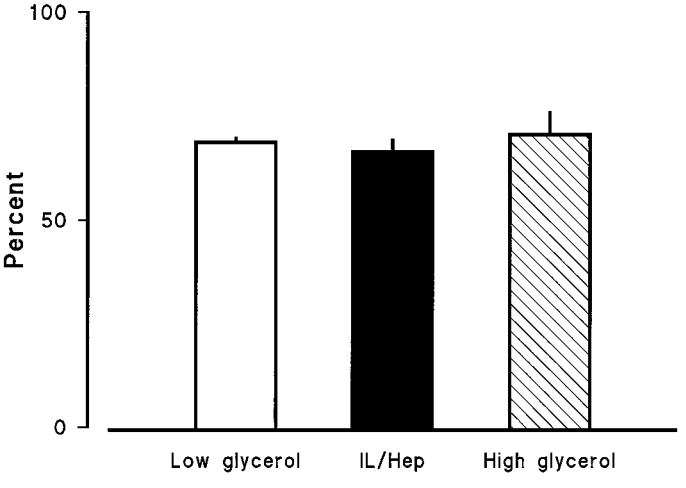

FIG. 5. Hepatic insulin extraction observed during the final 30 min of the IL/Hep and low- and high-glycerol infusions.

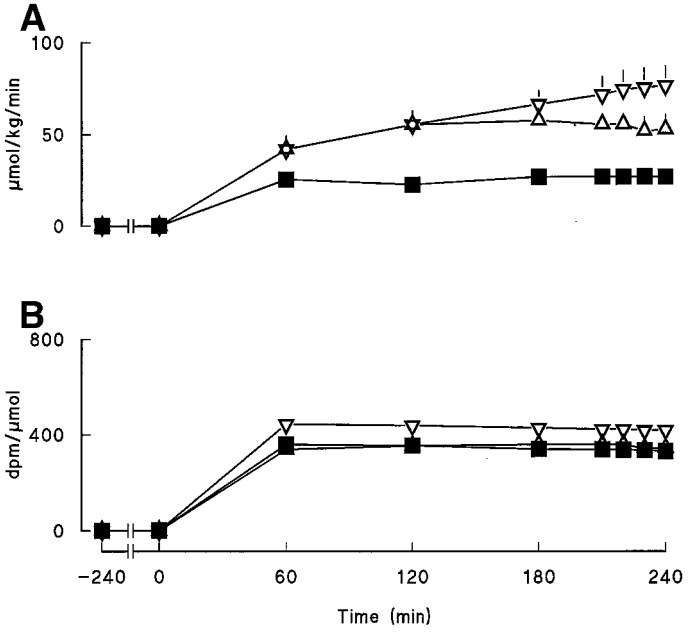

FIG. 6. Glucose infusion rate (*A*) required to maintain plasma glucose concentrations at target levels and plasma [3-^{3}H]glucose specific activity (*B*). The IL/Hep and low- and high-glycerol infusions were started at −240 min, and exogenous insulin and somatostatin infusions were started at time 0. ▽, low glycerol; ■, IL/Hep; △, high glycerol.

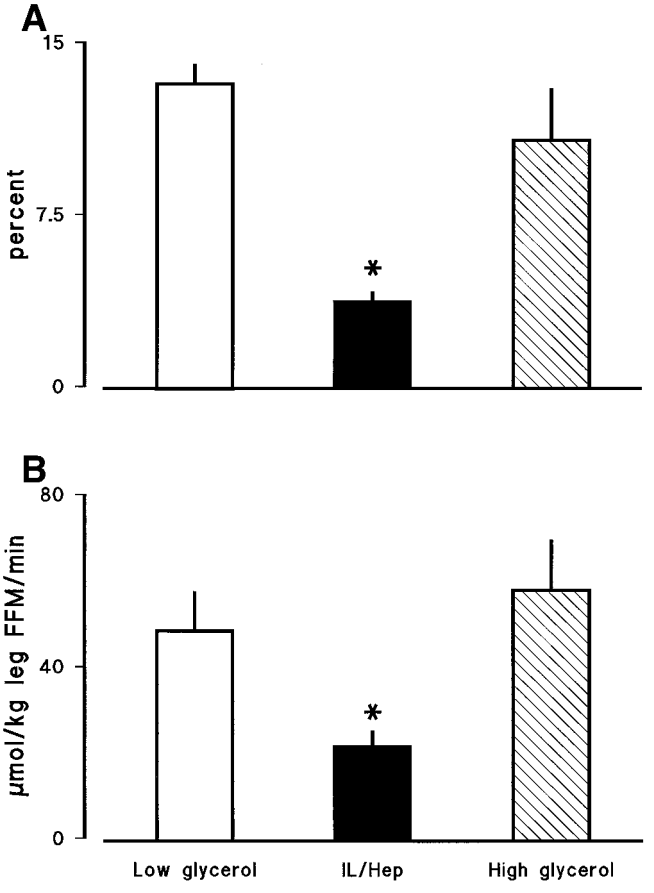

FIG. 7. Leg [3-^{3}H]glucose extraction (*A*) and leg glucose uptake (*B*) observed during the final 30 min of the IL/Hep and low- and high-glycerol infusions. **P* < 0.001 compared with low- and high-glycerol group. FFM, fat-free mass.

was markedly lower ($P < 0.0001$) from 120 min onward during the IL/Hep than either the low- or high-glycerol infusions (27.1 ± 2.9 vs. 75.2 ± 10.7 vs. 55.6 ± 5.1 μmol · kg^{-1} · min^{-1}). The glucose infusion rate did not differ significantly between low- and high-glycerol groups. Because all infused glucose contained [3-^{3}H]glucose, glucose specific activity remained constant from 60 min onward (coefficient of variation 3.5 ± 0.8%), permitting accurate measurement of glucose turnover in all groups (Fig. 6*B*).

Glucose appearance and disappearance. Both total glucose appearance (33.3 ± 2.8 vs. 80.5 ± 12.5 and 56.5 ± 5.1 μmol · kg^{-1} · min^{-1}) and glucose disappearance (33.3 ± 2.8 vs. 80.4 ± 12.5 and 56.6 ± 5.1 μmol · kg^{-1} · min^{-1}) were lower ($P < 0.001$) in the IL/Hep than in the low- or high-glycerol infusion groups. Endogenous glucose production did not differ between the three groups (6.1 ± 0.9 vs. 8.1 ± 4.0 vs. 6.2 ± 0.7 μmol · kg^{-1} · min^{-1}).

Leg glucose extraction and uptake. Leg glucose uptake, whether measured as tracer extraction (3.8 ± 0.4 vs. 13.2 ± 0.9 vs. 10.8 ± 2.3%) or leg glucose uptake (21.7 ± 3.5 vs. 48.3 ± 9.3 vs. 57.8 ± 11.7 μmol · kg^{-1} fat-free mass of the leg · min^{-1}) was less ($P < 0.001$) in the IL/Hep group than either the low- or high-glycerol infusion groups (Fig. 7). Neither leg tracer extraction nor leg glucose uptake differed between the low- and the high-glycerol groups.

Splanchnic glucose uptake and splanchnic glucose production. Splanchnic glucose uptake (10.3 ± 1.5 vs. 6.2 ± 1.4 vs. 8.6 ± 3.0 μmol · kg^{-1} · min^{-1}) did not differ among the IL/Hep and low- and high-glycerol groups (Fig. 8*A*). Splanchnic tracer extraction (4.7 ± 0.5 vs. 3.8 ± 0.6 vs. 3.9 ± 1.5%) also did not differ among the IL/Hep and low- and high-glycerol groups. Splanchnic glucose production in the IL/Hep group was higher ($P < 0.05$) than that present in either the high- or low-glycerol groups (7.6 ± 1.1 vs. 2.4 ± 1.3 vs. 4.0 ± 0.8 μmol · kg^{-1} · min^{-1}). Splanchnic glucose production did not differ between the low- and high-glycerol groups (Fig. 8*B*).

UDP-glucose flux. Flux through the UDP glucose pool (Fig. 9*A*) was higher ($P < 0.05$) in the IL/Hep and high-glycerol groups than the low-glycerol group (13.2 ± 1.7 vs. 12.5 ± 1.0 vs. 8.1 ± 0.5 μmol · kg^{-1} · min^{-1}) but did not differ between IL/Hep and high-glycerol groups. Flux through the direct pathway did not differ among the three groups (Fig. 9*B*). In contrast, flux through the indirect pathway was higher ($P < 0.05$) in both the IL/Hep and high-glycerol groups than in the low-glycerol group (8.4 ± 1.2 vs. 8.1 ± 0.6 vs. 4.8 ± 0.05 μmol · kg^{-1} · min^{-1}). On the other hand, flux through the indirect pathway did not differ between IL/Hep and high-glycerol groups (Fig. 9*C*).

DISCUSSION

The current experiments confirm that elevated FFAs impair the ability of insulin to stimulate leg (and therefore presumably muscle) glucose uptake. They demonstrate for the first time that elevated FFAs do not alter either splanchnic glucose uptake or the contribution of the extracellular (direct) pathway to hepatic UDP-glucose flux, implying no effect on hepatic glucokinase activity. On the other hand, although elevated FFAs and high glycerol comparably increase both total UDP-glucose flux and the contribution of the indirect (intracellular) pathway to UDP-glucose flux, only FFAs increase splanchnic glucose

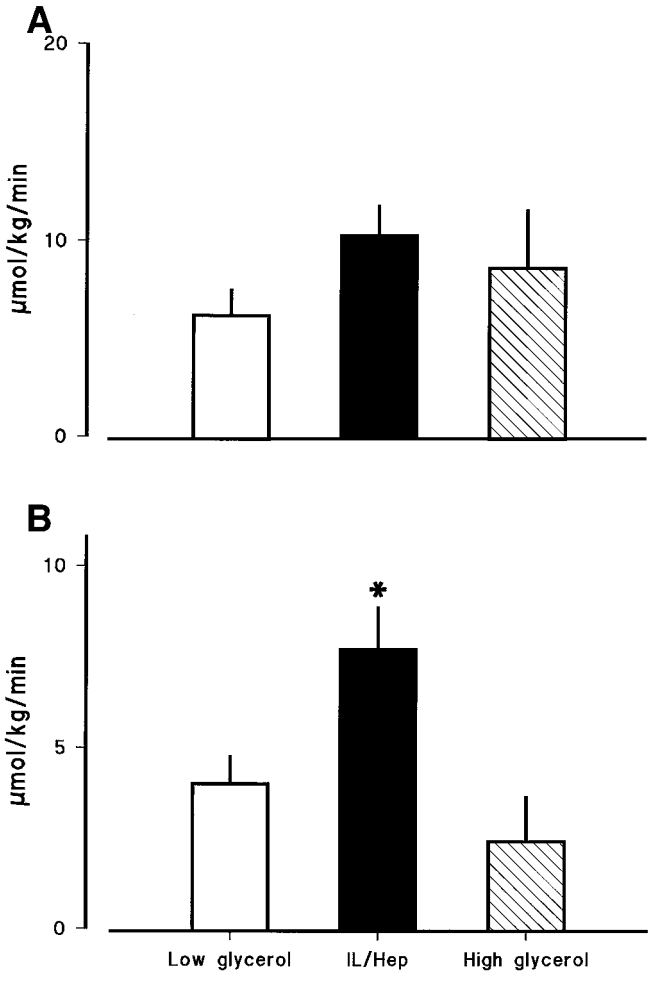

FIG. 8. Splanchnic [3-^{3}H]glucose uptake (*A*) and splanchnic glucose production (*B*) observed during the final 30 min of the IL/Hep and low- and high-glycerol infusions. *P < 0.01 compared with low- and high-glycerol group.

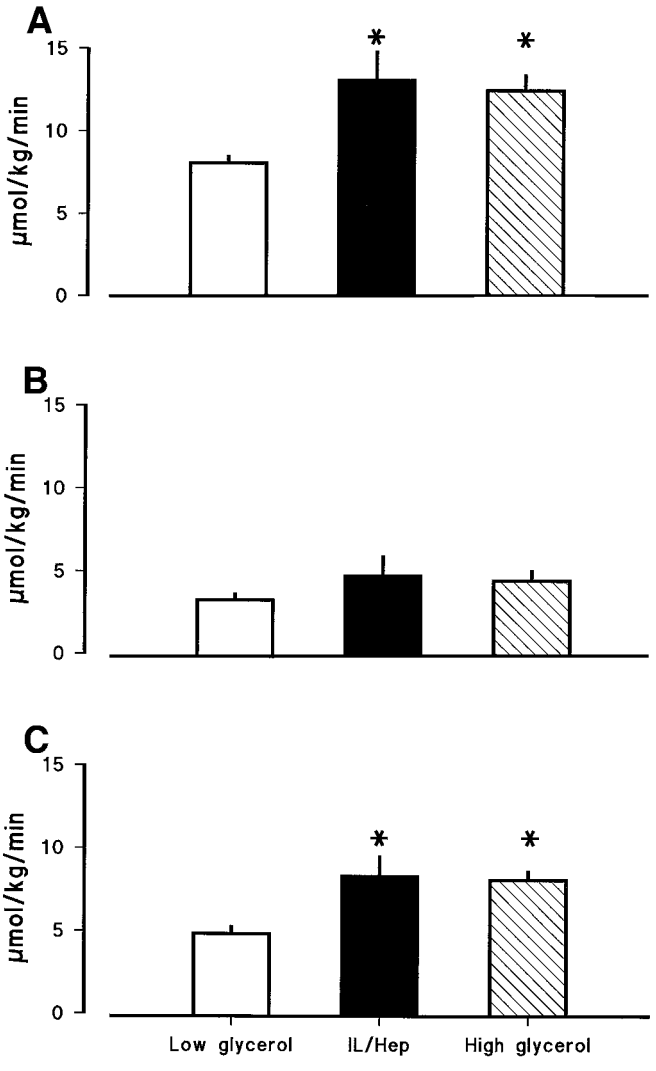

FIG. 9. Flux through UDP glucose pool (*A*) and contribution of the direct (*B*) and the indirect (*C*) pathways to glycogen synthesis observed during the final 30 min of the IL/Hep and low- and high-glycerol infusions.

production. Taken together, these data indicate that elevated FFAs impair normal hepatic glucose autoregulation by stimulating both the indirect pathway and hepatic glucose-6-phosphatase activity. Finally, elevated FFAs did not alter splanchnic insulin clearance, challenging the widely held belief that increased visceral FFA release can exacerbate systemic hyperinsulinemia by decreasing hepatic insulin extraction.

Effects of FFAs and glycerol on splanchnic glucose uptake. Plasma FFAs are commonly elevated in a variety of disease states, including type 2 diabetes and obesity (1,5,6,55). After the observation by Randle et al. (8) that increased FFAs decreased insulin-stimulated glucose uptake in perfused hearts, a number of studies have shown that they can also decrease either leg (and therefore presumably skeletal muscle) or whole-body glucose uptake in humans (7–17). The effect of FFAs on splanchnic glucose uptake has been less clear, likely due in large part to differences in the methods used in the previous studies. Tomita et al. (21) used the hyperinsulinemic clamp technique to demonstrate that elevated FFAs decreased the glucose infusion rate required to maintain euglycemia after glucose ingestion. Rigalleau et al. (20) reported that the exogenous glucose appearance rate after glucose ingestion was increased during lipid infusion, whereas Kruszynska et al. (23) reported no change. Although they were carefully performed, none of these studies directly measured splanchnic glucose uptake because all required assumptions regarding the rate of intestinal glucose absorption and completeness of suppression of hepatic glucose production. In subsequent experiments more closely resembling the present studies, Rigalleau et al. (22) sought to avoid the latter limitation by using the dual-tracer approach combined with a hyperglycemic-hyperinsulinemic clamp. Although this approach still required assumptions regarding completeness of glucose absorption and only measured initial (first pass) tracer extraction, the experiments were carried out in the presence of steady-state conditions, suggesting that errors due to these assumptions were likely small. The observation by Rigalleau et al. (22) that elevated FFAs did not decrease initial splanchnic glucose clearance is consistent with the demonstration in the present experiments showing that elevated FFAs did not alter either splanchnic glucose uptake or splanchnic tracer extraction.

The lack of an effect of FFAs on splanchnic glucose uptake is intriguing. There is a substantial literature indicating that elevated FFAs can decrease both GLUT4 translocation and hexokinase activity (9,11,18,56). Because glucose transport and phosphorylation are rate-limiting for glucose metabolism in muscle, it is not surprising that elevated FFAs decrease overall muscle glucose uptake (19). In contrast, glucose phosphorylation via glucokinase is believed to be rate limiting for hepatic glucose uptake (31). Tippett et al. (35) have shown that rat liver glucokinase is inhibited by long-chain fatty acyl coA. If elevated FFAs produce a similar effect in humans, then it would be anticipated that both splanchnic glucose uptake and the contribution of the direct pathway (i.e., extracellular glucose) to hepatic glycogen synthesis would be decreased. The lack of difference in either of these independently measured parameters in present experiments strongly argues against a primary effect of elevated FFAs on glucokinase activity. On the other hand, FFAs were only elevated to ~0.8 mmol/l. It is therefore possible that higher levels of FFAs may inhibit splanchnic glucose uptake. If so, this indicates that the effect(s) of FFAs on muscle and hepatic glucose metabolism is substantially different because the plasma FFA concentrations achieved in the present experiments were sufficient to cause a twofold reduction in leg (and therefore presumably muscle) glucose uptake. In addition, plasma FFA concentrations are rarely >0.8 mmol/l in the postprandial state in patients with type 2 diabetes.

The present experiments evaluated the effects of FFAs in the presence of combined hyperinsulinemia and hyperglycemia. We chose these conditions to ensure that glucose uptake was increased in both muscle and liver in order to be able to detect inhibition of uptake, if it were to occur. It is therefore possible that elevated FFAs impair insulin-induced stimulation of hepatic glucose uptake, but the effect was offset by hyperglycemia. If so, this would imply that the inhibitory effects of FFAs on splanchnic glucose uptake are likely to be of little significance under the conditions of daily living because insulin concentrations are rarely, if ever, elevated in the absence of a concomitant increase in glucose concentration. Furthermore, the current data indicate that the decrease in both splanchnic glucose uptake and uptake of extracellular glucose that we have previously observed under similar conditions in people with type 2 diabetes is unlikely to be caused by the higher FFAs that were present in those individuals during the study (1,2). On the other hand, because the present experiments examined the effects of a transient (~8 h) elevation of FFAs, it remains possible that a more chronic elevation alone or in association with hepatic steatosis may have a more marked effect on hepatic glucose uptake and glycogen synthesis.

We raised FFA concentrations by infusion of IL/Hep. The predominant FFAs in plasma are palmitic acid and stearic acid, whereas the predominant FFAs in Intralipid are linoleic acid and oleic acid. It can therefore be argued that this as well as other experiments (17–23,36–40) that used lipid and heparin infusions to raise FFAs may be unphysiological. However, we found in pilot experiments that heparin alone was unable to overcome insulin-induced suppression of lipolysis. Therefore, it remains possible that the metabolic effects of elevated FFAs demonstrated in the present experiments may not fully reflect those associated with a chronic elevation of plasma FFAs that result from endogenous causes.

Effects of elevated FFAs and glycerol on splanchnic glucose production. Splanchnic glucose production was higher during the IL/Hep infusion than either of the glycerol infusions. This indicates that the impaired ability of insulin to suppress endogenous glucose production observed in some (10,15,16,22,40), but not all (39), previous studies is due least in part to an increase in hepatic glucose release. The IL/Hep infusion and the high-glycerol infusion led to a comparable increase in both UDP-glucose flux and flux via the indirect pathway. However, only IL/Hep infusion increased splanchnic glucose production. The rate of hepatic glucose release is determined by the net balance between glucose entering and leaving the glucose-6-phosphate pool, which in turn is determined by the relative activities of glucokinase and glucose-6-phosphatase. Comparable rates of splanchnic glucose uptake and glucose flux through the direct extracellular pathway during the IL/Hep and high-glycerol infusions argue against an FFA-induced decrease in glucokinase activity. Rather, the data are entirely consistent with the demonstration by Massillon et al. (36) in rats that lipid infusion rapidly increases hepatic glucose-6-phosphatase gene expression and protein levels.

The lack of increase in glucose production during the high-glycerol infusion is also consistent with previous reports in rats (57,58), mice (59), dogs (60), and humans (41,43) that glycerol increases gluconeogenesis but not glucose release. The increase in both total UDP-glucose flux and flux via the indirect pathway observed during the high-glycerol infusion adds to a growing body of evidence that increased flux through the glucose-6-phosphate pool in itself is not sufficient to increase hepatic glucose release. An additional alteration, such as an FFA-induced increase in glucose-6-phosphatase activity, is required.

We did not directly measure gluconeogenesis, but rather the contribution of the indirect pathway to UDP-glucose flux. The contribution of the indirect pathway is calculated by subtracting the contribution of the direct (or extracellular) pathway from total UDP-glucose flux. The indirect (or intracellular) pathway reflects the sum of several factors, including the contributions of glucose derived from gluconeogenesis and ongoing glycogenolysis. [3-^{3}H]glucose is detritiated when it passes through the triose phosphate pool (61). Therefore, new glucose-6-phosphate synthesis resulting from the entry of substrates at the level of either phosphoenol-pyruvate (e.g., pyruvate) or glycerol-3-phosphate (e.g., glycerol) would lead to an increase in the contribution of the indirect pathway when assessed with this tracer. Although we did not directly measure glycogenolysis, because insulin concentrations in the range used in the present experiments previously have been shown to fully suppress the contribution of glycogenolysis to glucose production (and therefore presumably to glucose-6-phosphate) in humans (62), and because other studies have established that elevated FFAs stimulate gluconeogenesis (37–39), we assume that the increase in the indirect pathway observed during the IL/Hep and high-dose glycerol infusions was caused by an increase in

gluconeogenesis. However, additional studies in which gluconeogenesis is directly measured will be required to confirm this assumption.

Of interest, endogenous glucose production did not differ among the IL/Hep and glycerol groups, whereas splanchnic glucose production did. This may be due to imprecision of measurement of endogenous glucose production, which is calculated by subtracting the exogenous glucose infusion rate required to maintain target glucose concentrations from the total rate of glucose appearance. Alternatively, it could be due to offsetting effects of FFAs on the liver and the kidneys (i.e., stimulating splanchnic but inhibiting renal glucose production). In any case, these data emphasize the importance of directly measuring glucose production by the splanchnic bed when the effects of FFAs on splanchnic glucose metabolism are being evaluated.

Effects of FFAs and glycerol on hepatic insulin clearance. The current studies also afforded us the opportunity to directly measure the effects of elevated FFAs on hepatic insulin clearance. In vitro studies have shown that increased FFAs can decrease hepatocyte insulin binding and degradation (45,46). Furthermore, Wiesenthal et al. (47) have reported that an IL/Hep infusion decreases calculated hepatic insulin extraction in dogs. On the other hand, Boden et al. (63) have shown that plasma insulin concentrations achieved during a continuous insulin infusion were the same in the presence and absence of IL/Hep infusion, indicating no difference in total-body insulin clearance. We are unaware of any previous studies that have directly assessed splanchnic insulin extraction in humans in the presence of elevated FFAs. This is probably due in large part to the technical difficulty of this measurement. To remove the confounding effects of non-steady state, we only have reported hepatic insulin clearance measured during the final 30 min of the experiments. However, these values differed minimally from those present during the preceding 3 h. C-peptide concentrations were suppressed to undetectable levels in both groups, removing the confounding effect of persistent endogenous insulin secretion. Under these conditions, splanchnic insulin extraction was virtually identical in the IL/Hep and low- and high-glycerol groups (68 ± 3 vs. 69 ± 1 vs. 70 ± 6%). Of note, arterial insulin concentrations also were the same in all three groups. Because the exogenous insulin infusion rate also was the same, this indicates that peripheral insulin clearance also did not change. The present experiments used a conventional radioimmunoassay by Linco to measure arterial and hepatic insulin concentrations. It is possible that partially degraded insulin is still measured with this assay. However, we doubt this is the case because comparable results were observed when we used a two-site chemiluminescence assay to measure insulin in a subset of subjects. It remains possible that more marked and more prolonged elevations of FFAs may decrease hepatic insulin extraction. In any case, the present experiments indicate that an increase in FFAs to a level that is sufficient to stimulate splanchnic glucose production and inhibit muscle glucose uptake does not alter hepatic insulin clearance in nondiabetic humans.

In summary, the experiments demonstrate that elevated FFAs impair insulin and glucose-induced stimulation of muscle glucose uptake but do not alter splanchnic glucose uptake. Elevated FFAs and glycerol comparably stimulate the indirect pathway and flux through the hepatic UDP-glucose pool. However, only FFAs increase splanchnic glucose production. The lack of change in either splanchnic glucose uptake or the contribution of the direct extracellular pathway to glycogen synthesis argues against an effect of FFAs on hepatic glucokinase activity. The concurrent increase in both splanchnic glucose production and flux through the indirect pathway strongly imply that FFAs accelerate both gluconeogenesis and glucose-6-phosphatase activity. On the other hand, we found no evidence that FFAs decrease hepatic insulin extraction. These data indicate that transient (~8 h) elevations of FFAs differentially regulate hepatic glucose production and hepatic glucose uptake as well as muscle glucose metabolism. They suggest that the elevated FFAs that are commonly present in people with type 2 diabetes may contribute to impaired suppression of glucose production but are unlikely to be the cause of the decrease in splanchnic glucose uptake that is typically observed in these individuals. Finally, these data call into question the widely held assumption that elevated FFAs exacerbate systemic hyperinsulinemia by decreasing hepatic insulin clearance.

ACKNOWLEDGMENTS

This study was supported by the U.S. Public Health Service (DK-29953, RR-00585, DK-40484, and DK-50456) and the Mayo Foundation. P.S. was supported by a research fellowship from Novo-Nordisk and R.B. by an American Diabetes Association Mentor–based fellowship.

We thank C. Etter, C. Stacey-Oda, C. Nordyke, and B. Dicke for technical assistance; J. Feehan and B. Norby and the staff of the Mayo General Clinical Research Center for assistance in performing the studies; and M. Davis for assistance in the preparation of the manuscript.

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