In Normal Men, Free Fatty Acids Reduce Peripheral but Not Splanchnic Glucose Uptake

Vincent Rigalleau, Christophe Binnert, Kaori Minehira, Nathalie Stefanoni, Phillippe Schneiter, Emmanuel Henchoz, Oscar Matzinger, Christine Cayeux, Eric Jéquier, and Luc Tappy

Raising plasma free fatty acid (FFA) levels reduces muscle glucose uptake, but the effect of FFAs on splanchnic glucose uptake, total glucose output, and glucose cycling may also be critical to producing lipid-induced glucose intolerance. In eight normal volunteers, we measured glucose turnover and cycling rates ([$^2H_7$]glucose infusion) during a moderately hyperglycemic (7.7 mmol/l) hyperinsulinemic clamp, before and after ingestion of a labeled (dideuterated) oral glucose load (700 mg/kg). Each test was performed twice, with either a lipid or a saline infusion; four subjects also had a third test with a glycerol infusion. As shown by similar rates of exogenous glucose appearance, the lipid infusion did not reduce first-pass splanchnic glucose uptake (saline 1.48 ± 0.18, lipid 1.69 ± 0.17, and glycerol 1.88 ± 0.17 mmol/kg per 180 min; NS), but it reduced peripheral glucose uptake by 40% ($P < 0.01$ vs. both saline and glycerol infusions). Before oral ingestion of glucose, total glucose output was similarly increased by the lipid and glycerol infusions. Total glucose output was significantly increased by FFAs after oral ingestion of glucose (saline 3.68 ± 1.15, glycerol 3.68 ± 1.70, and lipid 7.92 ± 0.88 μmol · kg^{-1} · min^{-1}; $P < 0.01$ vs. saline and $P < 0.05$ vs. glycerol). The glucose cycling rate was ~2.7 μmol · kg^{-1} · min^{-1} with the three infusions and tended to decrease all along the lipid infusion, which argues against a stimulation of glucose-6-phosphatase by FFAs. It is concluded that in situations of moderate hyperinsulinemia-hyperglycemia, FFAs reduce peripheral but not splanchnic glucose uptake. Total glucose output is increased by FFAs, by a mechanism that does not seem to involve stimulation of glucose-6-phosphatase.
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From the Institut de Physiologie, Lausanne, Switzerland.

Address correspondence and reprint requests to Dr. V. Rigalleau, Service de Nutrition et Diabétologie, USN, Hôpital Haut-lévêque, Avenue de Magellan, 33600 Pessac, France. E-mail: vincent.rigalleau@iphysiol.unil.ch.

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FFA, free fatty acid; R_aE, rate of exogenous appearance; R_aT, rate of total appearance; R_dT, rate of total disappearance.

Plasma free fatty acids (FFAs) are often elevated in obesity and type 2 diabetes (1). Their potential role in inducing insulin resistance, as initially proposed by Randle et al. (2), is supported by the effects of lipid infusions on peripheral and hepatic glucose metabolism during hyperinsulinemic-euglycemic clamp experiments. An experimental elevation of plasma FFA levels reduces insulin-mediated muscle glucose uptake (3) and muscle glycogen synthesis (4). Impaired suppression of endogenous glucose production has also been reported during hyperinsulinemic-euglycemic clamps with lipid infusions (5,6), although it was sometimes attributed to the high glycerol content of the lipid infusion (7,8).

Recent studies using oral glucose tolerance tests with doubly labeled glucose have shown that a defect in suppression of endogenous glucose production plays a significant role in lipid-induced glucose intolerance (9,10). Moreover, under these more physiological conditions, the liver is a major site to determine glucose tolerance because it also takes up an important part of the oral glucose load (11). Unchanged (9) or even increased (10) exogenous glucose appearance rates were observed during oral glucose tolerance tests with lipid infusion, suggesting that lipids may alter splanchnic glucose uptake. Plasma glucose and insulin levels during oral glucose tolerance tests increased more rapidly under lipid infusions during these experiments, which is expected to increase splanchnic glucose uptake (12), as they indeed increased peripheral glucose uptake (9,10). The effects of a lipid infusion on splanchnic glucose uptake must therefore be studied in controlled conditions, at the same moderately hyperglycemic level, which can be performed by the combination of a hyperinsulinemic-hyperglycemic clamp and the ingestion of a labeled oral glucose load.

The liver is able to first take up and then release glucose (i.e., glucose cycling); this process is undetected by usual isotopic dilution methods. This leads to underestimate overall glucose utilization and endogenous glucose production during oral glucose tolerance tests, and it may have a marked effect on the calculation of splanchnic glucose uptake (13). Because lipid infusion probably produces opposite effects on glucose production and utilization, a systematic error will arise if it also modifies glucose cycling. Glucose cycling rates can also provide information about the control of endogenous glucose production by glucokinase and glucose-6-phosphatase. In eight normal subjects, we have therefore studied the effect of

elevated FFAs (produced by a lipid infusion) on insulin-suppressed endogenous glucose production and total glucose output during moderately hyperinsulinemic-hyperglycemic clamps, with constant isotopic enrichment (hot-clamp) and determination of glucose cycling ([$^2H_7$]glucose infusion), before and after labeled (dideuterated) glucose ingestion. All subjects were studied twice, with and without the lipid infusion; four subjects also underwent the same experiment during a glycerol infusion.

RESEARCH DESIGN AND METHODS

Subjects

Eight subjects were studied and underwent two tests (with a saline or a lipid infusion in random order); in addition, four subjects also underwent a third test with a glycerol infusion. Subjects were healthy men (age 25 ± 0.6 years; BMI 22.4 ± 0.6 kg/m^2). None of them had a personal or familial history of diabetes or obesity or were receiving any medication. Subjects gave their written consent to the study after being informed of its nature, purpose, and potential risks. The protocol was approved by the ethical committee of Lausanne University Faculty of Medicine.

Experimental protocol

All subjects were studied in the postabsorptive state after a 12-h overnight fast. A retrograde catheter was inserted into a vein in the dorsal aspect of the hand; the hand was kept in a warming box (50°C) to collect arterialized venous blood. A forearm vein of the contralateral arm was catheterized to infuse isotonic saline (1 ml/min), Lipovenös 20 g/100 ml (1 ml/min), or glycerol 2.5 g/100 ml (1.25 ml/min) during 420 min (Lipovenös contains glycerol 2.5 g/100 ml).

One hour later, a hyperinsulinemic-hyperglycemic pancreatic clamp was started, with a concomitant infusion of somatostatin (49 nmol · kg^{-1} · min^{-1}), insulin (2.44 pmol · kg^{-1} · min^{-1}), and glucagon (0.5 ng · kg^{-1} · min^{-1}) for the next 360 min. Plasma glucose level was held constant at 7.7 mmol/l until the end of the test with a glucose 20 g/100 ml infusion, according to the glucose clamp technique (14). A primed infusion of [$^2H_7$]glucose (prime 12 μmol/kg; Cambridge Isotope Laboratories, Andover, MA) was begun simultaneously. For 180 min, the infusion rate was proportional (0.5%) to the "cold" glucose infusion rate, according to the hot-clamp technique (15). Thereafter, it was kept constant at the level reached at time 240 min, when the oral glucose load (700 mg/kg glucose, 5% enriched with D-[6,6-$^2H_2$]glucose) was ingested.

Blood samples were drawn at time 0, 60, 120, and every 30 min thereafter for determination of plasma metabolites, hormones, and isotopic enrichments.

Analytical procedures

Plasma glucose was measured with a Beckman glucose analyzer II (Beckman Instruments, Fullerton, CA). Plasma FFA concentrations were measured with a colorimetric method using a kit from Wako (Freiburg, Germany). Plasma insulin, C-peptide, and glucagon were measured using radioimmunoassays.

For isotopic analysis, plasma was deproteinized with 6% perchloric acid, neutralized with 3.2 mol/l K_2CO_3, and partially purified over sequential cation anion exchange resins (AG 50W-X8 and AG1X8; Bio Rad, Richmond, CA). Pentacetyl glucose derivatives were analyzed by gas chromatography-mass spectrometry (GC 5890/MS 5971; Hewlett Packard, Palo Alto, CA) in chemical ionization mode with selective monitoring of m/z 331, 333, 337, and 338 to determine concentrations of D-[6,6-$^2H_2$]glucose, [$^2H_6$]glucose, and [$^2H_7$]glucose, respectively. Using [$^2H_7$]glucose and cold glucose, two calibration curves were established by measuring abundances of m/z 331, 337, and 338 on samples with increasing [$^2H_7$]glucose isotopic enrichments (0, 0.001, 0.0023, 0.0045, 0.0084, 0.0125, 0.0202, and 0.0385 molar ratios). The first calibration curve:

$338/331 = a * ([^2H_7]\text{glucose/cold glucose}) - b$

was used to calculate [$^2H_7$]glucose isotopic enrichments from 338/331 abundance ratios. The second calibration curve:

$337\text{imp}/331 = a' * (338/331) - b'$

was used to correct 337 abundances for the contribution of [$^2H_7$]glucose to mass 337.

Plasma glycerol concentration was determined on the same sample by selective monitoring of m/z 159 from glycerol and 162 from U-^{13}C glycerol used as an internal standard.

Calculations

Rates of total glucose appearance (R_aT) and disappearance (R_dT) were calculated from [$^2H_7$]glucose isotopic enrichments using non–steady-state equations, with a pool fraction of 0.75 and a distribution volume of 0.2 l/kg (16). Using D-[6,6-$^2H_2$]glucose enrichments, non–steady-state calculations were also performed for determination of rates of exogenous glucose appearance (R_aE), as proposed by Proietto et al. (17). Total glucose output was calculated as R_aT – (glucose infusion rate, M) before oral glucose ingestion and as R_aT – (M + R_aE) after oral glucose ingestion. The same calculations were also performed using ([$^2H_7$]+[$^2H_6$]) glucose isotopic enrichments to obtain net endogenous glucose production.

First-pass splanchnic glucose uptake was calculated as:

Ingested glucose – 180 min cumulated R_aE.

The rate of glucose cycling was calculated as:

total glucose output – endogenous glucose production

Statistical analysis

Results are shown as means ± SE. The changes in hormones, substrate concentrations, and turnover rates over time were analyzed by analysis of variance for repeated measurements and paired Student's t tests. Comparisons between results from saline, lipid, and glycerol infusions were performed by two-way analysis of variance and paired Student's t tests, corrected for multiple comparisons. $P < 0.05$ was considered significant.

TABLE 1
Metabolic parameters at the postabsorptive state

	Saline	Lipid	Glycerol
Glucose (mmol/l)	5.0 ± 0.1	5.1 ± 0.1	4.8 ± 0.1
FFA (μmol/l)	323 ± 45	401 ± 87	405 ± 133
Glycerol (μmol/l)	52 ± 3	46 ± 7	39 ± 8
Insulin (pmol/l)	60.5 ± 6.2	65.4 ± 6.2	49.4 ± 9.8
Cpeptide (nmol/l)	0.66 ± 0.03	0.69 ± 0.06	0.56 ± 0.06
Glucagon (ng/l)	65 ± 4	62 ± 3	61 ± 2

RESULTS

Metabolites

Postabsorptive levels of plasma glucose, FFAs and glycerol did not differ at the onset of the three tests (Table 1). As shown by Fig. 1 plasma glucose levels were similarly clamped at 7.7 mmol/l during the three tests, and this level was well maintained after oral glucose ingestion at time 240 min. FFA and glycerol levels were reduced all during the clamps with saline infusion (both $P < 0.001$ from time 120 to 420 min vs. time 0 min). The lipid infusion prevented the decrease of FFAs during the clamp: FFA levels were not significantly different from time 0 min until 300 min, and then values were slightly increased as compared with time 0 ($P < 0.05$). At every time after 0 min, FFA levels were higher during the lipid infusion than during the saline and glycerol infusions ($P < 0.01$). Glycerol levels were increased by the lipid infusion ($P < 0.0001$ at every time vs. time 0 min) and were higher than during the saline infusion at every time after 0 min ($P < 0.001$). As shown on Fig. 1, most of this increase was mimicked by the glycerol infusion, although glycerol levels were slightly lower than during the lipid infusion after time 210 min ($P < 0.05$).

Hormones

Postabsorptive plasma levels of insulin, C-peptide, and glucagon did not differ at the onset of the three tests. C-peptide was totally suppressed during all clamps. Glucagon levels were lower all during the clamps than before (time 120 min 42.8 ± 1.8 ng/l vs. time 0 min 62.8 ± 2.1 ng/l; $P < 0.01$); results were similar for the three tests. Plasma insulin increased to a mean level of 235 ± 17 pmol/l during the saline infusion, 262 ± 13 pmol/l during the lipid infusion ($P < 0.05$ vs. saline), and 271 ± 13 pmol/l during the glycerol infusion (NS vs. saline and lipid) (Fig. 1).

Turnover rates

[$^2H_7$]glucose enrichments were stable after time 120 min during all tests, although there was a slight tendency to progressive increase during saline tests (time 420 min 0.47 ± 0.03 mmol/l vs. time 120 min 0.42 ± 0.01 mmol/l;

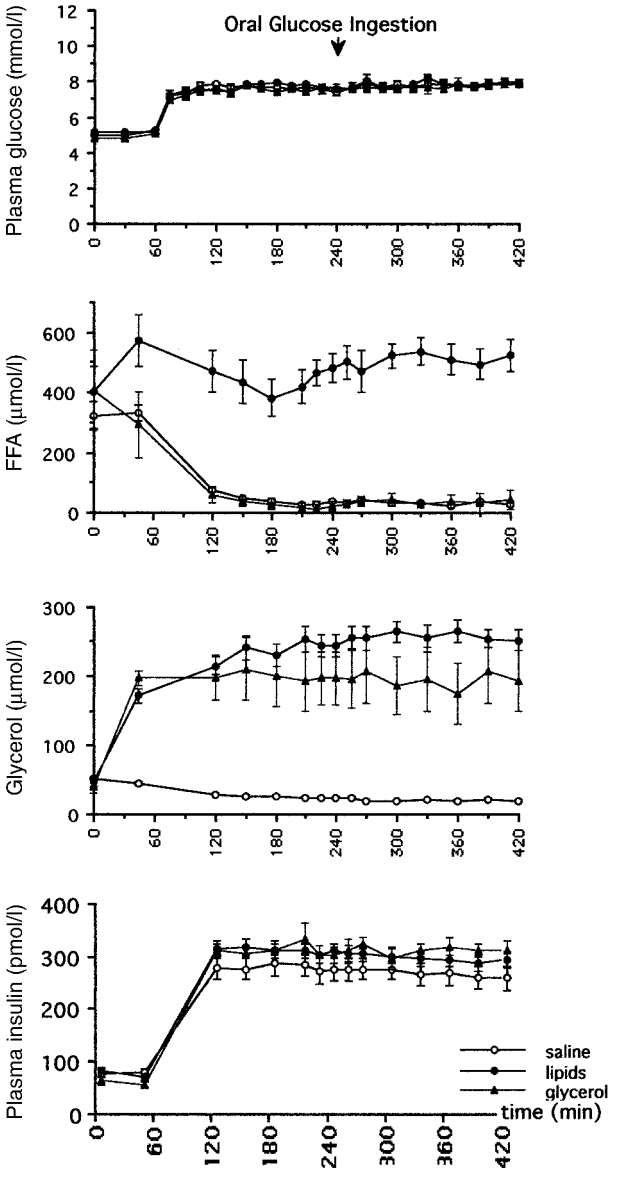

FIG. 1. Time course of plasma glucose (mmol/l), FFA (μmol/l), glycerol (μmol/l), and insulin (pmol/l). Open circles represent saline tests, closed circles represent lipid tests, and triangles represent glycerol tests.

$P = 0.06$). D-[6,6-$^2H_2$]glucose appeared 15 min after oral ingestion (Figs. 2 and 3). D-[6,6-$^2H_2$]glucose enrichments were still significantly elevated at the end of the tests, but they did not change significantly during the last hour of the tests. The glucose infusion rates required to maintain moderate hyperglycemia were reduced after oral ingestion of glucose during the three tests, more acutely during the lipid test (from 23.6 ± 2.7 μmol · kg^{-1} · min^{-1} at time 180–240 min to 8.8 ± 2.2 μmol · kg^{-1} · min^{-1} at time 240–420 min; $P < 0.0001$) than during the saline test (from 39.6 ± 2.2 μmol · kg^{-1} · min^{-1} at time 180–240 min to 30.8 ± 3.3 μmol · kg^{-1} · min^{-1}at time 240–420 min; $P < 0.01$) and glycerol (from 48.9 ± 3.8 μmol · kg^{-1} · min^{-1} at time 180–240 min to 40.1 ± 3.3 μmol · kg^{-1} · min^{-1} at time 240–420 min; P = NS) infusions.

Glucose R_dT did not differ significantly between saline, lipid, and glycerol tests at time 120–180 min (Fig. 2). Thereafter, R_dT increased progressively during the saline

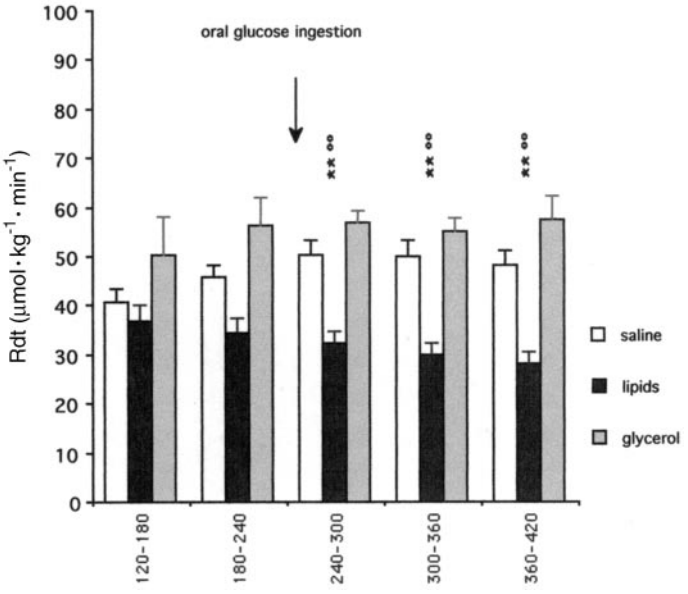

FIG. 2. Time course of glucose R_dT (μmol · kg^{-1} · min^{-1}). ** $P < 0.01$ for lipid vs. saline tests at the same time. °° $P < 0.01$ for lipid vs. glycerol tests at the same time.

infusion, reaching higher values ($P < 0.05$ vs. time 120–180 min) at time 240–300 min and stabilizing later on. It decreased progressively during the lipid infusion, reaching lower values ($P < 0.05$ vs. time 120–180 min) at time 300–360 min and later on. No significant change occurred during the glycerol infusion. As a consequence, R_dTs were lower during the lipid infusion than during the saline and glycerol infusions during the last 3 h (both $P < 0.01$).

During all tests, glucose R_aE increased from the first to the second hour after glucose ingestion ($P < 0.05$) and was stable during the third hour (Fig. 3). For the first hour, it was higher during the saline than during the lipid infusion ($P < 0.05$); thereafter, this difference disappeared and overall 3-h cumulated R_aE did not differ between the three tests. First-pass splanchnic glucose uptake did not differ between the three tests (saline 1.48 ± 0.18, lipid 1.69 ± 0.17, or glycerol 1.88 ± 0.17 mmol/kg per 180 min; NS).

Before the oral glucose ingestion, total glucose output and endogenous glucose production did not differ significantly between saline, glycerol, and lipid infusions, although values tended to be higher with lipids ($P = 0.10$ vs. saline). After the oral glucose ingestion, total glucose output and endogenous glucose production were higher with lipid than with the saline and glycerol infusions (both $P < 0.05$) (Fig. 3).

Glucose cycling rates were not different between saline, lipid, and glycerol tests at any time (Fig. 3). During the saline infusion, the glucose cycling rate increased progressively from 2.47 ± 0.33 μmol · kg^{-1} · min^{-1} at time 120–180 min to a maximum of 3.08 ± 0.55 μmol · kg^{-1} · min^{-1} at time 240–300 min ($P < 0.05$ vs. time 120–180 min). The increase was halted after time 300 min, and then values tended to decrease. Cycling rates followed the same time course during the glycerol infusion, without reaching significance. Cycling rates decreased progressively during the lipid infusion from 2.53 ± 0.38 μmol · kg^{-1} · min^{-1} at time 120–180 min to a minimum of 2.20 ± 0.38 μmol · kg^{-1} · min^{-1} at time 360–420 min ($P = 0.12$ vs. time 120–180 min).

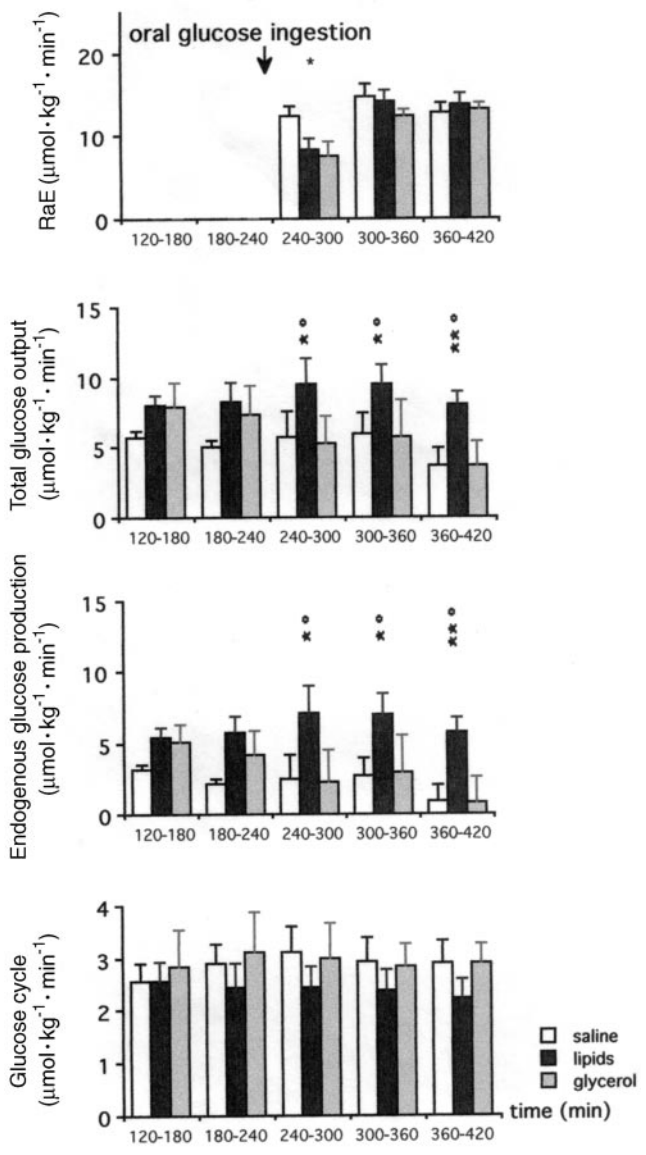

FIG. 3. Time course of glucose cycling rates, endogenous glucose production, total glucose output, and exogenous glucose R_aE (μmol · kg^{-1} · min^{-1}). White bars represent saline tests, black bars represent lipid tests, and shaded bars represent glycerol tests. * $P < 0.05$, ** $P < 0.01$ for lipid vs. saline tests at the same time, and ° $P < 0.05$ for lipid vs. glycerol tests at the same time.

DISCUSSION

Although the effect of FFAs on peripheral insulin sensitivity are well established by clamp experiments, their relevance to the physiological postprandial situation is more uncertain: during oral glucose tolerance tests, the increase in plasma glucose and insulin levels results in an increase of total glucose disappearance (9,10) and glucose metabolic clearance rate (9), despite the lipid infusion. In this more physiological situation, lipids deteriorate glucose tolerance by increasing endogenous glucose production (9,10). To further delineate the mechanisms by which lipids disturb hepatic glucose metabolism after an oral glucose ingestion, we addressed the following questions: *1*) Is first-pass splanchnic glucose uptake reduced by a lipid infusion? *2*) Is total glucose output modified by FFAs when glucose comes through the oral route? *3*) Is glucose cycling modified by lipids before or after oral glucose ingestion? As hyperglycemia exerts its own influence on glucose production (18), utilization (19), cycling (20), and splanchnic uptake (12), we performed moderately hyperinsulinemic-hyperglycemic clamps to mimic the postprandial state, with identical plasma glucose levels of 7.7 mmol/l before and after the oral ingestion of glucose.

Due to reduced insulin clearance as previously described (21), plasma insulin levels were slightly higher during the lipid infusion, which does not modify the interpretation of the results. To avoid underestimation of glucose turnover rate during the clamp (15) and to limit errors by use of non–steady-state kinetics, we maintained constant [$^2H_7$]glucose enrichments throughout the tests. Because the glycerol content of the lipid infusion may interfere with glucose metabolism, four subjects also received a glycerol infusion. Intravascular lipolysis of infused triglycerides also releases glycerol, and we had previously experienced that infusion of the same rate of glycerol as contained in the lipid infusion leads to a lower plasma glycerol concentration (10), so we empirically used a 25% higher rate of infusion. It was not sufficient to exactly match glycerol levels obtained during the lipid infusion; however, the difference was not important.

Under our conditions of identical and moderate hyperglycemia, the elevation of FFAs significantly reduced the glucose R_dT. The reduction of R_dT was more pronounced (−40%) after rather than before (−25%) glucose ingestion, probably due to a time effect: during a euglycemic clamp, a lipid infusion reduced glucose uptake two times more during the fourth than during the second hour of infusion (22). This result contrasts with the increased glucose utilization reported during lipid-modified oral glucose tolerance tests (9,10), which is therefore not due to the oral route itself, nor to the slightly higher peripheral insulin levels (the difference concerning insulin levels was quite similar during our experiments and previously reported oral glucose tolerance tests). Higher peripheral glucose (23), and maybe higher portal insulin levels, are the most plausible explanations for this differing effect of lipids.

The dramatic influence of FFAs on R_dT (−40% at the end of the test) did not involve first-pass splanchnic glucose uptake, as evidenced by similar R_aE during the saline and the lipid infusions. Our calculation of splanchnic glucose uptake assumes that the 700-mg/kg oral glucose charge was completely absorbed after 180 min, according to Ludvik et al. (24). Preserved splanchnic glucose uptake is in good agreement with normal glucose R_aE during lipid-modified oral glucose tolerance tests (9) and confirms our hypothesis that the high R_aE observed during our previous experiments reflected increased recycling of oral [^{13}C]glucose (10). The fact that splanchnic glucose uptake was not reduced by FFAs contrasts with their influence on peripheral uptake; however, this is not surprising because uptake of glucose occurs differently in the liver, by GLUT2 transporters, and in the muscle, by GLUT4 transporters (25). This probably has important consequences on further steps of glucose metabolism: reduction of muscle glycogen synthesis by FFAs has been attributed to the reduction of muscle glucose uptake, as demonstrated by reduced intramuscular glucose-6-phosphate content (4). Because FFAs repeatedly have been reported to increase gluconeogenesis (26–28), unchanged splanchnic glucose uptake may lead to a different intrahepatic glucose-6-phosphate con-

tent, and hepatic glycogen synthesis is probably not inhibited by FFAs, as is muscle glycogen synthesis. This was already suggested by results from Chakley et al. (29): after 5 hours of lipid infusion in rats, the glycogen content was reduced in muscles but not in liver cells. Splanchnic glucose uptake recently has been reported to be increased in obese subjects (24) and decreased in type 2 diabetic subjects (30). Our results show that these abnormalities cannot be directly attributed to high FFA levels.

We found that total glucose output and endogenous glucose production were significantly higher with the lipid infusion. Previous euglycemic clamp experiments have already shown that lipids alter the sensitivity of endogenous glucose production to the suppressive effect of moderate hyperinsulinemia (5,6). However, the relevance of these results to the physiological postprandial state, with moderate hyperglycemia and oral glucose ingestion, remained questionable, because a high glucose concentration suppresses endogenous glucose production on its own (18). During hyperglycemic clamps in normal subjects (7) and isoglycemic clamps in type 2 diabetic subjects (8), a lipid infusion did not increase endogenous glucose production more than glycerol alone, as we found before oral glucose ingestion. However, we can attribute elevated total glucose output and endogenous glucose production to FFAs after oral glucose ingestion, because they were also significantly higher than during the glycerol infusion at this time, and oral glucose probably increased portal glycemia comparably during the lipid and saline experiments. Total glucose output may have decreased after oral glucose ingestion during the glycerol infusion, because oral glucose ingestion redirected glucose-6-phosphate from total glucose output to hepatic glycogen synthesis, as proposed by Shikama et al. (31). By contrast, the lipid infusion increased endogenous glucose production both before and after oral ingestion of glucose. This could result from increased hepatic gluconeogenesis or glycogenolysis. FFAs do increase gluconeogenesis in the postabsorptive state, more than glycerol alone (27), and our previous observation that they increased recycling of oral [^{13}C]glucose (10) suggests that this takes place also after oral glucose ingestion. On the other hand, this effect is balanced by a reduced hepatic glycogenolysis at the postabsorptive state (26–28), as recently confirmed by nuclear magnetic resonance studies (32). The effect of FFAs on hepatic glycogen metabolism during oral glucose ingestion has not yet been specifically explored, to our knowledge.

Glucose cycling did not differ between saline, lipid, and glycerol infusions. Although it tended to decrease during the lipid infusion, this did not reach significance and was weak (-0.27 μmol · kg^{-1} · min^{-1}) compared with the effects of lipids on R_dT on the same time interval (-7.5 μmol · kg^{-1} · min^{-1}). Lipid-modified glucose cycling is therefore not a significant cause of error in the determination of glucose turnover rates. When glucose is infused or ingested, the liver switches from net glucose production to net glucose uptake (12), and then glucose cycling depends on the reverse flux, which is a function of glucose-6-phosphatase activity (33). The total flux through glucose-6-phosphatase was increased by lipids (increased total glucose output), but stable glucose cycling therefore argues against a stimulation of glucose-6-phosphatase in this situation. Although a lipid infusion induces expression of the glucose-6-phosphatase gene (34), short-term effects are not so clear-cut, depending on the concentration, the length and saturation of the chain, and the binding to intracellular proteins (35). Normal glucose cycling differentiates the short-term effect of lipids from other states of hepatic insulin resistance due to glucocorticoids (36), fructose infusion (37), or type 2 diabetes (33) and suggests that the high glucose-6-phosphate availability from increased gluconeogenesis and glycogenolysis is the cause of the high glucose-6-phosphatase flux during the lipid infusion, by a "push" mechanism.

In summary, we found that a lipid infusion did not reduce first-pass splanchnic glucose uptake in conditions of controlled hyperinsulinemia-hyperglycemia at physiological (postprandial) levels, as evidenced by similar exogenous glucose appearance rates contrasting with a 40% reduction of total glucose uptake. Total glucose output and endogenous glucose production were significantly increased by the lipids after glucose ingestion, both as compared with a saline and a glycerol infusion. Lipids did not modify glucose cycling rates, which argues against a short-term stimulatory effect on glucose-6-phosphatase activity.

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