Hyperglycemia-induced inhibition of splanchnic fatty acid oxidation increases hepatic triacylglycerol secretion

LABROS S. SIDOSSIS, BETTINA MITTENDORFER, ERIC WALSER, DAVID CHINKES, AND ROBERT R. WOLFE
Metabolism Unit, Shriners Burns Institute; and Departments of Surgery, Anesthesiology, and Internal Medicine, University of Texas Medical Branch, Galveston, Texas 77550

Sidossis, Labros S., Bettina Mittendorfer, Eric Walser, David Chinkes, and Robert R. Wolfe. Hyperglycemia-induced inhibition of splanchnic fatty acid oxidation increases hepatic triacylglycerol secretion. Am. J. Physiol. 275 (Endocrinol. Metab. 38): E798–E805, 1998.—The effect of hyperglycemia (~8 mmol/l) on splanchnic fatty acid oxidation and triacylglycerol (TG) secretion rates was investigated in five healthy men. U-13C-labeled fatty acids were infused to estimate fatty acid kinetics and oxidation across the splanchnic region, and in vivo labeled very low density lipoprotein (VLDL)-TG was infused to estimate TG secretion rate. Plasma fatty acid carbon enrichment and concentration were maintained constant by infusion of lipids and heparin in the hyperglycemia experiments. Fatty acid uptake by the splanchnic region was 1.4 ± 0.2 and 2.2 ± 0.9 µmol·kg⁻¹·min⁻¹ in the basal and clamp experiments, respectively, whereas fatty acid oxidation decreased from 0.4 ± 0.04 to 0.2 ± 0.05 µmol·kg⁻¹·min⁻¹ (P < 0.05). Hepatic TG secretion increased from 0.35 ± 0.07 to 0.53 ± 0.11 µmol·kg⁻¹·min⁻¹ after 15 h of hyperglycemia (P < 0.05). Similarly, plasma VLDL-TG concentration increased from 0.28 ± 0.06 to 0.43 ± 0.05 mmol/l during the clamp (P < 0.05). In summary, hyperglycemia attenuates fatty acid oxidation in the splanchnic region in human volunteers, even when fatty acid availability is constant. This adaptation results in a significant increase in the VLDL-TG secretion rate and concentration in plasma.

Hypertriglyceridemia is the most common abnormality associated with insulin resistance (11, 25) and a strong predictor of coronary heart disease mortality in non-insulin-dependent diabetes mellitus (NIDDM) patients (18, 26). Other pathological responses, such as alterations of the coagulation system (3) and pancreatitis (6), have also been attributed to hypertriglyceridemia. However, the mechanisms responsible for the development of hypertriglyceridemia in insulin-resistant states remain unclear.

Fatty acid availability to the liver is an important regulator of very low density lipoprotein (VLDL)-triacylglycerol (TG) production (12). Studies in vitro (2, 4) and in humans in vivo (8, 14) have suggested that fatty acids directly stimulate TG production. However, fatty acid concentration in plasma, and therefore fatty acid availability for TG production, are very sensitive to changes in serum insulin, making the independent effects of fatty acids and insulin on TG metabolism in vivo studies difficult to interpret. Furthermore, no study has directly assessed the role of hepatic free fatty acid (FFA) availability on VLDL-TG production in human volunteers during hyperglycemia.

Results from studies in dogs suggest that hyperglycemia per se may directly regulate VLDL-TG secretion (29). Wolfe et al. (29) reported that infusion of glucose into dogs significantly increased VLDL-TG secretion, independently of changes in insulin and glucagon (29). However, the mechanism via which hyperglycemia may affect TG production is unknown. We have recently reported that hyperglycemia inhibits fatty acid oxidation at the whole body level independently of FFA availability (22, 23). If the same is true for the liver, then FFA availability for TG production would increase severalfold when oxidation is inhibited. Under normal conditions, this would not be expected to be a problem, because peripheral lipolysis is inhibited by the insulin response to hyperglycemia, and thus uptake of FFA by the liver would be suppressed as a consequence of decreased availability. However, plasma FFA concentration is generally normal or elevated in insulin-resistant states, such as type II diabetes (7) and severe burn injury (28). An inhibition of hepatic fatty acid oxidation in the setting of an elevated plasma FFA concentration would thus cause further increase in FFA availability for TG synthesis if hepatic uptake of FFA is a function of delivery rather than oxidation.

To simultaneously address the effect of hyperglycemia-hyperinsulinemia on hepatic fatty acid oxidation and triacylglycerol secretion, we studied five male volunteers in the basal state and during moderate hyperglycemia-hyperinsulinemia (blood glucose = ~8 mmol/l, insulin = ~35 µU/ml) created by infusion of glucose. Plasma FFA concentration was maintained constant in the two experiments via exogenous infusion of lipids and heparin during hyperglycemia-hyperinsulinemia. With this approach, we created an experimental model in human volunteers with a similar metabolic profile to insulin-resistant states with respect to blood glucose, insulin, and FFA concentration. Our findings are also pertinent to the common circumstance of intravenous glucose infusions for nutritional purposes in hospitalized patients.

A femoral artery and a hepatic vein were catheterized to obtain glucose balance and FFA uptake and oxidation rates across the splanchnic region. Hepatic TG secretion was estimated using a newly developed methodology involving in vivo VLDL-TG labeling and reinfusion as a tracer on a separate occasion. Our

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results suggest that the observed hypertriglyceridemia during moderate hyperglycemia-hyperinsulinemia is at least partly due to increased efficiency of incorporation of plasma fatty acids into TG, mediated primarily by the glucose-induced inhibition of hepatic fatty acid oxidation and enhanced non-plasma-derived fatty acid availability.

METHODS

Five male volunteers (age 29 ± 2 yr, weight 76 ± 5 kg, height 177 ± 5 cm) participated in this study. All volunteers were healthy, as indicated by comprehensive history, physical examination, and standard blood and urine tests, and all had maintained stable weights for ≥3 mo before the studies. The Institutional Review Board and the General Clinical Research Center (GCRC) of the University of Texas Medical Branch at Galveston approved the experiments. Informed consent was obtained for all procedures.

Volunteers were studied twice: once in the basal state (overnight fast) and once during 15 h of moderate hyperglycemia. Teflon catheters were placed in the femoral artery and hepatic vein to measure fatty acid kinetics and oxidation across the splanchnic region. Labeled VLDL-TG, produced in vivo in the same volunteers 3–4 days earlier, were infused to determine the rate of VLDL-TG secretion. To maintain FFA availability constant, we infused lipids and heparin during the clamp experiments. A detailed description of the study design follows.

Experimental Design

Each volunteer was admitted to the GCRC at the University of Texas Medical Branch at Galveston and received a light meal at 5 PM. The meal contained 30% of energy as fat, 20% as protein, and 50% as carbohydrate. At 9 PM, catheters were placed percutaneously into an antecubital vein for infusion and into a contralateral dorsal hand vein that was heated for sampling of arterialized blood. The catheters were kept patent by infusion of 0.9% NaCl. Background blood and breath samples were collected after the volunteers had rested for 30 min, and one of the following two randomly assigned experimental protocols was performed.

Protocol 1: Basal state. Infusion of a U-13C-labeled fatty acid mix (no prime, constant infusion = 0.035 μmol·kg⁻¹·min⁻¹) bound to human albumin was started through the arm vein and maintained until the end of the study ~15 h later. The bicarbonate pool was primed using NaH13CO3 (25 µmol), TG pool, and the remaining 40% was given at a constant rate different for each subject and depended on the amount of labeled TG produced. Sixty percent of the available tracer was given as a bolus at the beginning of the study to prime the TG pool, and the remaining 40% was given at a constant rate over the remaining 2 h of the study. Labeled VLDL-TG were produced in vivo, as will be described in Procedures. Blood samples were obtained from the artery and the hepatic vein at 100, 110, and 120 min after start of the labeled VLDL-TG infusion for measurement of hormone and substrate concentrations and carbon enrichment of blood CO2, VLDL-TG glycerol, and FFA. At the same time points, breath samples were collected for determination of CO2 carbon enrichment.

Whole body oxygen consumption and carbon dioxide production were measured over 30 min during the last hour of the study. The acetate correction factor, for use in the estimation of splanchnic fatty acid oxidation, was determined on a different occasion, as will be described in Procedures. Splanchnic blood flow was determined using a constant infusion of indocyanine green. After completion of the study, all catheters were removed and the volunteers remained in the hospital for observation until the next morning.

Protocol 2: Hyperglycemic clamp. The protocol was the same as in protocol 1, except that a continuous infusion of 15% dextrose was started at the same time as the labeled fatty acid infusion (i.e., 9 PM) and maintained until the end of the study. Dextrose was infused at a rate designed to increase blood glucose concentration to ~8 mmol/l. Blood glucose levels were measured every 30 min, and dextrose infusion was appropriately adjusted to maintain blood glucose concentration at ~8 mmol/l. The average glucose infusion rate was 8 mg·kg⁻¹·min⁻¹. The concentration of hormones, including insulin, was allowed to change freely in response to dextrose infusion. Blood potassium concentration was measured every 2 h, and a variable infusion of potassium chloride was given to maintain a constant plasma potassium concentration. Lipids (0.7 ml·kg⁻¹·h⁻¹) and heparin (bolus of 7.0 U/kg; continuous infusion of 7.0 U·kg⁻¹·h⁻¹) were infused only during the clamp experiments to prevent the expected insulin-induced decline in plasma FFA concentration.

Materials

The mixture of U-13C-labeled fatty acids, 86.2% enriched, and [13C3]glycerol, 99% enriched, was obtained from Cambridge Isotope Laboratories (Andover, MA). The composition of the lipid mixture was: palmitic acid (16:0), 51.7%; palmitoleic acid (16:1), 9.3%; stearic acid (18:0), 5.5%; oleic acid (18:1), 17.9%; linoleic acid (18:2), 14.1%; and linolenic acid (18:3), 1.5%. Human albumin 5% was purchased from Baxter Healthcare (Glendale, CA). The lipid emulsion (Intralipid, 20%), containing linoleic (58%), oleic (26%), palmitic (10%), linolenic (9%), and stearic (3.5%) acids, was obtained from Kabi (Clayton, NC), heparin was from Elkins Sinn (Cherry Hill, NJ), and indocyanine green came from Ceton Dickinson Microbiology Systems (Cockeysville, MD).

Procedures

Catheter placement. On the morning of the study, volunteers were brought to a vascular radiology suite, where the right groin was prepared and draped in a sterile fashion. A lead glove was placed over the genitalia before the procedure. After patient preparation, the right common femoral vein was punctured and a 6-Fr sheath was inserted. Through this sheath, a straight 5-Fr catheter with several side holes near its tip was manipulated into the right or middle hepatic vein. This catheterization was performed by using a deflecting-tip 0.035” guidewire within the straight catheter. After the catheter was positioned into the hepatic vein, a digital venogram was performed to verify placement, and both the sheath and catheter were infused with heparinized saline to maintain patency. The position of the catheter was confirmed...
again by a plain view abdominal X ray immediately after the end of the study. A short, straight 4-Fr catheter connected to a pressurized flush setup was then placed retrograde into the right common femoral artery. After both catheters and the sheath had been sutured in place, a sterile transparent dressing was used to cover the vascular entry sites.

In vivo VLDL-TG tracer production. The effect of hyperglycemia on the absolute rate of VLDL-TG secretion was determined using a primed-constant infusion of VLDL-TG labeled with [13C3]glycerol. Production of the VLDL-TG tracer was performed in vivo 3–4 days before the infusion studies, because an appropriate tracer is not available commercially. Because of the in vivo production procedure, only autologous infusions were used. The VLDL-TG labeling was performed under exactly the same conditions as the infusion studies (i.e., in the postabsorptive state for the basal experiments and during hyperglycemia for the clamp experiments) to account for possible changes in the properties of the VLDL-TG due to the metabolic state of the individual. Volunteers ingested 1 g of [13C3]glycerol, and, ~4 h later, plasmapheresis (400 ml) was performed, and the VLDL-TG (which contained the [13C3]glycerol) were isolated from plasma under sterile conditions by means of ultracentrifugation. First, chylomicrons and exogenous TG (Svedberg flotation constant (Sf) > 400) were isolated by overlaying with a density (d) = 1.006 g/ml solution and spinning in a SW 28 rotor (model L-7–55, Beckman Instruments, Palo Alto, CA) for 30 min at 45,000 g and 15°C. After ultracentrifugation, the top part of the solution, containing the chylomicrons and exogenous TG, was removed, and the remaining solution was spun for isolation of VLDL (Sf = 20–400). This was accomplished by overlaying the solution with 0.9% NaCl (d = 1.006 g/ml) and spinning in a SW 28 rotor for 16 h at 78,000 g. The newly labeled VLDL-TG were checked for sterility and subsequently stored at 4°C until used as a tracer 2–3 days later. This procedure does not deplete subjects of any red blood cells and produces enough tracer for 2–3 h of infusion plus priming dose.

Blood flow. Blood flow was determined using a constant infusion of indocyanine green dissolved in 0.9% saline. The dye was infused through the femoral artery catheter at the rate of 0.5 mg/min for 55 min, starting 60 min before the end of the tracer infusion, and blood samples were taken at 40, 45, 50, and 55 min simultaneously from the hepatic vein and a peripheral vein. The average plasma flow of the four time points was used to calculate arteriovenous balances. The concentrations of the dye in the infusate and in serum samples were determined using a spectrophotometer set at 805 nm. Splanchnic plasma flow was determined by dividing the fatty acid enrichment (6 ml) were collected into prechilled tubes containing 120 µl of 0.2 M EGTA to prevent in vitro lipolysis (Sidossis and Wolfe, unpublished observation). Plasma was separated by centrifugation shortly after sampling and was stored at 4°C until further processing. Chylomicrons and exogenous lipid-derived TG (Sf > 400) were isolated from 3 ml of plasma by overlaying with a d = 1.006 g/ml solution and spinning in a 70.1 Ti rotor for 30 min, 45,000 g at 15°C (1). After ultracentrifugation, the top part of the solution, containing the chylomicrons and exogenous TG, was removed, and the remaining solution was spun for isolation of VLDL (Sf = 20–400). This was accomplished by overlaying the solution with 0.9% NaCl (d = 1.006 g/ml) and spinning in a 70.1 Ti rotor for 22 h, 110,000 g at 15°C. After ultracentrifugation, VLDL were carefully removed from the top portion of the tube by slicing the tube and were stored at 4°C until further processing (1). TG concentration in the VLDL fraction was determined enzymatically (Sigma Diagnostics, St. Louis, MO).

For determination of the VLDL-TG bound fatty acid and glycerol carbon enrichment, TG in the VLDL fraction were isolated by thin-layer chromatography and hydrolyzed, and the fraction containing the fatty acids and glycerol was collected in separate tubes. FFA were combusted (Elemental Analyzer, Carlo Erba 1500) and the 13C-to-12C ratio was determined by IRMS. Glycerol was isolated using HPLC on a carbohydrate Aminex HPX-87P column. The recovered glycerol was then combusted, and the 13C-to-12C ratio was determined by IRMS.

Samples for determination of substrate concentration and enrichment (6 ml) were collected into prechilled tubes containing 120 µl of 0.2 M EGTA to prevent in vitro lipolysis, and plasma was immediately separated by centrifugation and frozen until further processing. For determination of plasma fatty acid enrichment, the fatty acids were extracted from plasma, isolated by thin-layer chromatography, and combusted (Elemental Analyzer, Carlo Erba 1500), and the 13C-to-12C ratio was determined by IRMS. Plasma β-hydroxybutyrate (β-OHB) concentration was determined enzymatically (Sigma Diagnostics). Blood glucose and plasma lactate concentrations were measured on a 2300 STAT analyzer (Yellow Springs Instruments, Yellow Springs, OH). Plasma insulin concentration was determined using a radioimmunoassay method (INCSTAR, Stillwater, MN). Individual and total plasma fatty acid concentrations were determined by gas chromatography (GC; Hewlett Packard 5890), with heptadecanoic acid as internal standard.

Calculations

Whole body kinetics. The rate of appearance of fatty acids in plasma (Rn) was determined by dividing the fatty acid
tracer infusion rate \( I \) by the arterial fatty acid enrichment \( E_A \)

\[
R_s = \frac{I}{E_A}
\]

Splanchnic region substrate kinetics and oxidation. The fatty acid kinetic parameters for the splanchnic region were calculated using femoral artery and hepatic venous measurements and splanchnic blood flow.

The percentage of FFA taken up by the splanchnic region \( \%FFA_{\text{ox}} \) that is released as CO2 was calculated by dividing the arteriovenous labeled CO2 concentration difference by the arteriovenous FFA tracer concentration difference

\[
\%FFA_{\text{ox}} = \left( E_{\text{CHV}} \times C_{\text{CHV}} - E_{\text{CA}} \times C_{\text{CA}} \right) / \left( E_A \times C_A - E_{\text{HV}} \times C_{\text{HV}} \right)
\]

where \( E_{\text{CA}} \) and \( E_{\text{CHV}} \) are the respective enrichments of CO2 in the artery and hepatic vein, \( C_{\text{CA}} \) and \( C_{\text{CHV}} \) are the concentrations of CO2 in the artery and hepatic vein, \( E_A \) and \( E_{\text{HV}} \) are the enrichments of FFA in the artery and hepatic vein, and \( C_A \) and \( C_{\text{HV}} \) are arterial and hepatic venous concentrations of FFA.

The fractional extraction of labeled FFA by the splanchnic region \( \%FFA_{\text{extr}} \) was determined by dividing the uptake of label by the arterial concentration of label

\[
\%FFA_{\text{extr}} = \left( E_A \times C_A - E_{\text{HV}} \times C_{\text{HV}} \right) / \left( E_A \times C_A \right)
\]

The absolute rate of uptake of FFA by the splanchnic region \( \text{FFA}_{\text{Rd}} \) was determined by multiplying the fractional extraction of labeled FFA by the arterial (unlabeled) FFA concentration times the blood flow

\[
\text{FFA}_{\text{Rd}} = \%FFA_{\text{extr}} \times C_A \times BF
\]

Plasma fatty acid oxidation across the splanchnic region \( \text{FFA}_{\text{ox}} \) was calculated by multiplying the absolute rate of FFA uptake across the region by the percentage of FFA uptake that is released as CO2. This value was then divided by the acetate correction factor \( \text{(ac)} \) obtained after 15 h of labeled acetate infusion

\[
\text{FFA}_{\text{ox}} = \text{FFA}_{\text{Rd}} \times \%FFA_{\text{ox}} / \text{ac}
\]

VLDL-TG kinetics. Rate of appearance of VLDL-TG was calculated by dividing the rate of infusion of labeled VLDL-TG-glycerol by the arterial VLDL-TG-glycerol carbon enrichment. The contribution of labeled and unlabeled VLDL-TG infused was taken into account in the estimation of VLDL-TG kinetics by subtracting the VLDL-TG infusion rate from the estimated VLDL-TG secretion. The percentage of plasma VLDL-TG that was derived from plasma FFA (as opposed to non-plasma-derived FFA) was obtained by dividing the plasma VLDL-TG-FFA carbon enrichment by the plasma FFA carbon enrichment at plateau. The percentage of plasma VLDL-TG that did not derive from plasma FFA was calculated by subtracting the percentage of VLDL-TG that was derived from plasma FFA from 100%. VLDL-TG clearance was calculated by dividing the VLDL-TG secretion rate by arterial VLDL-TG concentration.

Statistical Analysis

Results are reported as means ± SE. The effects of glucose infusion on the various parameters were evaluated using a two-tailed paired Student’s t-test. Significance was set at the 0.05 level.

RESULTS

Labeled Carbon Content

Isotopic plateau for fatty acid and CO2 carbon enrichment in the basal and clamp experiments was reached when the measurements of labeled carbon content were made. The mean plasma \(^{13}\text{C}\) fatty acid content (plasma fatty acid carbon enrichment times plasma fatty acid concentration) in the artery and hepatic vein is presented in Fig. 1; the mean plasma \(^{13}\text{CO}_2\) content (plasma CO2 carbon enrichment times plasma CO2 concentration) in the artery and hepatic vein is presented in Fig. 2.

Substrate and Hormone Concentrations

Plasma glucose concentration decreased overnight, but it remained constant during the last 8 h of the study in the basal state (Fig. 3, Tables 1 and 2). During the clamp, glucose concentration increased shortly after the start of dextrose infusion, and it remained relatively constant during the last 6 h of the study (Fig. 3, Tables 1 and 2). To achieve the desired blood glucose levels, dextrose was infused during the clamp experiments at an average rate of 8 mg·kg\(^{-1}\)·min\(^{-1}\). Insulin concentration increased significantly in response to dextrose infusion (Table 1). Despite the significant increase in insulin, plasma FFA concentrations were maintained constant in the basal and clamp experiments by the infusion of lipid and heparin (Tables 1 and 2). The concentration of \(^{1}-\)hydroxybutyrate decreased to undetectable levels during the clamp (Tables 1 and 2).

Splanchnic Fatty Acid Kinetics and Oxidation

We were able to maintain constant the rate of appearance of fatty acids in the basal and clamp studies by means of lipid and heparin infusion. This is crucial, because the primary goal of the present study was to...
isolate the effects of hyperglycemia on splanchnic fatty acid kinetics and oxidation. Oxidation of plasma-derived fatty acids by the splanchnic region decreased significantly during the clamp (Fig. 4). The decrease in splanchnic fatty acid oxidation was not related to a decline in FFA availability to the splanchnic region, as the absolute FFA uptake by the splanchnic region was similar in the basal and clamp experiments (0.7 ± 0.3 and 0.7 ± 0.5 µmol·kg⁻¹·min⁻¹, respectively). Ketone production was 1.5 ± 0.3 µmol·kg⁻¹·min⁻¹ in the basal state but dropped to undetectable levels during the clamp, suggesting complete inhibition of ketone body production during hyperglycemia. Interestingly, ketone body production was nearly completely inhibited during hyperglycemic hyperinsulinemia, even though splanchnic fatty acid oxidation to CO₂ was proceeding at a significant rate (Fig. 4).

VLDL-TG Concentration and Kinetics

VLDL-TG-glycerol carbon enrichment was at a steady state during the last 20 min of the experiments (Fig. 5). The average value of three measurements (i.e., 100, 110, and 120 min) was used for calculating VLDL-TG rate of appearance. The rate of VLDL-TG appearance in plasma increased from 0.35 ± 0.07 in the basal state to 0.53 ± 0.11 µmol·kg⁻¹·min⁻¹ during the clamp (P < 0.05, Fig. 6). Plasma-derived fatty acids contributed 91 ± 6% of the fatty acids for VLDL-TG formation in the basal state and 70 ± 22% during the clamp (P < 0.05). Non-plasma-derived fatty acids contributed the remaining FFA. Plasma VLDL-TG concentration increased significantly during the clamp (Tables 1 and 2). The increase in plasma VLDL-TG concentration during the clamp appears to be mainly due to the increased VLDL-TG secretion, because the clearance of VLDL-TG did not change significantly (Table 3).

Effect of Exogenous Glucose Infusion on Blood and Breath CO₂ Carbon Enrichment

Infusion of unlabeled "cold" glucose to increase blood glucose concentration during the clamp experiments is expected to increase blood and breath CO₂ carbon enrichment (19, 30). To minimize the possibility that the infused glucose could affect the tracer estimation of fatty acid oxidation, we infused the fatty acid tracer at a high rate to increase the breath and blood CO₂ carbon enrichment enough so that the contribution of naturally occurring ¹³C in glucose would become negligible. Thus, whereas the average breath CO₂ carbon enrichments in the present study were 1.4 ± 0.3 ± 1.0e⁻⁰⁴, glucose infused at rates similar to those used in the present experiment is expected to contribute no more than 7.0e⁻⁰⁵ to 7.0e⁻⁰⁵ to CO₂ carbon enrichment (22).

Effect of Exogenous Glucose Infusion on Blood and Breath CO₂ Carbon Enrichment

Table 1. Substrate and hormone concentrations in the artery in the basal state and during hyperglycemic hyperinsulinemia

<table>
<thead>
<tr>
<th></th>
<th>Basal</th>
<th>Clamp</th>
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<tbody>
<tr>
<td>Glucose, mmol/l</td>
<td>4.3 ± 0.2</td>
<td>7.9 ± 0.4*</td>
</tr>
<tr>
<td>Insulin, µU/ml</td>
<td>5.5 ± 1.1</td>
<td>35.8 ± 11.4*</td>
</tr>
<tr>
<td>FFA, mmol/l</td>
<td>0.45 ± 0.1</td>
<td>0.41 ± 0.1</td>
</tr>
<tr>
<td>β-OHB, mmol/l</td>
<td>0.3 ± 0.02</td>
<td>Undetectable*</td>
</tr>
<tr>
<td>VLDL-TG, mmol/l</td>
<td>0.28 ± 0.06</td>
<td>0.43 ± 0.05*</td>
</tr>
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</table>

Values are means ± SE for 5 volunteers. FFA, free fatty acid; β-OHB, β-hydroxybutyrate; VLDL-TG, very low density lipoprotein-triglyceride(s). *P < 0.05 vs. basal.

Table 2. Substrate concentrations in the hepatic vein in the basal state and during hyperglycemic hyperinsulinemia

<table>
<thead>
<tr>
<th></th>
<th>Basal</th>
<th>Clamp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>4.8 ± 0.1</td>
<td>6.3 ± 0.5*</td>
</tr>
<tr>
<td>FFA</td>
<td>0.38 ± 0.02</td>
<td>0.34 ± 0.03</td>
</tr>
<tr>
<td>β-OHB</td>
<td>0.45 ± 0.02</td>
<td>Undetectable*</td>
</tr>
<tr>
<td>VLDL-TG</td>
<td>0.31 ± 0.03</td>
<td>0.49 ± 0.07*</td>
</tr>
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</table>

Values are means ± SE for 5 volunteers expressed in mmol/l. *P < 0.05 vs. basal.
DISCUSSION

This is the first time that the effect of moderate hyperglycemia on splanchnic fatty acid uptake and oxidation has been determined simultaneously with the absolute rate of VLDL-TG secretion in human volunteers. We found that hyperglycemia decreased hepatic fatty acid oxidation by ~50%. However, fatty acid uptake by the splanchnic region remained unchanged, and, thereby, the availability of hepatic fatty acids for TG synthesis significantly increased. Non-plasma-derived fatty acids also contributed to TG synthesis during hyperglycemic hyperinsulinemia, because ~30% of total VLDL-TG coming out of the liver did not originate from plasma FFA. The channeling of fatty acids toward TG synthesis instead of oxidation in combination with increased non-plasma-derived fatty acid availability resulted in a significant increase in VLDL-TG secretion during hyperglycemia. Furthermore, the rate of VLDL-TG catabolism or clearance from the circulation was not decreased during hyperglycemia. The channeling of fatty acids into hepatic TG synthesis rather than oxidation may be a mechanism for explaining the observed hypertriglyceridemia in insulin-resistant states in which hyperglycemia and hyperinsulinemia occur concomitantly (e.g., upper body obesity, NIDDM, high-carbohydrate diets).

Our model measures splanchnic region oxidation of plasma FFA rather than splanchnic fatty acid oxidation by all sources (i.e., plasma, visceral fat, gut). One would presume that release of fatty acids from visceral fat would be greater in the fasted state than after the glucose infusion. Thus the drop in total splanchnic fatty acid oxidation observed during hyperglycemia is likely even greater than the drop in plasma fatty acid oxidation measured in this experiment. Likewise, we are measuring splanchnic uptake of plasma FFA rather than splanchnic uptake of fatty acid by all sources. One would expect that, because extraction of plasma FFA by the splanchnic region was the same in fasted and glucose-infused subjects, the uptake of fatty acids by the splanchnic region that were released by visceral fat would have been higher in the fasted subjects.

Fatty acid oxidation across the splanchnic region decreased significantly during hyperglycemia. This finding is similar to our observation at the whole body level.

Table 3. Lipid kinetics in the basal state and during hyperglycemic hyperinsulinemia determined isotopically

<table>
<thead>
<tr>
<th></th>
<th>Basal</th>
<th>Clamp</th>
</tr>
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<tbody>
<tr>
<td>Systemic rate of FFA appearance (µmol·kg⁻¹·min⁻¹)</td>
<td>4.8 ± 0.6</td>
<td>4.4 ± 0.6</td>
</tr>
<tr>
<td>FFA uptake by splanchnic region (µmol·kg⁻¹·min⁻¹)</td>
<td>1.4 ± 0.2</td>
<td>2.2 ± 0.9</td>
</tr>
<tr>
<td>Rate of VLDL-TG secretion (µmol·kg⁻¹·min⁻¹)</td>
<td>0.35 ± 0.07</td>
<td>0.53 ± 0.11*</td>
</tr>
<tr>
<td>Rate of VLDL-TG clearance, ml·kg⁻¹·min⁻¹</td>
<td>1.4 ± 0.3</td>
<td>1.3 ± 0.3</td>
</tr>
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</table>

Values are means ± SE for 5 volunteers, expressed in µmol·kg⁻¹·min⁻¹. *P < 0.05 vs. basal.
(22, 23). Although the exact mechanism mediating this response was not elucidated by our study, we have recently presented evidence suggesting that hyperglycemia-hyperinsulinemia decreases fat oxidation by direct inhibition of fatty acid entry into the mitochondria (22).

In this study we infused only glucose, and we allowed the concentration of insulin (as well as that of other hormones and mediators) to change spontaneously. It is thus not possible to specifically assign responsibility to hyperglycemia, per se, for the observed changes, as opposed to changes caused by secondary alterations in the concentration of other hormones or mediators (e.g., insulin). Nonetheless, it is likely that hyperglycemia per se, and not the resulting hyperinsulinemia, most probably mediated the increase in TG secretion in the present study. In previous studies, it was found that acute hyperinsulinemia inhibits, rather than stimulates, VLDL-TG secretion when glucose concentration is maintained at a normal level by use of the euglycemic clamp technique (13, 14). The inhibitory effect of insulin on TG secretion is in part due to decreased FFA availability, resulting from the insulin-induced inhibition of peripheral lipolysis (14). In the current study, the infusion of lipids prevented a change in FFA delivery during hyperglycemia. We can extrapolate from the findings of the present study to suggest a mechanism to explain the observed hypertriglyceridemia in insulin-resistant states. Systemic hyperglycemia enhances glucose uptake and oxidation in the liver but does not inhibit hepatic FFA uptake. Increased hepatic glucose oxidation in turn inhibits fatty acid oxidation and diverts fatty acids toward TG synthesis, resulting in accelerated TG synthesis and secretion into the systemic circulation. According to our model, an increase in plasma glucose concentration is required for TG production to increase. This notion is supported by the finding of the Diabetes Control and Complications Trial that, when hyperglycemia is well controlled via intensive insulin treatment, serum TG concentration significantly decreases (1).

Using fatty acid tracers and arteriovenous balance across the splanchnic region, we calculated that, during hyperglycemia, ~0.95 µmol TG·kg⁻¹·min⁻¹ could have been formed from FFA available to the liver that was not oxidized to CO₂ or ketones. This estimation significantly exceeds the measured VLDL-TG secretion rates during the clamp (i.e., 0.53 µmol TG·kg⁻¹·min⁻¹). If all of the fatty acids available for TG synthesis were incorporated into TG, then it seems logical to suggest that a significant amount of TG was deposited in the liver of the volunteers in our study, thereby significantly increasing hepatic TG content. However, under normal circumstances, little net hepatic fat accumulation occurs in healthy subjects. Thus TG that were not secreted and presumably accumulated in the liver of the volunteers during hyperglycemia should exit the liver at a later time. In fact, our observation that in the postabsorptive state TG secretion (0.35 mmol TG·kg⁻¹·min⁻¹) exceeded the availability for TG synthesis (0.25 mmol TG·kg⁻¹·min⁻¹) suggests that TG that accumulate in the liver during excess FFA availability slowly exit the liver when both FFA and glucose levels return to normal. This would suggest that the liver lacks the ability to effectively and timely match the rate of TG production and secretion, resulting in temporary imbalances in the hepatic TG pool.

The present findings may aid our understanding of the pathophysiology of hepatic steatosis. Accumulation of TG in the liver is a phenomenon common in many disease states, such as thermal injury (9, 10), obesity (24), diabetes (5), hepatitis C infection (15), and critical illness (9, 10, 27), and in pregnancy (17). Unlike steatohepatitis, common in alcoholic fatty liver that eventually leads to cirrhosis, in hepatic steatosis observed in the above disease states (also called nonalcoholic or nutritional hepatic steatosis), the condition is reversible on patient improvement. In all of the above-mentioned conditions, hyperglycemia occurs concomitantly with normal or elevated systemic fatty acid concentration, which, according to the findings of the present study, favors increased TG production and accumulation in the liver. A period of normal glucose and FFA concentration is required for the accumulated hepatic TG to be secreted. However, the patient is constantly in a hyperglycemic state, and thereby TG continue to accumulate in the liver. Return to normoglycemia after patient improvement increases hepatic fatty acid oxidation, thereby decreasing hepatic fatty acid availability for TG synthesis. Gradually the liver secretes the stored TG and returns to its normal predisease state.

In summary, the results of the present study suggest that glucose infusion at a rate sufficient to induce moderate hyperglycemia attenuates fatty acid oxidation in the splanchnic region in human volunteers, even when fatty acid availability is maintained constant via exogenous lipid administration. This adaptation results in a significant increase in the VLDL-TG secretion rate and concentration in plasma and could, therefore, provide a mechanism for the observed systemic hypertriglyceridemia and hepatic steatosis in insulin-resistant states.

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Address for reprint requests: L. S. Sidossis, Metabolism Unit, Shriners Burns Institute, Univ. of Texas Medical Branch, Galveston, TX 77550.

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