The operation of the glucose-fatty acid cycle has been postulated to occur in various tissues, particularly the heart (16). Since its introduction, the 'glucose-fatty acid cycle' has been studied extensively in human volunteers. We have recently shown that intracellular glucose availability directly regulates fatty acid oxidation in the setting of constant free fatty acid (FFA) availability (25). This notion represents the exact opposite of the concept of fatty acid-mediated regulation of glucose oxidation originally proposed by Randle et al. (15), namely, that fatty acid availability and oxidation control the rate of glucose disposal. In subsequent papers, we presented evidence suggesting that glucose elicits its effect on fat oxidation by controlling the rate of fatty acid entry into the mitochondria (23, 24). According to those findings (23, 24), an increase in glycolytic flux inhibits long-chain fatty acid entry into the mitochondria, possibly via inhibition of carnitine palmitoyltransferase I (CPT I).

The traditional "glucose-fatty acid cycle" hypothesis was developed from studies performed in rat diaphragm and heart muscle (16). Since its introduction, the operation of the glucose-fatty acid cycle has been extensively examined in resting human volunteers, with conflicting results (see Refs. 1, 2, 7, 9, 26, 27, 29). It may be that the difficulty in confirming the findings of Randle and co-workers (15, 16) at the whole body level is that only specific tissues respond as predicted by the in vitro studies, and that the contribution of those tissues does not account for a sufficient percentage of resting substrate metabolism to be detected at the whole body level. In this regard, although skeletal muscle comprises a large percentage of the lean body mass, at rest it is less metabolically active than the splanchnic region. It is thus possible that whole body responses that have been previously observed predominantly reflected the splanchnic region metabolism, and that different responses in muscle tissue were obscured.

In our previous studies (24, 25), the effect of glycolytic flux on fatty acid oxidation was determined at the whole body level. This precluded drawing any conclusions as to the nature of glucose-fatty acid interaction in specific tissues, because whole body measurements reflect the sum of kinetics and oxidation of the various regions of the body. Therefore, in the current study we examined the effect of dextrose infusion, causing moderate hyperglycemia (~8 mM) and hyperinsulinemia (35.8 ± 11.4 µU/ml) on fatty acid kinetics and oxidation simultaneously at the whole body level, across the splanchic region, and across the leg in five healthy volunteers. Plasma FFA concentration was maintained constant via exogenous infusion of lipids and heparin during dextrose infusion, whereas insulin concentration was allowed to change in response to dextrose infusion. 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 concentrations. Our findings are also pertinent to the common circumstance of intravenous glucose infusions for nutritional purposes in hospitalized patients.

METHODS
Volunteers
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 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.

Materials
U-13C-labeled fatty acids, 86.2% enriched, were obtained from Cambridge Isotope Laboratories (Andover, MA). The
composition of the fatty acid 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 (50%), oleic (26%), palmitic (10%), linolenic (9%), and stearic (3.5%) acids, was obtained from Kabi (Clayton, NC). Heparin came from Elkins Sinn (Cherry Hill, NJ), and indocyanine green came from Ceton Dickinson Microbiology Systems (Cocksville, MD).

Experimental Design

Volunteers were studied in the basal state (overnight fast) and, on a different occasion, during 15 h of hyperglycemia-hyperinsulinemia. The order of the studies was randomized. The volunteers were 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 of the same day, catheters were placed percutaneously into an antecubital vein for infusion and into a contralateral dorsal hand vein for sampling. The catheters were kept patent by infusion of 0.9% NaCl. After the volunteers had rested for 30 min, background blood samples were collected, and one of the following two randomly assigned experimental protocols was performed.

Protocol 1: basal state. Infusion of the 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. The bicarbonate pool was primed using NaH13CO3 (25 μmol/kg). At 7 AM the following morning, the volunteers were transferred to the Angiography and Interventional Radiology Center of the University of Texas Medical Branch, in the same building as the GCRC, where femoral artery, hepatic vein, and femoral vein catheters were inserted, as described in Procedures. Thirty to sixty minutes after catheter placement, the volunteers were transferred back to the GCRC for completion of the study.

The U-13C-labeled fatty acid infusion continued uninterrupted during catheter placement by use of a battery-operated pump and for two more hours after volunteers returned to the GCRC. Blood samples were drawn from the femoral artery, femoral vein, and hepatic vein at 100, 110, and 120 min after volunteers returned to the GCRC. At the same time points, breath samples were collected for determination of breath CO₂ carbon enrichment.

Whole body oxygen consumption (VO₂) and carbon dioxide production (VCO₂) were measured over 30 min during the last hour of the study. Splanchnic and leg blood flow were determined using a constant infusion of indocyanine green, as described in Procedures. After completion of the study all catheters were removed, and the volunteers remained in the hospital for several hours for observation.

Protocol 2: glucose infusion (clamp). Protocol 2 was the same as 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. 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) were infused together with heparin (bolus of 7.0 U/kg; continuous infusion of 7.0 U·kg⁻¹·h⁻¹) to prevent the expected insulin-induced decline in plasma FFA concentration.

Procedures

Catheter placement. In 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 placed. 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 using a deflecting-tip 0.035" guidewire within the straight catheter. After the catheter had been 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 was then placed retrograde into the right common femoral artery, and it was also connected to a pressurized flush setup. After both catheters and the sheath were sutured in place, a sterile transparent dressing was used to cover the vascular entry sites.

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 during the last hour of the study, and blood samples were taken at 40, 45, 50, and 55 min simultaneously from the hepatic vein, the femoral vein, and an antecubital vein. The concentrations of the dye in the infusate and in serum samples were determined using a spectrophotometer set at 805 nm. Splanchnic plasma flow (SPF) was calculated via the formula SPF = I/(PV – HV), where I is the infusion rate of the dye, PV is the concentration of dye in an antecubital vein, and HV is the concentration of dye in the hepatic vein. Splanchnic blood flow (SBF) was calculated via the formula SBF = SPF/(1 – Hct), where Hct is the hematocrit. Leg plasma flow (LPF) was calculated via the formula LPF = I/(FV – PV), where I is the infusion rate of the dye, PV is the concentration of dye in an antecubital vein, and FV is the concentration of dye in the femoral vein. Splanchnic blood flow (SBF) was calculated via the formula SBF = SPF/(1 – Hct), where Hct is the hematocrit. Leg plasma flow (LPF) was calculated via the formula LPF = I/(FV – PV), where I is the infusion rate of the dye, PV is the concentration of dye in an antecubital vein, and FV is the concentration of dye in the femoral vein of the leg into which the dye is infused. Leg blood flow (LBFW) was calculated via the formula LBF = LPF/(1 – Hct).

Determination of acetate correction factor. On a different occasion, a 15-h primed (45 mg/kg), continuous infusion (1.5 μmol·kg⁻¹·min⁻¹) of [U-13C]acetate was performed in the postabsorptive state to determine the “acetate correction factor,” i.e., the acetate-carbon recovery rate, for use in the calculation of fatty acid oxidation rate (21). Unlike the traditional “bicarbonate correction factor,” the acetate correction factor fully accounts for label fixation occurring at any point between the entrance of labeled acetyl-CoA into the tricarboxylic acid cycle and the appearance of labeled CO₂ in breath (21, 22). It was not necessary to determine acetate carbon recovery across the splanchnic region and the leg in this study, because we have recently shown that acetate recovery across these sites is similar to whole body acetate recovery (13).

Assays

Breath. Ten milliliters of expired air were injected into evacuated tubes for determination of the 13CO₂-to-12CO₂
ratio. Briefly, CO₂ was isolated from the breath samples before analysis by isotope ratio mass spectrometry (IRMS; SIRA VG Isotech, Cheshire, UK) by passage through a water trap followed by condensation in a liquid nitrogen trap to allow other gases to be evacuated. The ¹³C₂O₂-to-¹²C₂O₂ ratio was then determined by IRMS. Results are presented as percent enrichment, i.e., ¹³C₂O₂-to-¹²C₂O₂ ratio times 100.

Blood. Blood CO₂ analysis. All blood samples for CO₂ analysis were collected into prechilled tubes containing sodium heparin. Blood CO₂ concentration was measured immediately using a 965 Ciba Corning CO₂ Analyser, and the remaining blood samples were kept frozen until further analysis. For the determination of blood CO₂ enrichment, 5–10 ml of hyperphosphoric acid were added to 1 ml blood in a sealed tube to release the CO₂. The ¹³C-to-¹²C ratio in the headspace was then determined by IRMS. Results are presented as percent enrichment, i.e., ¹³C-to-¹²C ratio times 100.

PLASMA FFA ENRICHMENT AND SUBSTRATE CONCENTRATIONS. Blood samples (6 ml) for determination of substrate concentration and enrichment 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. Individual and total plasma fatty acid concentrations were determined by gas chromatography (GC; Hewlett-Packard 5890), with heptadecanoic acid as internal standard. Plasma β-hydroxybutyrate concentration was determined enzymatically (Sigma Diagnostics, St. Louis, MO). Plasma glucose concentration was measured on a 2300 STAT analyzer (Yellow Springs Instruments, Yellow Springs, OH). Plasma insulin concentration was determined using a radioimmunoassay method (INCSTAR, Stillwater, MN).

Calculations

The rate of appearance of fatty acids in plasma (Rₘ) was determined by dividing the fatty acid tracer infusion rate (I) by the arterial fatty acid carbon enrichment (EA)

\[ R_m = I/EA \]

The fatty acid kinetic parameters for the splanchnic region were calculated using femoral artery and hepatic venous venous measurements and splanchnic blood flow, whereas the kinetic parameters for the leg were determined using femoral artery and femoral venous measurements and leg blood flow. All values calculated for the leg represent kinetics and oxidation across one leg only. The net rate of FFA uptake or release across the splanchnic region (NBspl) and the leg (NBleg) was determined by multiplying the arteriovenous FFA concentration difference times the plasma flow, that is

\[ NB_{spl} = (CA - CHV) \times HPF \]
\[ NB_{leg} = (CA - CFV) \times LPF \]

where CA, CHV, and CFV are arterial, hepatic venous, and femoral venous concentrations of FFA, respectively, and HPF and LPF are hepatic and leg plasma flow, respectively.

The percentage of FFA taken up by either the splanchnic region (%FFAoxspl) or leg (%FFAoxleg) that is released as CO₂ was calculated by dividing the arteriovenous labeled CO₂ concentration difference by the arteriovenous FFA tracer concentration difference

\[ \%FFA_{ox} = \frac{(EHVCO₂ - CHVCO₂ - EACO₂ - CACO₂)}{(EA \cdot CA - EHV \cdot CHV)} \]

%FFAoxleg

\[ \%FFA_{ox} = \frac{(EFVCO₂ - CFVCO₂ - EACO₂ - CACO₂)}{(EA \cdot CA - EFV \cdot CFV)} \]

where EACO₂, EHVCO₂, and EFVCO₂ are the enrichments of CO₂ in the artery, hepatic vein, and femoral vein, respectively, and CACO₂, CHVCO₂, and CFVCO₂ are the concentrations of CO₂ in the artery, hepatic vein, and femoral vein, respectively, and EA, EHV, and EFV are the enrichments of FFA in the artery, hepatic vein, and femoral vein, respectively.

The fractional extraction of labeled FFA by the splanchnic region (%FFAoxspl) and the leg (%FFAoxleg) was determined by dividing the uptake of label by the arterial concentration of label

\[ %FFA_{ox} = \frac{(CA \times EHV \times CHV)}{(EA \times CA)}/\frac{(EACO₂ \cdot CACO₂)}{(EACO₂ \cdot CACO₂)} \]

The absolute rate of uptake of FFA by the splanchnic region (FFARdspl) and leg (FFARdleq) was determined by multiplying the fractional extraction of labeled FFA by the arterial (unlabeled) plasma FFA concentration times the plasma flow

\[ FFAR_{dspl} = \%FFA_{oxspl} \times CA \times HPF \]
\[ FFAR_{dleq} = \%FFA_{oxleq} \times CA \times LPF \]

Plasma fatty acid oxidation across the splanchnic region (FFAoxspl) and the leg (FFAoxleg) was subsequently calculated by multiplying the absolute rate of FFA uptake across each region by the percentage of FFA uptake that is released as CO₂. This value was then divided by the acetate correction factor (ar) obtained using labeled acetate infusion.

\[ FFA_{dspl} = FFAR_{dspl} \times \%FFA_{oxspl}/ar \]
\[ FFA_{dleq} = FFAR_{dleq} \times \%FFA_{oxleq}/ar \]

The percentage of FFA taken up by either the splanchnic region (%FFAketspl) or leg (%FFAketleg) that is released as ketones was calculated by dividing the release of ketones by the uptake of FFA

\[ %FFA_{ket} = \frac{(CKHV - CKA) \times HPF}{FFAR_{dspl}} \]
\[ %FFA_{ket} = \frac{(CKA - CKFV) \times LPF}{FFAR_{dleq}} \]

where CKA, CKHV, and CKFV are the ketone concentrations in the artery, hepatic vein, and femoral vein, respectively.

The percentage of Rₘ FFA which is taken up by either the splanchnic region (%RₘFFAoxspl) and by the leg (%RₘFFAoxleg) is calculated by dividing the absolute uptake of FFA by Rₘ FFA.

\[ %RₘFFA_{dspl} = \frac{FFAR_{dspl}}{Rₘ FFA} \]
\[ %RₘFFA_{dleq} = \frac{FFAR_{dleq}}{Rₘ FFA} \]
Statistical Analysis

Results are reported as means ± SE. The effects of the 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
Systemic and Regional Substrate Concentrations

Plasma glucose concentrations were significantly higher during the clamp compared with the basal state (Fig. 1A). To achieve the desired plasma glucose levels in the clamp experiments, dextrose was infused at an average rate of 608 ± 49 mg/min. In response to dextrose infusion, insulin concentration increased from 5.5 ± 1.1 in the basal to 35.8 ± 11.4 µU/ml during the clamp (P < 0.05).

We were able to maintain arterial plasma FFA concentration similar in the basal and clamp experiments via infusion of lipids and heparin (Fig. 1B). This was important, because if we had allowed fatty acid availability to the splanchnic region and leg to vary between the two trials, we would not have been able to differentiate the direct effects of hyperglycemia-hyperinsulinemia from the effect of decreasing fatty acid availability on regional fatty acid kinetics and oxidation.

The concentration of β-hydroxybutyrate (β-OHB) was significantly higher in the hepatic vein than in the artery and the femoral vein in the basal experiments (Fig. 1C). During hyperglycemia, β-OHB concentration decreased to levels not different from zero (Fig. 1C).

Whole Body and Regional Fatty Acid Kinetics and Oxidation

Plasma fatty acid carbon enrichment was constant during the last 20 min of labeled fatty acid infusion (Table 1). These values were used in the calculation of fatty acid kinetics and oxidation.

The rates of appearance of fatty acids in plasma coming from endogenous (basal state) or endogenous plus exogenous (clamp) sources were 4.8 ± 0.6 and 4.4 ± 0.6 µmol·kg⁻¹·min⁻¹, respectively. Net uptake of fatty acids across the splanchnic region was similar in the basal state and the clamp experiments (Fig. 2). We did not observe significant net fatty acid uptake or release across the leg in either state (i.e., basal vs. clamp).

Fatty acid extraction was similar across the splanchnic region and the leg both in the basal state and during the clamp (Fig. 3). In the basal state, the splanchnic region and the leg accounted for 28.7 ± 2.7 and 12.5 ± 2.4% of total FFA disappearance, respectively, whereas during hyperglycemia-hyperinsulinemia, the splanchnic region and the leg accounted for 36.6 ± 16.9 and 10.3 ± 3.5% of total FFA disappearance (Fig. 4).

Plasma CO₂ carbon enrichment was constant over the last 20 min of labeled fatty acid infusion (Table 2). These values were used to calculate fatty acid oxidation. The decrease in enrichment during the clamp experiments compared with the basal reflects a decrease in labeled fatty acid oxidation and/or increased unlabeled substrate oxidation (e.g., glucose).

In the basal state, splanchnic region and leg fatty acid oxidation accounted for 15 ± 2 and 11 ± 1.5% of whole body fatty acid oxidation, respectively (Fig. 5). Approximately 50% of the fatty acids taken up by the
leg were oxidized to CO₂ under these conditions, a figure similar to that calculated for the whole body level (Fig. 6). In contrast, only 30% of the fatty acids taken up by the splanchnic region were oxidized to CO₂ (Fig. 6), with an additional 20% of the fatty acids appearing as ketones in the hepatic vein, bringing the total percentage of fatty acids taken up by the splanchnic region that were oxidized to levels similar to those observed across the leg and the whole body.

Compared with the basal state, hyperglycemia-hyperinsulinemia significantly impaired whole body and regional fatty acid oxidation (Table 3), whereas it did not affect the relative contribution of the various regions on total fatty acid oxidation (17 ± 3 and 8 ± 1% across the splanchnic region and the leg, respectively; Fig. 5). The decrease in whole body and regional fatty acid oxidation was independent of FFA availability, as the availability of fatty acids at the whole body level and across the tissues was similar in the basal and clamp experiments (Figs. 1B and 2). Hyperglycemia-hyperinsulinemia similarly decreased the percentage of fatty acids taken up that were oxidized to CO₂ by ~50% at the whole body level, across the splanchnic region, and across the leg (Fig. 6).

Splanchnic and Leg Blood Flow

Splanchnic blood flow increased significantly during the clamp from 1,121 ± 63 ml/min at basal to 1,231 ± 65 ml/min in the clamp (P < 0.05). Leg blood flow was

Table 1. Plasma fatty acid carbon enrichment

<table>
<thead>
<tr>
<th></th>
<th>100 min</th>
<th>110 min</th>
<th>120 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Artery</td>
<td>0.657 ± 0.092</td>
<td>0.628 ± 0.073</td>
<td>0.650 ± 0.074</td>
</tr>
<tr>
<td>Hepatic vein</td>
<td>0.467 ± 0.050</td>
<td>0.474 ± 0.055</td>
<td>0.474 ± 0.050</td>
</tr>
<tr>
<td>Femoral vein</td>
<td>0.428 ± 0.054</td>
<td>0.443 ± 0.058</td>
<td>0.429 ± 0.050</td>
</tr>
<tr>
<td>Hyperglycemia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Artery</td>
<td>0.711 ± 0.104</td>
<td>0.718 ± 0.119</td>
<td>0.715 ± 0.121</td>
</tr>
<tr>
<td>Hepatic vein</td>
<td>0.403 ± 0.055</td>
<td>0.405 ± 0.067</td>
<td>0.407 ± 0.065</td>
</tr>
<tr>
<td>Femoral vein</td>
<td>0.446 ± 0.091</td>
<td>0.447 ± 0.083</td>
<td>0.468 ± 0.106</td>
</tr>
</tbody>
</table>

Values are means ± SE for 5 volunteers, expressed in % \(\left(\frac{13C}{12C}\right)\)·100. Decrease in fatty acid carbon enrichment between artery and hepatic vein and between artery and femoral vein resulted from release of fatty acids across splanchnic region and leg, respectively.

Fig. 2. Net fatty acid uptake (positive value) or release (negative value) across splanchnic region and leg in basal state (open bars) and during hyperglycemia (hatched bars). Values are means ± SE for 5 volunteers.

Fig. 3. FFA extraction (%) across splanchnic region and leg in basal state (open bars) and during hyperglycemia (hatched bars). Values are means ± SE for 5 volunteers.

488 ± 20 ml/min in the basal and 443 ± 41 ml/min during the clamp (not significant).

Effect of Exogenous Glucose 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 breath and blood CO₂ carbon enrichment (18, 28). To account for the “unlabeled” glucose contribution to CO₂ carbon enrichment, it is usually necessary to repeat the clamp experiments without tracer infusion, measure breath CO₂ carbon enrichment, and use this number as the background enrichment for calculation of fatty acid oxidation during the tracer infusion experiments (24). Because of the invasiveness of the experimental design in the present studies, we decided instead to infuse the fatty acid tracer at a high rate to increase the breath and blood CO₂ carbon enrichments enough so that the contribution of naturally occurring \(^{13}C\) in glucose would become negligible. Thus, whereas the average breath CO₂ carbon enrichments in the present study were 1.4e-03 ±
infused glucose is expected to contribute no more than $5 \times 10^{-5}$ to $7 \times 10^{-5}$ to CO$_2$ carbon enrichment (24). Furthermore, any effect of the infused glucose on $^{13}$C enrichment would have caused us to underestimate the extent to which the fatty acid oxidation was suppressed by glucose infusion.

**DISCUSSION**

We had hypothesized that the difficulty in confirming the findings of Randle and co-workers (15, 16) at the whole body level at rest may be because only specific tissues respond as predicted by the in vitro studies. It is thus possible that the contribution of the tissues that respond, as suggested by Randle and co-workers, does not account for a sufficient percentage of resting substrate metabolism to be detected at the whole body level. The findings of the present study, that hyperglycemia-hyperinsulinemia affects regional (i.e., muscle and splanchnic) fatty acid oxidation similarly, may be indicative of the fact that glucose-fatty acid interactions are not regulated as would be predicted by the Randle hypothesis in resting postabsorptive individuals.

Despite the significant decrease in fatty acid oxidation across both the leg and the splanchnic region

**Table 2. Plasma CO$_2$ carbon enrichment**

<table>
<thead>
<tr>
<th></th>
<th>Basal</th>
<th>Basal</th>
<th>Basal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>100 min</td>
<td>110 min</td>
</tr>
<tr>
<td>Artery</td>
<td>0.142 ± 0.010</td>
<td>0.143 ± 0.011</td>
<td>0.143 ± 0.010</td>
</tr>
<tr>
<td>Hepatic vein</td>
<td>0.142 ± 0.011</td>
<td>0.141 ± 0.011</td>
<td>0.141 ± 0.011</td>
</tr>
<tr>
<td>Femoral vein</td>
<td>0.144 ± 0.010</td>
<td>0.144 ± 0.010</td>
<td>0.144 ± 0.010</td>
</tr>
<tr>
<td>Hyperglycemia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Artery</td>
<td>0.060 ± 0.009</td>
<td>0.052 ± 0.013</td>
<td>0.059 ± 0.008</td>
</tr>
<tr>
<td>Hepatic vein</td>
<td>0.060 ± 0.008</td>
<td>0.059 ± 0.008</td>
<td>0.059 ± 0.008</td>
</tr>
<tr>
<td>Femoral vein</td>
<td>0.062 ± 0.010</td>
<td>0.062 ± 0.009</td>
<td>0.061 ± 0.009</td>
</tr>
</tbody>
</table>

Values are means ± SE for 5 volunteers, expressed in % [$^{12}$C/$^{13}$C] 100. Decrease in CO$_2$ carbon enrichment from basal to hyperglycemia represents a decrease in labeled fatty acid oxidation and/or an increase in oxidation of unlabeled substrates (e.g., glucose).

(Table 3), fatty acid uptake was not affected in either leg or splanchnic region during hyperglycemia-hyperinsulinemia (Fig. 2), suggesting increased channeling of fatty acids toward an alternative pathway, e.g., reesterification into triacylglycerols (TG). It is possible that, in the postabsorptive state, glycerol 3-phosphate availability is rate limiting for reesterification into TG and that the increased glucose uptake during hyperglycemia increases its availability to serve as a backbone for TG synthesis. In fact, the possibility cannot be excluded that, during hyperglycemia-hyperinsulinemia, fatty acid oxidation decreased because of preferential channeling of fatty acids toward TG instead of oxidation.

The rate of fatty acid oxidation across the splanchnic region and the leg did not correlate with fatty acid uptake (Fig. 7), suggesting that fatty acid oxidation is not a function of uptake, at least with FFA concentration $\sim$0.5 mmol/l. This observation is in accord with the finding of the present study that glucose availability determines the rate of fatty acid oxidation. If this is true, then it should not be expected that fatty acid uptake influences the rate at which fatty acids are oxidized.

The percentage of fatty acid uptake that was oxidized at the whole body level, across the splanchnic region, and across the leg decreased similarly in response to hyperglycemia-hyperinsulinemia (Fig. 6). The fact that the magnitude of the decrease in fatty acid oxidation in the present study ($\sim$50%) is similar to the decrease observed after 5 h of hyperinsulinemia-hyperglycemia

**Table 3. Plasma fatty acid oxidation at whole body level, across splanchnic region, and across leg in basal state and during hyperglycemia**

<table>
<thead>
<tr>
<th></th>
<th>Whole Body</th>
<th>Splanchnic Region</th>
<th>Leg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>2.5 ± 0.3</td>
<td>0.4 ± 0.04</td>
<td>0.3 ± 0.07</td>
</tr>
<tr>
<td>Hyperglycemia</td>
<td>1.2 ± 0.2*</td>
<td>0.2 ± 0.05*</td>
<td>0.1 ± 0.02*</td>
</tr>
</tbody>
</table>

Values are means ± SE for 5 volunteers expressed in µmol·kg$^{-1}$·min$^{-1}$. *P < 0.05 vs. basal.
induced via exogenous infusion of insulin and glucose (24) suggests that there is a limit to the effect that glucose may have on fatty acid oxidation. In other words, when fatty acid availability is maintained constant, fatty acid oxidation persists at a significant rate even when glucose uptake and oxidation are maximally stimulated. Thus, whereas we envision glucose availability as the predominant regulator of substrate oxidation, variations in FFA availability have some effect. This is because, if we had allowed fatty acid concentration to decrease in the present study, then there would probably have been a further decrease in fatty acid oxidation. Therefore, even in the setting of hyperglycemia-hyperinsulinemia, changes in fatty acid concentration also exert a modest effect on the rate of fatty acid oxidation. This is consistent with the notion of glucose acting via suppression of CPT I activity, because at even a suppressed level of activity, one would expect a residual effect of fatty acid concentration on the rate of acylcarnitine formation and entry into the mitochondria.

The mechanism by which accelerated glucose metabolism may directly inhibit fatty acid oxidation in humans is not yet entirely clear. We have previously shown that hyperglycemia-hyperinsulinemia inhibits long-chain but not medium-chain fatty acid oxidation and decreased muscle long-chain acylcarnitine concentration in resting healthy volunteers, even in the setting of constant plasma fatty acid availability (24). Those data suggest that hyperglycemia-hyperinsulinemia inhibits fatty acid oxidation by limiting long-chain fatty acid entry into the mitochondria (24).

In the present study, we infused only glucose and allowed the concentration of insulin (as well as that of other hormones and mediators) to change spontaneously. Indeed, infusion of glucose to induce hyperglycemia in the clamp experiments is expected to result in changes in the secretion of various hormones, including insulin. It is well established that increased glucose and insulin concentration in plasma inhibits peripheral lipolysis and fatty acid release, thereby decreasing FFA concentration in plasma (3–5, 8, 14, 20). A significant decrease of plasma FFA is expected to decrease fatty acid oxidation. To prevent the insulin-induced decline in fatty acid availability, fatty acid concentration was maintained similar in the basal and clamp experiments via infusion of lipids and heparin. This was important, because if we had allowed fatty acid availability to vary between the two trials, we would not have been able to differentiate the direct effects of hyperglycemia-hyperinsulinemia from the effect of decreasing fatty acid availability on regional fatty acid kinetics and oxidation.

Even though the insulin effect on fatty acid availability was controlled, a possible other direct effect of insulin on fatty acid oxidation cannot be excluded by the present study, as insulin concentration was allowed to change freely in response to dextrose infusion. Insulin stimulates pyruvate dehydrogenase activity, which results in increased acetyl-CoA formation. An increase in acetyl-CoA-to-CoA ratio may directly decrease β-oxidation via feedback inhibition of 3-ketoacyl-CoA thiolase (19). Furthermore, it has been proposed that insulin activates acetyl-CoA carboxylase (11), which is the enzyme that catalyzes malonyl-CoA formation. Malonyl-CoA, in turn, may decrease fatty acid oxidation by decreasing CPT I activity and therefore inhibiting fatty acid entry into the mitochondria (10, 12). However, glucose needs to be present for insulin to decrease fatty acid oxidation, because insulin alone cannot alter malonyl-CoA concentration (6). On the other hand, glucose alone may also not be able to affect fatty acid oxidation, at least in the insulin-sensitive tissues, because of limited entry into the cells. In any case, in physiological circumstances the concentrations of insulin and glucose change simultaneously. Therefore, from the present experimental design, it is not possible to specifically assign responsibility to hyperglycemia, per se, for the observed changes, as opposed to changes caused as a consequence of secondary alterations in the concentration of other hormones or mediators (e.g., insulin).
On the basis of findings from our previous experiments at the whole body level (23, 24), we have advocated the view that intracellular glucose availability and glycolytic flux directly regulate fatty acid oxidation via control of fatty acid entry into the mitochondria. Our present findings of glucose-induced inhibition of fatty acid oxidation across the splanchic region and the leg support our view that, under the present experimental conditions, glucose is the primary regulator of glucose-fatty acid interactions in most tissues of the body. Similar findings have been obtained across the rat heart (17).

In summary, the results of the present study indicate that an increase in glucose availability, in combination with changes in other hormones and/or mediators (e.g., insulin), inhibits fatty acid oxidation similarly across the leg and the splanchic region in the setting of constant fatty acid availability. The decrease in fat oxidation observed across these tissues is similar to the decrease observed at the whole body level, suggesting a uniform effect of glucose and/or insulin on fatty acid oxidation across most tissues of the body.

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