Effects of portal free fatty acid elevation on insulin clearance and hepatic glucose flux

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Submitted 5 July 2005; accepted in final form 29 December 2005

Numerous studies have shown an association between obesity and cardiovascular disease (39), and recent studies have also shown associations between obesity and some types of cancer (18). A crucial factor that has been implicated in these associations (7, 11) is insulin resistance and the concomitant hyperinsulinemia (20). In obesity, peripheral hyperinsulinemia is a consequence of both insulin hypersecretion (48), usually secondary to insulin resistance, and decreased insulin clearance (47, 48). One factor that can account for impaired insulin clearance in obesity, particularly abdominal obesity (34), is the elevated plasma level of free fatty acids (FFAs) (34). In Balent et al. (2), decreased insulin clearance was completely responsible for the hyperinsulinemia induced by FFA elevation. There is an undisputed relationship between “central” fat distribution (i.e., fat in the visceral and subcutaneous abdominal region) and features of the metabolic syndrome (20), although the causal nature of this relationship (15), as well as the role of FFAs vs. adipokines (25) and of visceral vs. subcutaneous abdominal fat (35, 41) in the pathogenesis of insulin resistance, remains a matter of debate. There are important metabolic differences between fat stores. Visceral fat has a higher lipolytic activity than subcutaneous fat (50) and is less responsive to the antilipolytic action of insulin (52). In addition to greater whole body FFA flux due to greater lipolysis, the portal route of this flux may determine the insulin resistance/hyperinsulinemia of visceral obesity by elevating the hepatic FFA load to a greater extent than peripheral FFA delivery, thus impairing hepatic insulin action and clearance (“portal factor” theory; portal factor describes the portal-to-peripheral gradient of FFA with portal infusion, which is due to less dilution of FFA in the portal vs. peripheral blood and to first-pass hepatic uptake of portally delivered FFA) (5). This theory is still controversial because, in the postabsorptive state, the contribution of visceral fat lipolysis to the measured (dog) (24) or calculated hepatic FFA load (humans) (44) is low in nonobese subjects; however, this contribution increases with increased visceral fat and may account for more than 30% of hepatic FFA load in some obese humans (44). It is likely that this contribution is greater at the higher insulin levels seen in the postprandial state, given the insulin resistance of visceral fat (42). Thus there is support for the concept of greater hepatic FFA load in visceral than in nonvisceral obesity (26), although portal FFA cannot be measured in humans. There are no published data about portal FFA in the high-fat-fed model of visceral obesity in the dog, although this model has a high visceral vs. subcutaneous lipolytic activity and liver triglyceride accumulation (25).

To investigate the role of FFA in the pathogenesis of insulin resistance/hyperinsulinemia, we elevated circulating FFA by peripheral infusion of Intralipid plus heparin in nonobese dogs and found peripheral and hepatic insulin resistance and decreased hepatic insulin clearance (62). With Intralipid plus heparin, the elevation of plasma FFA is due to lipoprotein lipase, an enzyme present mostly in muscle and fat. The FFA release is therefore peripheral, independent of the route (portal or peripheral) of Intralipid plus heparin delivery. Thus direct

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infusion of FFAs, rather than Intralipid plus heparin, is needed to examine the selective effect of portal FFA on hepatic insulin action and clearance.

The aim of the present study was to test the hypothesis that portal delivery of FFAs achieving a greater elevation in portal FFA and hepatic FFA load vs. peripheral FFA delivery, as is thought to occur in visceral vs. nonvisceral obesity, also impairs hepatic insulin action and clearance to a greater extent. We performed a direct infusion of oleate by the portal or peripheral venous route in dogs. Oleate is, with palmitate, the most prevalent circulating FFA. It is more soluble than palmitate, which allows for greater rates of FFA infusion. Insulin action on hepatic glucose metabolism was assessed by measuring endogenous glucose production (EGP), total glucose output (TGO, flux from glucose 6-phosphate to glucose), and glucose cycling (GC, backflow of glucose to glucose 6-phosphate) by tracer methods. Hepatic insulin clearance was calculated via a combination of portal and peripheral insulin infusions during a euglycemic moderately hyperinsulinemic clamp with somatostatin and glucagon replacement.

MATERIALS AND METHODS

Animals

This study was performed on eight nondiabetic male mongrel dogs weighing 20–35 kg. The dogs underwent vessel cannulation, which was performed under general anesthesia and assisted ventilation. Anesthesia was induced with thiopental sodium (25 mg/kg) and maintained with 0.5% halothane in carrier gas containing 60% nitric oxide and 40% oxygen.

A sampling cannula was inserted into the aortic arch through the carotid artery. Cannulae were also inserted into the superior vena cava through the jugular vein (for peripheral infusion) and into the splenic and jejunal veins (for portal infusion). Infusion through two sites (splenic and jejunal) facilitates mixing of infusates into the portal circulation. This allowed us to infuse high rates of portal oleate (up to 13 μeq·kg⁻¹·min⁻¹ in pilot experiments) without any untoward effects (i.e., erythrocyte damage at the infusion site, resulting in hemolysis, or elevation in plasma levels of hepatic enzymes). Sampling cannulae were also inserted into the portal and hepatic veins to directly determine hepatic insulin extraction. The gastroduodenal vein was ligated, and Doppler flow probes were placed around the hepatic artery and portal vein. All catheters and lines of Doppler flow probes were exteriorized at the back of the neck through a subcutaneous tunnel. The cannulae were filled with 1,000 U/ml heparin and were regularly flushed with saline to maintain patency.

The dogs received 15 g·kg⁻¹·day⁻¹ chow mixed with 500 g of beef, folic acid, and iron. Only dogs that did not lose weight, ate regularly, and had normal temperature and a hematocrit >35% underwent experiments. All procedures were in accordance with the Canadian Council of Animal Care Standards and were approved by the Animal Care Committee of the University of Toronto.

Experimental Design

Treatments. Experiments were performed after at least a 10- to 15-day recovery from surgery in overnight-fasted, conscious dogs. All infusates were prepared under sterile conditions. The experimental design (Fig. 1) consisted of three 5-h treatments carried out in random order, i.e., portal oleate infusion (POR OLE; 10 μeq·kg⁻¹·min⁻¹), equidose peripheral oleate infusion (PER OLE), or saline control treatment (CONT). The oleate infusion was an emulsion of oleate (Sigma cat. no. O-1630) prepared in 5.5% fatty acid-free bovine serum albumin (BSA) in saline, according to the method of Bezman-Tarcher (4), as modified by Miles et al. (40). Because dogs can develop allergic reactions to BSA after repeated administrations, we avoided performing three repeated BSA exposures in the same dog by infusing saline, rather than BSA, in control experiments. We have previously found no difference in metabolic parameters at rest and during hyperglycemic clamps after 48-h infusions of fatty acid-free BSA or saline in rats (36). In the dogs, no allergic reactions were apparent with two exposures to BSA.

Three of the eight dogs underwent all three treatments in experiments performed 2–3 wk apart, three dogs underwent two treatments, and two dogs underwent only one treatment. The reason for failure to complete the three experiments was catheter occlusion or catheter-related complications. In total, we performed six POR OLE, five PER OLE, and six CONT experiments.

Protocol. The experimental protocol consisted of a 5-h hyperinsulinemic euglycemic clamp. The modalities of insulin infusion are described below. Throughout the clamp, a peripheral infusion of somatostatin (0.8 μg·kg⁻¹·min⁻¹; Bachem, Torrance, CA) inhibited endogenous insulin secretion, while basal glucagon (Eli Lilly, Indianapolis, IN) was replaced intraperitoneally (0.65 ng·kg⁻¹·min⁻¹). Throughout the experiments, the insulin-induced decrease in plasma glucose was prevented by exogenous infusion of 25% glucose at variable rates according to frequent (every 5 min) plasma glucose determinations (euglycemic clamp technique). Insulin action on hepatic glucose metabolism was assessed by measuring EGP, TGO (flux from glucose 6-phosphate to glucose), and GC (backflow of glucose to glucose 6-phosphate) by tracer methods. Insulin action on peripheral glucose metabolism was also assessed with tracers. To this end, a primed (50 μCi) peripheral infusion (0.5 μCi/min) of a 50% mixture of HPLC-purified [2-³H]- and [6-³H]glucose (New England Nuclear, Boston, MA) was given to enable measurement of hepatic and peripheral glucose flux. The 50% tracer mixture was also added to the 25% glucose infusion to a specific activity of 5.6 μCi/g “Hot Gua” technique, as in Finegood et al. (14).

Arterial blood samples were taken in the basal fasting state (i.e., before all infusions) and in the last 40 min of each period. Portal blood samples were also taken; however, the portal vein catheter remained patent in only four portal oleate experiments, two peripheral oleate experiments, and one control experiment. Portal and hepatic vein catheters were both patent in only three experiments.

Experimental Design

Treatment:

PORTAL OLEATE OR PERIPHERAL OLEATE OR CONTROL

Protocol:

Hyperinsulinemic euglycemic clamp with somatostatin infusion and portal glucagon replacement

Insulin infusion:

<table>
<thead>
<tr>
<th>PERIOD 1</th>
<th>PERIOD 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 min</td>
<td>180 min</td>
</tr>
<tr>
<td>0.5mU/kg·min</td>
<td>OFF</td>
</tr>
<tr>
<td>Portal Insulin</td>
<td></td>
</tr>
<tr>
<td>0.2mU/kg·min</td>
<td>0.45mU/kg·min</td>
</tr>
<tr>
<td>Peripheral Insulin</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1. Experimental design as described in MATERIALS AND METHODS.
Insulin infusion. The hyperinsulinemic euglycemic clamp was divided into two periods, period 1 (0–180 min) and period 2 (180–300 min), which differed in the dose and route of insulin infusion. The combinations of portal and peripheral insulin infusion were designed to maintain constant peripheral insulin levels in the postprandial range. Two periods were necessary to calculate hepatic insulin clearance, as period 2 allowed us to obtain the plasma clearance rate of insulin, which was necessary to derive hepatic insulin clearance in period 1 (see Calculations).

In period 1, intraportal insulin infusion was 0.5 mU·kg⁻¹·min⁻¹, and peripheral insulin infusion was 0.2 mU·kg⁻¹·min⁻¹. In period 2, intraportal insulin infusion was stopped, and peripheral insulin infusion was 0.45 mU·kg⁻¹·min⁻¹. Thus portal insulin levels were higher in period 1 than in period 2 by experimental design. All of these levels were in the physiological range. In period 1, we used a combination of portal and peripheral insulin infusions, rather than portal infusion alone, to obtain hepatic sinusoidal levels that were still compatible with linearity of the insulin system (12, 57). All insulin infusions were prepared using regular porcine insulin (Eli Lilly) in saline containing ~3% of the dog’s own plasma.

Laboratory Methods

Plasma glucose was measured with a Beckman Glucose Analyzer II (Fullerton, CA), and plasma FFAs and glycerol were measured with colorimetric kits (Wako Industrials, Neun, Germany and Roche Diagnostics, Basel, Switzerland). Plasma palmitate and oleate (indexes of endogenous and exogenous FFAs, respectively) were determined by HPLC (40). Plasma insulin and glucagon were measured by radioimmunoassay kits from Diagnostic Products (Los Angeles, CA). The coefficients of variations were 7% and 16%.

Calculations

EGB, TGO, and GC. A modified one-compartmental model (14) was used for calculation. EGP and TGO (flux from glucose 6-phosphate to glucose) are the endogenous rates of appearance of glucose measured with [6-³H]- and [2-³H]glucose, respectively; GC (glucose to glucose 6-phosphate and back) is the difference between TGO and EGP. Glucose utilization is the rate of disappearance (R₄) of glucose measured with [6-³H]glucose. R₄ corresponds to glucose utilization, and plasma clearance rate of glucose (i.e., R₄/glycemia) corresponds to glucose metabolic clearance (MCR) because euglycemia was maintained (therefore, renal glucose clearance was assumed to be zero).

Plasma insulin clearance and hepatic insulin clearance. Throughout the experiments, endogenous insulin secretion was inhibited by somatostatin. We have shown that, when the same dose of somatostatin in dogs is used under similar conditions, plasma C-peptide is undetectable (62). Thus endogenous insulin secretion is effectively inhibited and does not affect calculations of insulin clearance.

Hepatic insulin clearance was derived from the peripheral insulin levels obtained during portal infusion in period 1 combined with the measurement of plasma insulin clearance in period 2 of the same experiment. Therefore, the extrahepatic component of plasma insulin clearance was taken into account and did not affect the calculated value of hepatic insulin clearance. The following approach was used, which was based on standard linear methods on insulin kinetics and was previously used by others (13) to calculate posthepatic insulin delivery (RSYS in our model):

\[
C_{IH} = F_{IH} \times FE_{IH}
\]

Hepatic insulin clearance (\(C_{IH}\)) is the product of hepatic plasma flow (\(F_{IH}\)) and hepatic fractional extraction (\(FE_{IH}\)). Hepatic plasma flow was derived from the implanted Doppler probes and from the dog’s hematocrit.

During period 1, \(F_{IH}\) of portally derived insulin is the rate of hepatic extraction of portally derived insulin (REIH) divided by the rate of portal insulin infusion (\(R_{POR}\)) (i.e., how much is extracted over how much is there). Therefore,

\[
C_{IH} = F_{IH} \times RE_{IH}/R_{POR} \quad (2)
\]

The rate of hepatic extraction of portally infused insulin (\(RE_{IH}\)) is the difference between the portal infusion rate (\(R_{POR}\), i.e., the flux of insulin into the liver) and the rate of systemic appearance of portally infused insulin (\(R_{SYS}\), i.e., the flux of insulin out of the liver and into the systemic circulation, also called posthepatic insulin delivery).

\[
C_{IH} = F_{IH} \times R_{POR} - R_{SYS} \quad (3)
\]

\(RSYS\) is the product of the peripheral insulin concentration derived from portal infusion in period 1 (\(I_{POR-1}\), in period 1 there are both portal and peripheral insulin infusions) and the clearance of insulin from the systemic circulation, i.e., plasma insulin clearance (ClP). ClP is also sometimes called peripheral insulin clearance because it has an extrahepatic component. However, it also incorporates a hepatic component (the hepatic clearance of insulin that is not first pass, i.e., the hepatic clearance of the insulin that has reached the systemic circulation). Thus, for period 1,

\[
C_{IH} = F_{IH} \times R_{POR} = (I_{POR-1} \times Cl_{P})/R_{POR} \quad (4)
\]

Assuming linearity of the insulin system at our insulin levels and time-invariance of insulin clearance (12, 57), ClP is equal in periods 1 and 2 and can thus be calculated during the peripheral insulin infusion alone (period 2) as

\[
Cl_{P} = R_{PER-2}/I_{PER-2} \quad (5)
\]

where \(R_{PER-2}\) is the peripheral insulin infusion rate in period 2 and \(I_{PER-2}\) is the peripheral insulin level in period 2. [Note that \(I_{PER-2}\) corresponds to the total peripheral insulin concentrations \((I_{TOT-2})\) because there is no portal infusion in period 2]

In contrast to period 2, where there is only peripheral infusion, in period 1 there are both portal and peripheral insulin infusions. Therefore, the total peripheral insulin levels \((I_{TOT-1})\) can be separated into two components, one derived from the portal \((I_{POR-1})\) and the other derived from peripheral infusion \((I_{PER-1})\). Assuming that the peripheral insulin levels obtained with peripheral infusion are proportional to the peripheral infusion rate (linearity of the insulin system and time-invariance of insulin clearance as above), \(I_{PER-1}\) can be calculated from the data in period 2 as \(I_{PER-2}\) multiplied by the ratio between the peripheral infusions in periods 1 and 2 \((R_{PER-1}/R_{PER-2})\). Thus

\[
I_{PER-1} = I_{TOT-1} - R_{PER-2} \times R_{PER-1}/R_{PER-2} \quad (6)
\]

In brief, Eqs. 4, 5, and 6 were combined to derive \(Cl_{P}\).

\(Cl_{P}\) is independent of extrahepatic extraction processes. However, it still depends on hepatic plasma flow. To obtain a flow-independent parameter related to the metabolic hepatic insulin extraction processes, we calculated the intrinsic hepatic insulin clearance (\(Cl_{HI}^{int}\)), which takes into account the effect of hepatic plasma flow (\(F_{IH}\) on hepatic fractional extraction (\(FE_{IH}\) (63). We calculated \(Cl_{HI}^{int}\) according to the parallel-tube model (63) on the data of period 1, i.e.,

\[
FE_{IH} = 1 - e^{-Cl_{HI}^{int}F_{IH}} \quad \text{or} \quad Cl_{HI}^{int} = -F_{IH}/\ln(1 - FE_{IH}) \quad (7)
\]

The parallel-tube model is the most used model of hepatic insulin clearance; however, there is little difference in the calculated effects of \(F_{IH}\) on \(FE_{IH}\) between different models (63).

Statistical Analysis

Data are expressed as means ± SE and represent averages of the last 40 min of each period. Two-way ANOVA for repeated measure-
ments, followed by Tukey’s t-test, was used to compare treatments and periods. Data within periods were also analyzed with one-way ANOVA, using treatment as the independent variable, as some of the experiments were unpaired. When only the three paired experiments from the same dogs were analyzed, two-way ANOVA with dog and treatment as independent variables was used. In case of unequal variances, data were logarithmically transformed. Calculations were performed using SAS (Cary, NC).

RESULTS

Metabolites and Hormones

In CONT, peripheral FFAs (Fig. 2A) were lower during hyperinsulinemia than in the basal fasting state (i.e., before all infusions were started). With both PER OLE and POR OLE, peripheral FFAs were similar to basal and were 3-fold higher than with CONT (P < 0.001). There was no difference between POR OLE and PER OLE. With POR OLE, portal FFAs were 1.5- to 2-fold above the levels in PER OLE (Fig. 2B). Hepatic FFA load in POR OLE and PER OLE was 12.7 ± 1.7, n = 4 and 10.5 µeq·kg⁻¹·min⁻¹, n = 2, respectively.

During somatostatin infusion, peripheral glucagon was maintained at basal levels (Table 1) by glucagon replacement, and plasma glucose was maintained at euglycemia (Table 1). During the clamp, plasma glucose was slightly but significantly maintained at basal levels (Table 1) by glucagon replacement, 

Table 1. Plasma glucagon and metabolite levels

<table>
<thead>
<tr>
<th></th>
<th>Period</th>
<th>POR OLE</th>
<th>PER OLE</th>
<th>CONT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucagon, ng/ml</td>
<td>1</td>
<td>56.8±11</td>
<td>63.8±8</td>
<td>58.4±4</td>
</tr>
<tr>
<td>Glucose, mM</td>
<td>1</td>
<td>6.4±0.2*</td>
<td>6.1±0.1</td>
<td>5.7±0.3</td>
</tr>
<tr>
<td>Glycerol, µM</td>
<td>1</td>
<td>254±58</td>
<td>156±21</td>
<td>108±20</td>
</tr>
<tr>
<td>Palmitate, µM</td>
<td>1</td>
<td>123±18</td>
<td>76±22</td>
<td>66±9</td>
</tr>
<tr>
<td>Oleate, µM</td>
<td>1</td>
<td>423±81</td>
<td>453±95*</td>
<td>58±11</td>
</tr>
</tbody>
</table>

Values are means ± SE. POR OLE, portal oleate infusion; PER OLE, peripheral oleate infusion; CONT, saline control treatment. *P < 0.001 vs. CONT; †P < 0.05 vs. CONT; ‡P < 0.01 vs. CONT. Basal fasting state before the hyperinsulinemic clamp (mg/mL): POR OLE 51 ± 6; PER OLE 58 ± 6; CONT 68 ± 12. *Basal fasting state before the hyperinsulinemic clamp (mM): POR OLE 6.2 ± 0.1; PER OLE 6.0 ± 0.1; CONT 5.9 ± 0.3.

Glucose Turnover

The glucose infusion rate necessary to maintain euglycemia (not shown) was stable throughout the experiments. Glucose utilization or MCR between POR OLE and PER OLE. EGP was not higher with POR OLE than PER OLE despite the activation by portal oleate (3, 19). Hematocrit, which is believed to be a marker of sympathetic drive (56), was 45 ± 3, 40 ± 2, and 35 ± 3% in POR OLE (P < 0.001 vs. PER OLE or CONT), PER OLE (P < 0.001 vs. CONT), and CONT, respectively. Basal levels were 37 ± 7, 39 ± 3, and 39 ± 4%.

Table 2. Ginf and MCR

<table>
<thead>
<tr>
<th>Period</th>
<th>POR OLE</th>
<th>PER OLE</th>
<th>CONT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ginf, µmol·kg⁻¹·min⁻¹</td>
<td>1</td>
<td>28.4±2.1*</td>
<td>26.7±3.2</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>22.0±5.8*</td>
<td>23.9±4.0</td>
</tr>
<tr>
<td>MCR, ml·kg⁻¹·min⁻¹</td>
<td>1</td>
<td>6.6±0.3</td>
<td>7.0±0.4</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>6.1±0.7†</td>
<td>6.5±0.7</td>
</tr>
</tbody>
</table>

Values are means ± SE. Ginf, glucose infusion rate; MCR, metabolic clearance rate. *P < 0.01 vs. CONT; †P < 0.001 vs. CONT.
repeated measurements, followed by Tukey’s test. Statistics were performed using ANOVA for repeated measurements, followed by Tukey’s t-test. The hepatic vein insulin concentrations in both periods were 20% higher in POR OLE (n = 2) than in one PER OLE experiment.

The plasma clearance rate of insulin, calculated in period 2 (peripheral insulin infusion only), was lowest with POR OLE (P < 0.001 vs. CONT, P < 0.01 vs. PER OLE), intermediate with PER OLE (P < 0.01 vs. CONT), and highest in CONT (Fig. 5A). Hepatic insulin clearance calculated in period 1 (portal plus peripheral insulin infusion) was also lowest with POR OLE (P < 0.001 vs. CONT or PER OLE), intermediate with PER OLE (P < 0.001 vs. CONT), and highest in CONT (Fig. 5B). Hepatic insulin clearance was correlated with hepatic plasma flow (r = 0.82, P < 0.001). This tended to be lower with POR OLE in both period 1 (where hepatic insulin clearance was calculated; ANOVA, P < 0.13) and period 2 (ANOVA, P < 0.11; Table 3). When data from both periods were analyzed together using two-way ANOVA, hepatic plasma flow was lower in POR OLE than in CONT (P < 0.01) or PER OLE (P < 0.05), whereas there was no significant difference between PER OLE and CONT. Hepatic artery to hepatic plasma flow ratio was not significantly different among treatment. Neither hepatic plasma flow nor hepatic artery to hepatic plasma flow ratio was affected by the order of the experiment in the same dog.

Hepatic insulin clearance obtained directly was 4.84 ml·kg⁻¹·min⁻¹ in two POR OLE experiments vs. a calculated average value of 4.75; in one PER OLE experiment it was 11.9 ml·kg⁻¹·min⁻¹ vs. a calculated value of 10.0.

Because flow can influence fractional extraction, and in particular fractional extraction is higher when flow is lower (see Eq. 7 in MATERIALS AND METHODS), we calculated the intrinsic hepatic clearance of insulin, which represents metabolic extraction processes independent of hepatic plasma flow (63). Intrinsic hepatic insulin clearance was lowest with POR OLE (P < 0.001 vs. CONT or PER OLE), intermediate with PER OLE (P < 0.01 vs. CONT), and highest in CONT (Fig. 5C). Thus, by taking into account the differences in flow, we could not eliminate the differences in hepatic insulin clearance between the two routes of oleate delivery.

CONT), and mainly due to a decrease in GC, because TGO (= EGP + GC) was not affected.

**Insulin Levels and Insulin Clearance**

Total peripheral insulin concentrations (derived by both portal and peripheral infusion in period 1 and by peripheral infusion alone in period 2) were elevated to moderate hyperinsulinemia and were maintained constant throughout the clamp (Fig. 4). Despite the same insulin infusion rates, the peripheral insulin concentrations were highest with POR OLE (P < 0.001 vs. PER OLE or CONT), intermediate with PER OLE (P < 0.001 vs. PER OLE or CONT) and lowest in CONT. The portal insulin concentrations were higher with portal than with peripheral oleate in period 1 (POR OLE: 831 ± 168, n = 4 vs. an average value of 733 pM in PER OLE, n = 2); however, the low n precluded meaningful statistical analysis. The portal insulin concentrations in period 2 (206 ± 61 vs. 291 pM) and the calculated hepatic insulin delivery in both periods (not shown) were not greater with POR OLE than PER OLE.
DISCUSSION

This study is the first to show that, during euglycemic moderately hyperinsulinemic clamps in dogs, the portal route of FFA delivery, which is relevant to visceral obesity, generates peripheral hyperinsulinemia to a greater extent than the peripheral route. We compared the effects of an identical amount of exogenous FFA infused by either route, which includes effects due to the first-pass hepatic delivery of FFAs from the portal circulation, resulting in greater hepatic FFA load with portal rather than peripheral FFA infusion, and specific effects of portal FFA, for example, previously described (3, 19) reflex effects on sympathetic activity affecting hepatic plasma flow and endogenous lipolysis.

Hepatic plasma flow decreased by 20–25% with portal vs. peripheral oleate, whereas the increase in endogenous lipolysis (as shown by the higher glycerol and palmitate levels) was ~20% of the rate of oleate infused, because it obliterated the difference in peripheral FFA concentrations that was expected on the basis of a 20% first-pass hepatic uptake of portally delivered FFA (58). Thus the changes in the two parameters were quantitatively similar and had offsetting effects on the hepatic load of FFA. In fact, the observed difference in the hepatic FFA load (2.2 μeq·kg⁻¹·min⁻¹) corresponded to what was expected on the basis of the “portal factor” only (2.46 μeq·kg⁻¹·min⁻¹), assuming that hepatic hemodynamics and endogenous lipolysis had been the same in the portal vs. peripheral oleate (see APPENDIX for this calculation).

Thus sympathetic activity did not result in greater hepatic FFA load but matched the peripheral appearance of FFAs and peripheral FFA concentrations in the portal vs. peripheral oleate treatment, resulting in a selective difference in portal FFA levels. By comparing the two treatments, we could therefore examine the selective effect of portal FFA on hepatic insulin action and clearance, as was previously done to investigate the selective effects of portal insulin on hepatic glucose production (1, 17, 54). As described above, in the present study the selective effects of portal FFA may be due to both the increased hepatic FFA load and to specific effects of portal FFA, for example, those on hepatic plasma flow influencing hepatic insulin clearance. However, we have shown that most of the differences in hepatic insulin clearance are independent of flow; therefore, we believe these differences are largely due to the increased hepatic FFA load.

As mentioned above, peripheral FFA concentrations were similar; however, peripheral insulin levels were higher with portal rather than peripheral oleate. Glucose MCR was not greater with portal oleate infusion, which suggests that either...

### Table 3. HPF and ratio of HA/HPF

<table>
<thead>
<tr>
<th>Period</th>
<th>POR OLE</th>
<th>PER OLE</th>
<th>CONT</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPF, ml·kg⁻¹·min⁻¹</td>
<td>10.7±1.0</td>
<td>14.9±2.2</td>
<td>16.0±2.3</td>
</tr>
<tr>
<td>2</td>
<td>13.1±0.5</td>
<td>17.7±2.6</td>
<td>19.1±2.3</td>
</tr>
<tr>
<td>HA/HPF</td>
<td>0.36±0.05</td>
<td>0.25±0.01</td>
<td>0.34±0.06</td>
</tr>
<tr>
<td>2</td>
<td>0.37±0.02</td>
<td>0.32±0.03</td>
<td>0.32±0.05</td>
</tr>
</tbody>
</table>

Values are means ± SE. HPF, hepatic plasma flow; HA/HPF, hepatic artery/hepatic plasma flow ratio. *With ANOVA there were treatment effects (see text), but post hoc analysis was unable to identify differences in specific periods.
the difference in insulin was too small to induce functional changes and/or that portal oleate induced a greater degree of peripheral insulin resistance. This may also have been due to sympathetic activation.

EGP (the flux of glucose out of the liver) was 2-fold higher with both portal and peripheral oleate than with control. Interestingly, EGP was not greater with portal than with peripheral oleate despite the greater hepatic FFA load (and perhaps also despite the greater sympathetic activity) with portal oleate. This result may partly be explained by the slightly greater GC (backflow of glucose to the liver glucose 6-phosphate pool) with portal oleate, which apparently maintained similar EGP despite slightly increased TGO (total flux from glucose 6-phosphate to glucose). The lack of difference in EGP and the only slight difference in TGO, despite the large difference in portal FFAs between the two oleate treatments, may in turn be explained as follows: 1) The effect of FFA on increasing TGO and EGP was already near maximal with peripheral oleate delivery. A lower oleate dose may have been required to uncover a route-dependent effect of FFA on hepatic glucose flux; 2) the small increase in peripheral insulin with portal oleate might have offset possible differences in hepatic glucose flux; and/or 3) neither the differences in hepatic FFA delivery nor those in peripheral insulin were of sufficient magnitude to have major effects on hepatic glucose flux. Regarding 1), peripheral insulin has indirect effects to suppress hepatic glucose flux via suppression of FFA and glucagon (which were, however, both controlled by exogenous infusion in our model) (16), suppression of gluconeogenic precursors, and hypothalamic insulin signaling (45). Portal insulin concentrations appeared to be similar between the two oleate treatments; however, the n was small, and we cannot exclude that portal insulin levels might have been greater with portal oleate and could have contributed to EGP suppression, for example, by increasing GC. Portal insulin did not appear to have major effects on hepatic glucose flux in our model, contrary to other models with lower insulin levels (55). This is because TGO did not increase after portal insulin infusion was switched off at the end of period 1 (it is recognized that an increase in TGO might have been counteracted by the prolongation of fasting). However, EGP did increase minimally from period 1 to period 2 due to a decrease in GC.

Peripheral insulin levels were increased in proportion to portal FFA and were greater with portal than with peripheral oleate. Consistent with the differences in the peripheral insulin levels, plasma insulin clearance, obtained in period 2, and hepatic insulin clearance, calculated in period 1, were reduced in proportion to the portal FFA levels. Our calculations of hepatic insulin clearance, which are based on previously published standard methods of insulin kinetics (13), rely on the assumption of linearity of insulin extraction and on time-invariance of insulin clearance across periods. Linearity of extraction is generally (12, 57), although not universally (43), accepted within the range of our portal and peripheral insulin concentrations. The assumption of time-invariance is more difficult to judge, as some effects of FFA (for example, those on glucose MCR in our study) are time dependent. However, the present study was designed to underestimate, rather than overestimate, the effect of FFAs on the hepatic component of insulin clearance, because the determination of plasma insulin clearance followed that of hepatic insulin clearance. Thus, had plasma insulin clearance been higher in period 1 than the value obtained in period 2, this would have resulted in an even lower hepatic insulin clearance, as can be seen from Eq. 4. Hepatic plasma flow, which is one of the determinants of hepatic insulin clearance, did not significantly change across periods (P = 0.08), and although it tended to rise over time, the rise was similar in all groups and thus did not likely affect the comparison among groups.

Insulin clearance is mostly hepatic and is a receptor-mediated event (10). Insulin receptor binding is followed by internalization and degradation of insulin, although at high insulin levels some of the extracted insulin may be released without being degraded (33). Studies in rat hepatocytes showed that FFAs decrease insulin binding due to a decreased receptor number (60), and recent studies demonstrated that FFAs can decrease insulin degradation by inhibiting insulin-degrading enzyme (21). However, in other in vitro studies, FFAs did not affect hepatocyte insulin uptake (27). In the in situ perfused rat liver, FFAs decreased hepatic insulin extraction (61). The majority (2, 8, 9, 22, 30, 62), but not all, of the in vivo studies (6, 53) suggest that FFAs impair insulin clearance. Factors such as portal insulin delivery (8, 9, 22), prolonged FFA elevation (8, 9, 30), and a relatively high basal insulin clearance (8) appear to facilitate detection of the impairing effect of FFA (8, 9, 22).

In the present study, part of the differences in hepatic insulin clearance between groups could be due to the differences in hepatic plasma flow. We are unaware of previous reports of the effect of portal FFA on hepatic hemodynamics; however, a number of studies (3, 19) have demonstrated that portal oleate increases sympathetic activity in rats, presumably because of stimulation of hepatic vagal afferents (46). Also, elevation in plasma norepinephrine (28) or sympathetic stimulation (29, 51) has been shown to decrease hepatic blood flow. In the present study, plasma catecholamine levels could not be determined because the samples had not been collected with antioxidants; however, the fact that hematocrit, glycerol, and palmitate levels were elevated with portal oleate infusion is consistent with stimulation of catecholamine release by portal FFA. Sympathetic activation also occurs during inflammation, and we cannot exclude that a mild inflammatory reaction, due to possible traces of endotoxin in the commercial preparation of BSA (vehicle for oleate) despite charcoalization, could explain some of the vascular and thus insulin clearance effects in both oleate groups (38). However, the decrease in hepatic plasma flow was confined to the portal oleate group, whereas both oleate groups received BSA in randomized experiments. Flow was not significantly different between peripheral oleate and controls, and the trend towards a decrease with peripheral oleate could be ascribed to the greater portal FFA alternatively or in addition to the BSA infusion.

To take into account the effect of flow on hepatic insulin clearance, we calculated the intrinsic hepatic insulin clearance, which is a flow-independent parameter. Not only hepatic insulin clearance but also intrinsic hepatic insulin clearance was very different between groups, which suggests that a large part of the difference in hepatic insulin clearance was not due to differences in flow but to effects of portal FFAs on metabolic processes of insulin extraction, i.e., insulin binding, internal-
ization, and/or degradation. The precise mechanisms of the FFA effects on these processes have not been clarified; however, the FFA-induced decrease in insulin binding in isolated rat hepatocytes has been linked to FFA oxidation in some studies (23, 59). In similar studies in isolated rat hepatocytes (31) and in in vivo studies in rats (32), we have implicated FFA-induced activation of PKCβ in the FFA-induced decrease in hepatocyte insulin binding and clearance. PKC appears to modulate insulin receptor internalization (10), a process that is enhanced by insulin via tyrosine phosphorylation of CEACAM1 (49). However, it is still unknown whether FFA-induced PKC activation involves CEACAM1. Other authors have suggested that fatty acids and their coenzyme A thiosteres directly bind to insulin-degrading enzyme, resulting in enzyme inhibition (21).

In conclusion, we have shown for the first time that selective elevation in portal FFAs, which is believed to characterize visceral obesity, generates peripheral hyperinsulinemia. This may initially compensate for hepatic insulin resistance, as shown by greater total glucose output and cycling with portal than with peripheral oleate. However, in the long term, hyperinsulinemia may aggravate insulin resistance (49) and have atherogenic (11) and carcinogenic effects (7).

APPENDIX

The difference in hepatic FFA load based on the “portal factor” only, had hepatic hemodynamics and endogenous lipolysis been the same in the portal vs. peripheral oleate, can be calculated from the hepatic sinusoidal FFA concentration with portal oleate (1.6, recalculated to 1.7 after subtraction of the contribution of endogenous lipolysis), the ratio of hepatic artery and portal plasma flow to hepatic sinusoidal FFA concentration (model developed for system delivery and uptake of NEFA) 

\[ \text{system delivery and uptake of NEFA} = 0.8 \times x/0.192 \times x + 0.8 \times 0.3 = 1.192x \]

Thus the difference in hepatic sinusoidal FFA concentration would be 1.192x – x = 0.192x, which, after substitution of x with a hepatic sinusoidal FFA concentration of ~800 µg/ml with peripheral oleate (as the observed portal and peripheral FFA) and multiplication by a hepatic plasma flow of 16 ml/kg⁻¹·min⁻¹, would result in a difference in hepatic FFA load of 2.46 µg/kg⁻¹·min⁻¹.

ACKNOWLEDGMENTS

We thank Loretta Lam for excellent technical assistance and the laboratory of Dr. M. D. Jensen at the Mayo Clinic, Rochester, MN, for the determination of plasma oleate and palmitate.

GRANTS

This study was supported by a grant to A. Giacca from the Heart and Stroke Foundation of Canada, the Canadian Institutes of Health Research (grants to A. Giacca and I. G. Fantus) and the Canadian Diabetes Association provided partial support for personnel. T. K. T. Lam was supported by a Doctoral Award from the Canadian Institutes of Health Research.

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