Splanchnic free fatty acid kinetics

Michael D. Jensen,1 Sylvain Cardin,2 Dale Edgerton,2 and Alan Cherrington2
1Endocrine Research Unit, Mayo Clinic, Rochester, Minnesota 55905; and 2Department of Molecular Physiology and Biophysics, and Diabetes Research and Training Center, Vanderbilt University School of Medicine, Nashville, Tennessee 37232

Submitted 18 June 2002; accepted in final form 18 February 2003

Jensen, Michael D., Sylvain Cardin, Dale Edgerton, and Alan Cherrington. Splanchnic free fatty acid kinetics. Am J Physiol Endocrinol Metab 284: E1140–E1148, 2003; 10.1152/ajpendo.00268.2002.—These studies were conducted to assess the relationship between visceral adipose tissue free fatty acid (FFA) release and splanchnic FFA release. Steady-state splanchnic bed palmitate ([9,10-3H]palmitate) kinetics were determined from 14 sampling intervals from eight dogs with chronic indwelling arterial, portal vein, and hepatic vein catheters. We tested a model designed to predict the proportion of FFAs delivered to the liver from visceral fat by use of hepatic vein data. The model predicted that 15 ± 2% of hepatic palmitate delivery originated from visceral lipolysis, which was greater (P = 0.004) than the 11 ± 2% actually observed. There was a good relationship (r2 = 0.63) between the predicted and observed hepatic palmitate delivery values, but the model overestimated visceral FFA release more at lower than at higher palmitate concentrations. The discrepancy could be due to differential uptake of FFAs arriving from the arterial vs. the portal vein or to release of FFAs in the hepatic circulatory bed. Splanchnic FFA release measured using hepatic vein samples was strongly related to visceral adipose tissue FFA release into the portal vein. This finding suggests that splanchnic FFA release is a good indicator of visceral adipose tissue lipolysis.

isotope tracers; lipolysis; kinetic model

INCREASED VISCERAL FAT, especially in the setting of obesity, is associated with a greater risk of dyslipidemia, insulin resistance, and type 2 diabetes mellitus. It has been proposed that active visceral adipose tissue lipolysis mediates the physiology of this association by disproportionately increasing the delivery of free fatty acids (FFAs) to the liver (4). This would occur because the venous drainage of omental and mesenteric (visceral) fat is directly into the portal venous circulation, and the FFAs released by visceral fat undergo no further uptake before reaching the liver. Increased hepatic FFA delivery results in insulin resistance with regard to the suppression of hepatic glucose output (3) and increased VLDL triglyceride production (8), some cardinal features of upper body/visceral obesity. We have suggested that the increased hepatic FFA delivery in upper body/visceral obesity is due to increased FFA release from upper body subcutaneous fat, not from visceral adipose tissue FFA release (5). Unfortu-
ceral fat, this could help address the question of the relative importance of visceral fat in affecting hepatic metabolism. For example, if a greater fraction of hepatic FFA delivery derives from visceral fat in conditions such as upper body obesity, this would argue for a more important role for visceral lipolysis than we have previously suggested.

An assumption of the proposed model is that the fractional hepatic uptake of FFAs is the same regardless of whether FFAs reach the liver via the portal vein or the hepatic artery; this assumption has not been tested. If the fractional uptakes of FFA delivered to the liver via the hepatic artery and via the portal vein are different, the proposed model may not be applicable.

To address this issue, splanchnic palmitate kinetics were measured in chronically catheterized, conscious dogs during studies that involved collecting blood samples from the arterial, portal venous, and hepatic venous circulation. Samples were collected under conditions that resulted in a wide range of plasma FFA concentrations to test the model under a variety of conditions. Measures of regional FFA concentration and specific activity (SA), combined with specific measures of regional blood flow, allowed us to test the proposed model for predicting the proportion of hepatic FFA delivery that originates from visceral fat. We also examined the relationship between direct measures of FFA release into the portal vein and FFAs appearing in the hepatic vein.

MATERIALS AND METHODS

Preparation of infusates. [9,10-3H]palmitate (New England Nuclear, Boston, MA) was prepared for intravenous infusion by binding it to albumin in plasma collected from the dogs used in the studies.

Assays. The concentration and SA of plasma palmitate were determined by a modification (7) of a previously published HPLC method (11). Portal venous and hepatic arterial blood flows were measured using Doppler flow probes (12).

Experimental design. Experiments were conducted in a total of 20 dogs participating in three different protocols for the collection of data over a wide range of FFA concentrations. Permanent, indwelling catheters were surgically placed in the hepatic vein, portal vein, and femoral artery in each dog 17 days before study. The catheters were tunneled to a pouch under the skin for future access. Doppler flow probes were placed around the portal vein and hepatic artery during surgery. No omental or mesenteric fat was removed. The dogs were allowed to recover fully before the experiments. These studies were approved by the Vanderbilt Institutional Animal Care and Use Committee and were designed primarily to address issues of glucose metabolism; the FFA tracer studies were a supplement to the protocols. In order not to impede the conduct of the glucose metabolism studies, no experimental design modifications were made specifically to achieve steady-state FFA concentrations and SA. Thus, in only a portion of the studies were satisfactory FFA steady-state conditions achieved. [3H]palmitate was infused into a peripheral vein at a rate of ~0.3 μCi/min in each animal throughout the duration of the study. The palmitate tracer infusion was begun 30 min before collection of the first blood sample to achieve isotopic steady state. For each of the 16 dogs studied, a series of blood samples was collected over 20–60 min during two study intervals. Approximately 1 ml of plasma was collected from arterial, hepatic venous, and portal venous blood for assay at each time point. Blood samples were immediately placed on ice, and the plasma was separated by spinning in a refrigerated centrifuge. The plasma was stored below or at −20°C until assayed. We have found that these procedures prevent ex vivo lipolysis of lipoprotein triglyceride provided that heparin is administered only in amounts sufficient to maintain catheters patent (0.01 U·kg⁻¹·min⁻¹). Steady state was considered to be present if arterial palmitate concentrations were stable in two to three samples over the 20- to 60-min period. Satisfactory steady-state conditions were obtained in a total of eight animals (14 time intervals).

Problems with non-steady-state conditions prevented the use of data from the other animals and other time intervals. In our previous regional FFA kinetics studies (15), we found that even moderate deviations from steady-state conditions create nonphysiological values for calculated rates of FFA uptake and release. We have therefore limited the analysis in subsequent human studies and in this study to truly steady-state conditions. The coefficients of variation of plasma palmitate concentrations and SA in these studies were 5 ± 1 and 7 ± 1%, respectively, for the steady-state intervals.

There were three different protocol conditions for the animals included in this study. The dogs in protocol A (a glucose phosphorylase inhibitor study) underwent an 18-h fast before the study. Concurrent with the glycogen phosphorylase inhibitor infusion, somatostatin was infused to suppress pancreatic hormone secretion and, simultaneously, glucagon and basal insulin infusions were administered into the portal vein. In dogs A-1 and A-3, basal insulin was given together with sufficient glucose to maintain euglycemia. In dog A-2 the study protocol was the same; however, after the first sample collection interval, the insulin infusion was discontinued, and therefore FFA concentrations were increased due to insulin deficiency. The dog from protocol B was also fasted for 18 h before the study, having received intracerebroventricular leptin on the previous day. As in protocol A, somatostatin was infused to suppress pancreatic hormone secretion and, simultaneously, glucagon and basal insulin infusions were administered into the portal vein. In dogs A-1 and A-3, basal insulin was given together with sufficient glucose to maintain euglycemia. After an initial sampling interval, the insulin infusion was increased to 0.6 mU·kg⁻¹·min⁻¹, and the glucose infusion rate was adjusted to maintain euglycemia. The dogs in protocol C were 18 h fasted and, after collection of the initial series of blood samples, glucose was infused into the portal vein at a rate of 2.5 mg·kg⁻¹·min⁻¹. Endogenous insulin secretion was allowed to increase naturally. There were 1- to 2-h intervals between sample collection time points to allow for reequilibration.

Calculations and statistical analysis. The mean plasma palmitate concentration and SA in the arterial plasma, portal venous plasma, and hepatic venous plasma for each time interval were used for the calculations of regional palmitate uptake and release. Hepatic arterial blood flow and portal blood flow values were converted to plasma flow by use of the measured hematocrit for each animal. Splanchnic plasma flow was the sum of the portal vein and hepatic artery plasma flow.
The following definitions all relate to subsequent Eqs. 1–16.

- $\text{artrial}_{\text{[palmitate]}}$: Concentration of palmitate (µmol/l) in arterial plasma
- $H_{V\text{[palmitate]}}$: Concentration of palmitate (µmol/l) in hepatic venous plasma
- $P_{V\text{[palmitate]}}$: Concentration of palmitate (µmol/l) in portal venous plasma
- $\text{arterial}_{\text{[3H\text{[palmitate]}}}$: Concentration of $^3$H palmitate (dpm/ml) in arterial plasma
- $H_{V\text{[3H\text{[palmitate]}}}$: Concentration of $^3$H palmitate (dpm/ml) in hepatic venous plasma
- $P_{V\text{[3H\text{[palmitate]}}}$: Concentration of $^3$H palmitate (dpm/ml) in portal venous plasma
- $P_{F\text{HA}}$: Plasma flow in hepatic artery
- $P_{F\text{PV}}$: Plasma flow in the portal vein
- $P_{F\text{SPL}}$: Splanchnic plasma flow

Palmitate delivery to the liver (µmol/min) from the hepatic artery is

$$\text{artrial}_{\text{[palmitate]}} \times P_{F\text{HA}} \quad (1)$$

Palmitate delivery to the liver (µmol/min) from the portal vein is

$$P_{V\text{[palmitate]}} \times P_{F\text{PV}} \quad (2)$$

Total hepatic palmitate delivery is the sum of Eqs. 1 and 2. The net uptake of palmitate by the splanchnic bed was calculated as

$$\text{ arterial}_{\text{[palmitate]}} - H_{V\text{[palmitate]}} \times P_{F\text{SPL}} \quad (3)$$

The net hepatic palmitate uptake was calculated as

$$\text{total hepatic delivery} - (H_{V\text{[palmitate]}} \times P_{F\text{SPL}}) \quad (4)$$

The fractional net hepatic palmitate uptake is

$$\frac{\text{[3H\text{[palmitate]}} \text{delivery to the liver (dpm/min) from the hepatic artery} \times P_{F\text{HA}}}{\text{total hepatic palmitate delivery}} \quad (5)$$

$$\text{[3H\text{[palmitate]}} \text{delivery to the liver (dpm/min) from the portal vein} \times P_{F\text{PV}} \quad (6)$$

Total hepatic $^3$H palmitate delivery is the sum of Eqs. 6 and 7. The uptake of $^3$H palmitate by the splanchnic bed was calculated as

$$\text{ arterial}_{\text{[3H\text{[palmitate]}} - H_{V\text{[3H\text{[palmitate]}}}} \times P_{F\text{SPL}} \quad (8)$$

The hepatic $^3$H palmitate uptake was calculated as

$$\text{total hepatic } ^3\text{H palmitate delivery} - (H_{V\text{[3H\text{[palmitate]}}}} \times P_{F\text{SPL}}) \quad (9)$$

The fractional hepatic $^3$H palmitate extraction is

$$\frac{\text{fractional hepatic } ^3\text{H palmitate extraction}}{\text{total hepatic } ^3\text{H palmitate delivery}} \quad (10)$$

Comparison of the net fractional palmitate uptake with fractional $^3$H palmitate extraction is used to assess the validity of the proposed model. The fractional hepatic extraction of $^3$H palmitate can also be used to estimate the amount of palmitate derived from visceral adipose tissue lipolysis that should appear in hepatic venous plasma.

Visceral fat palmitate release

$$\times (1 - \text{fractional hepatic } ^3\text{H palmitate extraction}) \quad (11)$$

The net release of palmitate by nonhepatic splanchnic tissues (presumably visceral adipose tissue) was calculated as

$$P_{V\text{[palmitate]}} - \text{arterial}_{\text{[palmitate]}} \times P_{F\text{PV}} \quad (12)$$

Splanchnic palmitate uptake measured isotopically (as opposed to net uptake) was calculated as

$$\text{(arterial}_{\text{[palmitate]}} \times P_{F\text{SPL}}) \quad (13)$$

This calculation assesses the uptake of palmitate by both hepatic and nonhepatic splanchnic tissues. Splanchnic palmitate release was calculated as

$$\text{[(HV}_{\text{[palmitate]}} \text{ - arterial}_{\text{[palmitate]}} \times P_{F\text{SPL}})} \quad (14)$$

+ splanchnic palmitate uptake

The uptake of palmitate by nonhepatic splanchnic tissues was calculated as

$$\text{arterial}_{\text{[palmitate]}} \times P_{F\text{PV}} \quad (15)$$

$\times \text{fractional nonhepatic splanchnic palmitate extraction}$

$$\text{(i.e., arterial}_{\text{[3H\text{[palmitate]}} - P_{V\text{[3H\text{[palmitate]}}}} \div \text{arterial}_{\text{[3H\text{[palmitate]}}}} \quad (16)$$

Hepatic palmitate uptake is calculated by subtracting nonhepatic splanchnic palmitate uptake from splanchnic palmitate uptake.

Visceral adipose tissue palmitate release was calculated as

$$P_{V\text{[palmitate]}} - \text{arterial}_{\text{[palmitate]}} \times P_{F\text{PV}} \quad (16)$$

+ nonhepatic splanchnic palmitate uptake

Release of palmitate into the hepatic circulation was calculated by subtracting the predicted hepatic vein palmitate appearance derived from visceral fat (Eq. 11) from splanchnic palmitate release (Eq. 14).

All values are expressed as means ± SE unless otherwise indicated. The model described in APPENDIX A was used to estimate the percentage of palmitate delivered to the liver from visceral adipose tissue lipolysis. Statistical comparisons between the model-predicted percent delivery of palmitate to the liver from visceral fat vs. the observed visceral palmitate delivery were made with a paired t-test. Linear regression analysis was used to assess the relationship between plasma palmitate concentrations and the model-derived error in the proportion of palmitate delivered to the liver from visceral adipose tissue lipolysis.

RESULTS

The average steady-state palmitate concentrations and SA in arterial plasma, hepatic venous plasma, and portal venous plasma for the 14 time intervals in the 8 dogs are presented in Table 1. Arterial concentrations were higher than hepatic venous concentrations and tended to be lower than portal palmitate concentrations.

AJP-Endocrinol Metab • VOL 284 • JUNE 2003 • www.ajpendo.org
The average splanchnic plasma flow was 410 ± 16 ml/min. Portal venous plasma flow averaged 330 ± 17 ml/min, and hepatic arterial plasma flow was 81 ± 8 ml/min. The body weight and portal and hepatic artery plasma flow values are provided in Table 2.

Net palmitate balance. Splanchnic palmitate delivery from the arterial circulation was 71.4 ± 10.5 µmol/min, and splanchnic palmitate exit via the hepatic vein was 62.6 ± 9.4 µmol/min, for a net splanchnic palmitate uptake of 8.9 ± 1.7 µmol/min. Palmitate delivery to the liver via the hepatic artery and portal vein averaged 13.5 ± 1.9 and 62.3 ± 10.2 µmol/min, respectively; total hepatic palmitate delivery was 75.8 ± 11.6 µmol/min. The net hepatic palmitate uptake was 13.4 ± 2.3 µmol/min, for a net fractional hepatic palmitate extraction of 16 ± 2%. The net release of palmitate across visceral fat was 4.2 ± 1.8 µmol/min.

Tracer-determined intrasplanchnic palmitate kinetics. Total splanchnic palmitate uptake was 17.4 ± 2.3 µmol/min (mean of all 14 values), of which the 4.3 ± 0.8 µmol/min portion was nonhepatic splanchnic uptake and the 13.1 ± 2.3 µmol/min portion was hepatic uptake. Individual values are provided in Table 3. Twenty-five ± 1% of [3H]palmitate was taken up traversing the splanchnic bed, 18 ± 2% in the hepatic bed and 8 ± 2% in nonhepatic splanchnic tissues. The fractional extraction of [3H]palmitate across the liver was 21 ± 2%, which was greater (P = 0.003) than the fractional extraction of unlabeled palmitate (from arteriovenous concentration differences alone) across the liver of 16 ± 2%.

Individual values for visceral and splanchnic palmitate release are provided in Table 4. The palmitate release from visceral adipose tissue was 7.9 ± 1.8 µmol/min, accounting for 11 ± 2% of the palmitate delivered to the liver. Splanchnic palmitate release was 8.7 ± 1.5 µmol/min. Because ~79% of [3H]palmitate delivered to the liver via the portal vein and hepatic artery appeared in the hepatic vein, we anticipated that palmitate release from the splanchnic bed would be 6.1 ± 1.4 µmol/min. This predicted value was less (P < 0.01) than the observed splanchnic palmitate release rate. To account for this discrepancy, 2.5 ± 0.6 µmol/min of palmitate would have been released into the hepatic circulation if there had been no differences in FFA extraction between the portal and arterial circulation.

Visceral adipose tissue palmitate release ranged from ~0 to 21 µmol/min (Table 3), and splanchnic palmitate release ranged from 0.6 to 22 µmol/min. There was a good agreement between visceral adipose FFA release measured by use of portal vein sampling and splanchnic FFA release measured with hepatic vein sampling (Fig. 1).

Model-predicted vs. measured hepatic palmitate delivery. For all time intervals for which steady-state data were available, the model (APPENDIX A) predicted that 15 ± 2% of hepatic palmitate delivery was occurred.

Table 1. Plasma palmitate concentrations and specific activities

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>A-1</td>
<td>80</td>
<td>251</td>
<td>73</td>
<td>13.7</td>
<td>5.3</td>
<td>9.8</td>
</tr>
<tr>
<td>A-2</td>
<td>134</td>
<td>74</td>
<td>139</td>
<td>114</td>
<td>4.2</td>
<td>8.1</td>
</tr>
<tr>
<td>B-1</td>
<td>217</td>
<td>148</td>
<td>244</td>
<td>162</td>
<td>8.8</td>
<td>7.5</td>
</tr>
<tr>
<td>C-1</td>
<td>227</td>
<td>86</td>
<td>211</td>
<td>195</td>
<td>15.9</td>
<td>7.1</td>
</tr>
<tr>
<td>C-2</td>
<td>358</td>
<td>229</td>
<td>421</td>
<td>284</td>
<td>3.6</td>
<td>4.6</td>
</tr>
<tr>
<td>C-3</td>
<td>275</td>
<td>57</td>
<td>291</td>
<td>55</td>
<td>1.5</td>
<td>6.0</td>
</tr>
<tr>
<td>C-4</td>
<td>213</td>
<td>199</td>
<td>173</td>
<td>5.3</td>
<td>6.8</td>
<td>16.3</td>
</tr>
</tbody>
</table>

Mean concentration and specific activity (SA) values from 8 dogs with acceptable steady-state data for the presented time intervals.

Table 2. Body weight and plasma flow

<table>
<thead>
<tr>
<th>Dog no.</th>
<th>Weight, kg</th>
<th>Interval:</th>
<th>Arterial Plasma flow, ml/min</th>
<th>Portal vein Plasma flow, ml/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-1</td>
<td>27.3</td>
<td>63</td>
<td>381</td>
<td></td>
</tr>
<tr>
<td>A-2</td>
<td>23.3</td>
<td>76</td>
<td>302</td>
<td>376</td>
</tr>
<tr>
<td>B-1</td>
<td>22.8</td>
<td>90</td>
<td>377</td>
<td>277</td>
</tr>
<tr>
<td>A-3</td>
<td>22.8</td>
<td>66</td>
<td>367</td>
<td>367</td>
</tr>
<tr>
<td>C-1</td>
<td>20.6</td>
<td>118</td>
<td>267</td>
<td>256</td>
</tr>
<tr>
<td>C-2</td>
<td>20.1</td>
<td>79</td>
<td>317</td>
<td>246</td>
</tr>
<tr>
<td>C-3</td>
<td>26.6</td>
<td>54</td>
<td>456</td>
<td>384</td>
</tr>
<tr>
<td>C-4</td>
<td>24.6</td>
<td>54</td>
<td>249</td>
<td></td>
</tr>
</tbody>
</table>

Plasma flow values from the 8 dogs with acceptable steady-state palmitate data.

Table 3. Tracer-determined intrasplanchnic palmitate uptake

<table>
<thead>
<tr>
<th>Dog no.</th>
<th>Nonhepatic FFA release, µmol/min</th>
<th>Hepatic FFA release, µmol/min</th>
<th>Splanchnic FFA release, µmol/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-1</td>
<td>6.8</td>
<td>2.7</td>
<td>4.1</td>
</tr>
<tr>
<td>A-2</td>
<td>5.7</td>
<td>2.7</td>
<td>4.1</td>
</tr>
<tr>
<td>B-1</td>
<td>2.7</td>
<td>2.7</td>
<td>4.1</td>
</tr>
<tr>
<td>C-1</td>
<td>2.1</td>
<td>2.1</td>
<td>4.1</td>
</tr>
<tr>
<td>C-2</td>
<td>1.1</td>
<td>1.1</td>
<td>4.1</td>
</tr>
<tr>
<td>C-3</td>
<td>10.7</td>
<td>10.7</td>
<td>4.1</td>
</tr>
<tr>
<td>C-4</td>
<td>5.5</td>
<td>5.5</td>
<td>4.1</td>
</tr>
</tbody>
</table>

Nonhepatic FFA release measured by use of portal vein sampling and splanchnic FFA release measured with hepatic vein sampling (Fig. 1).

Model-predicted vs. measured hepatic palmitate delivery. For all time intervals for which steady-state data were available, the model (APPENDIX A) predicted that 15 ± 2% of hepatic palmitate delivery was occurred.

AJP-Endocrinol Metab • VOL 284 • JUNE 2003 • www.ajpendo.org
Table 4. Tracer-determined intrasplanchnic palmitate release

<table>
<thead>
<tr>
<th>Dog no.</th>
<th>Interval:</th>
<th>Palmitate Release, μmol/min</th>
<th>Splanchnic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>A-1</td>
<td>3.2</td>
<td>5.4</td>
<td></td>
</tr>
<tr>
<td>A-2</td>
<td>9.1</td>
<td>5.5</td>
<td>7.1</td>
</tr>
<tr>
<td>B-1</td>
<td>7.6</td>
<td>3.6</td>
<td>5.6</td>
</tr>
<tr>
<td>A-3</td>
<td>12.8</td>
<td>8.9</td>
<td>10.0</td>
</tr>
<tr>
<td>C-1</td>
<td>-2.2</td>
<td>0.5</td>
<td>5.7</td>
</tr>
<tr>
<td>C-2</td>
<td>21.0</td>
<td>14.0</td>
<td>22.0</td>
</tr>
<tr>
<td>C-3</td>
<td>18.0</td>
<td>3.9</td>
<td>15.8</td>
</tr>
<tr>
<td>C-4</td>
<td>2.1</td>
<td>0.6</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1. Visceral adipose tissue palmitate release is plotted vs. splanchnic palmitate release measured during 14 study intervals from 8 dogs. Solid line, regression line between visceral and splanchnic palmitate release; dotted line, line of identity (shown for comparison purposes only). □, ●, and △, Data from protocols A, B, and C, respectively.

Fig. 2. Actual %hepatic palmitate delivery originating from visceral adipose tissue free fatty acid (FFA) release is plotted vs. %hepatic palmitate delivery from visceral free fatty acid predicted on the basis of the model presented in APPENDIX A. Dashed line, line of identity (shown for comparison purposes only). □, ●, and △, Data from protocols A, B and C, respectively.

Fig. 3. Error of model-predicted hepatic palmitate delivery from visceral fat is plotted as a function of the arterial palmitate concentration. Error is calculated by subtracting the observed %hepatic palmitate delivery from visceral fat from observed %hepatic palmitate delivery predicted by the model presented in APPENDIX A.

ring from visceral adipose tissue lipolysis. This is most readily apparent from Table 1; the hepatic vein palmitate SA averages 15 ± 2% less than the matching arterial palmitate SA. The model-predicted fractional delivery from visceral lipolysis was greater (P < 0.004) than the percentage of palmitate delivery to the liver from visceral adipose tissue lipolysis that was measured with the portal vein and hepatic artery data (11 ± 2%). Despite the discrepancy, there was a generally good relationship between the predicted percent visceral FFA release from adipose tissue and the actual percent visceral FFA delivery to the liver (Fig. 2). The relationship between the model-predicted and observed values for percent hepatic palmitate delivery was as follows: intercept = 3.6% [standard error of the estimate (SEE) = 2.8%]; β-coefficient = 1.11 (SEE = 0.25). Because the intercept of this relationship was not significantly different from 0, but the slope was >1, we examined factors that might relate to the error in the predicted hepatic palmitate delivery to the liver. Figure 3 describes the relationship between arterial plasma palmitate concentrations and the error in the model-predicted hepatic palmitate delivery. There was a significant (P < 0.05) inverse correlation between arterial plasma palmitate concentrations. At low plasma palmitate concentrations, the model significantly overpredicted the percentage of hepatic palmitate delivery originating from visceral fat, whereas at mid- and high-physiological concentrations there was reasonably good agreement between the model-predicted and the observed percentages.
DISCUSSION

These studies were undertaken to assess the relationship between visceral adipose tissue FFA release into the portal vein and splanchnic FFA release by use of hepatic vein sampling. One goal was to determine whether it is possible to predict the percentage of FFAs being delivered to the liver from visceral adipose tissue lipolysis. We tested a model that relied on the assumption that the FFAs delivered to the liver from the hepatic artery and the portal venous circulation are extracted equally and that there is no FFA released across the hepatic bed. If this model were correct, there should have been no difference between the observed and model-predicted percentages of FFAs delivered to the liver originating from visceral fat. Instead, we found that the model overestimated the portion of visceral adipose tissue FFAs being delivered to the liver. The error was greatest at lower FFA, with errors of 4–14% at plasma palmitate concentrations below 100 μmol/l.

There appear to be at least two possible general explanations for the discrepancy between the observed proportion of hepatic FFA delivery originating from visceral adipose tissue lipolysis and that predicted by the model. If FFAs delivered to the liver via the hepatic artery are cleared more readily than those delivered via the portal circulation, the observed splanchnic palmitate release would exceed that predicted by the measured visceral adipose tissue palmitate uptake and hepatic [3H]palmitate uptake data (see APPENDIX B). Alternatively, FFA release directly into intrahepatic circulation would give the same result. FFAs being released directly from the liver would be surprising, given that the liver does not seem equipped as a lipolytic organ. We could find no reports of expression of hormone-sensitive lipase in adult liver tissue. Likewise, liver does not express some of the other proteins important in the regulation of trafficking and modulation of fatty acid release (e.g., perilipin-A). An alternative possibility is that the hydrolysis of triglyceride-rich lipoproteins by hepatic lipase is not accompanied by quantitative uptake of the resulting fatty acids into hepatocytes. Some of the fatty acids generated by this process may escape into the hepatic venous circulation. We have observed the entry of chylomicron triglyceride fatty acids into the systemic FFA pool (15), indicating that such a phenomenon is possible. We cannot distinguish between these possibilities given the present data.

APPENDIX B uses a numeric example to depict a scenario of a model overestimate of the relative contribution of visceral adipose tissue lipolysis to hepatic FFA delivery. If the relative uptake of fatty acids delivered via the hepatic artery is greater than the uptake of fatty acids delivered via the portal vein, the model overestimates the percentage of FFAs delivered to the liver from visceral adipose tissue lipolysis. This error could not be distinguished from intrahepatic FFA release, and it is the pattern we observed in the present study. We also considered the possibility that differences in the ratio of arterial to total hepatic blood flow or errors in blood flow measurement could account for the apparent model errors. By use of regression analysis approaches similar to those described to generate Fig. 3, there was no statistically significant association between relative source of hepatic blood flow and the model error.

Although the model significantly overestimated the relative contribution of visceral adipose tissue lipolysis to hepatic FFA delivery, it is reassuring to note that the error was primarily observed at low plasma FFA concentrations. With plasma FFA concentrations closer to the overnight postabsorptive range, the systematic error was much reduced (see Fig. 2). A perhaps surprising observation is the good agreement between the FFAs released from visceral adipose tissue lipolysis and splanchnic FFA release (Fig. 1). The data points fall around the line of identity, except perhaps at very low rates of visceral FFA release, where splanchnic FFA release was systematically greater than visceral FFA release. We expected that splanchnic FFA release would be systematically less than visceral adipose tissue FFA release. We take some comfort from the fact that the model-derived values of the percentage of FFAs delivered to the liver from visceral fat was highly correlated with the actual percentage of FFAs delivered to the liver from visceral adipose tissue lipolysis.

Thus the splanchnic FFA release information obtained from hepatic vein catheterization studies seems to provide good, albeit not perfect, information about visceral adipose tissue FFA release, except at a time of marked suppression of visceral lipolysis.

In this regard, we must reconsider results of some of our published hepatic vein catheterization studies. We studied regional suppression of FFA release by insulin (10) and by meals (6) in humans (13). In some cases, we found less relative suppression of splanchnic FFA release than of systemic FFA release and concluded that visceral adipose tissue lipolysis was more resistant to suppression than leg or upper body subcutaneous adipose tissue. The results of the present study lead us to question this assertion. Although splanchnic FFA release may account for a greater share of systemic FFA release under insulin-suppressed conditions, we cannot be certain that the “excess” FFAs actually arise from visceral adipose tissue lipolysis. Despite this reservation, the finding that the splanchnic bed becomes a net producer of FFAs under hyperinsulinemic conditions (10) cannot be attributed to differences in the hepatic uptake of FFAs delivered from the hepatic artery vs. the portal vein. Only less suppression of visceral adipose tissue lipolysis or substantial generation of FFAs within the hepatic circulation could account for this observation.

Our results are in general concordance with the elegant studies of intrasplanchnic FFA metabolism published by Basso and Havel (2). An exception is their conclusion that, because hepatic extraction was not
different between the $^{14}$C-labeled FFAs and titratable FFAs, there was no release of FFAs across the hepatic circulatory bed. It is likely that the greater precision of HPLC, which was used to measure both palmitate concentration and SA in the present study, allowed us to detect the relatively small discrepancy between these two values.

These studies involved a number of different protocols and insulin conditions that allowed us to test the model over a range of FFA availability. One concern, however, is that the experimental design could affect factors that influence the FFA results, such as hepatic lipase activity. For example, leptin administration to ob/ob mice increases hepatic lipase activity (9), and one of the dogs received intracerebroventricular leptin before the study. The data points from this animal fell on the same regression lines (see Figs. 1 and 2) as those of the other animals, making it unlikely that the leptin intervention had an effect in this leptin-sufficient animal. Hepatic lipase activity has also been reported to change after 2 wk of growth hormone (GH) therapy in GH-deficient adult humans (14), but the short-term nature of these experiments makes it unlikely that changes in hepatic lipase would have occurred via a GH mechanism. It is also possible that the glycogen phosphorylase inhibitor affected hepatic lipase activity or fractional palmitate uptake from portal vs. arterial sources. The data presented in Figs. 1 and 2 do not indicate that animals in this protocol were different from those that received only a glucose infusion. Other studies have shown that insulin is not different from saline infusion as regards hepatic lipase activity (1), indicating that the insulin infusions likely did not affect this aspect of our study.

In summary, we performed studies of intrasplanchnic FFA kinetics in dogs with hepatic vein, portal vein, and arterial catheters to determine whether it is possible to model the fraction of FFAs delivered to the liver originating from visceral lipolysis. Studies were selected to include a wide range of FFA release rates to test the model over the range of FFA concentrations encountered in metabolic studies. We found that the model significantly overestimated the portion of FFAs delivered to the liver from visceral lipolysis. Fortunately, there was a good correlation between the model-predicted and observed visceral contribution to hepatic FFA delivery. In addition, we found that splanchnic FFA release rates were in good agreement with visceral adipose FFA release rates, except when lipolysis was suppressed. Under these circumstances, splanchnic FFA release may overestimate visceral adipose tissue FFA release due either to generation of a small amount of FFAs in the intrahepatic circulation or differential hepatic extraction of FFAs from the arterial vs. portal venous circulation, which are factors that do not appear to be proportional to the prevailing plasma FFA concentration. Awareness of this limitation to the result of hepatic vein catheterization studies should help avoid overinterpretation of results.

**APPENDIX A**

Known values are

- $A$  Arterial FFA entering the splanchnic bed ($\mu$mol/min)
- $A^*$  Arterial FFA exiting the splanchnic bed ($\mu$mol/min)
- $V^*$  Visceral FFA exiting the splanchnic bed ($\mu$mol/min)

$A$ is the product of arterial palmitate ($\mu$mol/l) and splanchnic plasma flow (l/min), both measured values.

$A^*$ is known, because the arterial palmitate SA (dpm/\mu mol) entering the splanchnic bed is measured and is not changed as FFAs pass through the splanchnic bed (there is no addition of $^3$H or selective removal of labeled or nonlabeled FFAs). Therefore, the appearance of $[^3]$Hpalmitate (dpm/min) into the hepatic vein divided by the arterial $[^3]$Hpalmitate SA (dpm/\mu mol) permits the calculation of arterial palmitate exiting the splanchnic bed ($\mu$mol/min).

$V^*$ is the FFAs (palmitate) exiting the splanchnic bed over and above those passing through from the arterial circulation. This value is equal to the exit of palmitate from the splanchnic bed ($HV[palmitate] \times$ splanchnic plasma flow) – $A^*$.

Unknown values are

- $A'$  Arterial FFAs delivered to the liver
- $V'$  Visceral FFAs delivered to the liver

Let $y$ = the fraction of arterial FFAs taken up by extrahepatic splanchnic tissues.

Let $z$ = the fraction of FFA taken up by the liver.

It follows that

$$A' = A(1 - y)$$
$$A^* = A(1 - y)(1 - z) = A'(1 - z)$$
$$V^* = V'(1 - z)$$

---

Fig. A1.
The proportion of FFAs delivered to the liver originating from visceral fat equals

\[ \frac{V^*}{V^*(400 \text{ nmol}) + A^*(400 \text{ nmol @ 1 dpm/nmol})} \]

50% from visceral lipolysis

Because both \( V^* \) and \( A^* \) are known values, the proportion of FFAs delivered to the liver that originates from visceral fat can theoretically be determined.

More simply stated, this model assumes that the SA (or enrichment in studies using stable isotopes) of FFAs appearing in the hepatic vein is equivalent to the average SA of FFAs delivered to the liver from the portal vein and hepatic artery relative to their respective plasma flows. Thus the fractional reduction in hepatic vein FFA SA relative to the arterial FFA SA reflects the fraction of hepatic FFA delivery originating from visceral lipolysis.

**APPENDIX B**

A numeric example is provided in Fig. B1 to depict how the model proposed in APPENDIX A might perform if the fractional extraction of FFAs delivered from the hepatic artery were greater than that delivered from the portal vein.

In this example, the arterial FFA SA is 1.0 dpm/nmol, and total splanchnic FFA delivery is 1,000 nmol/min (200 nmol/min from the hepatic artery and 800 nmol/min from the celiac, superior, and inferior mesenteric arteries). The non-hepatic splanchnic tissues take up 200 nmol/min (and thus 200 dpm/min) of the 800 nmol/min to which they are exposed, allowing 600 nmol/min and 600 dpm/min of arterially delivered FFAs to enter into the portal vein. In this example, visceral adipose tissue releases 600 nmol/min of FFAs (no radioactive FFAs) into the portal vein.

In this hypothetical example, hepatic uptake of arterially delivered FFAs is greater (100%, i.e., 200 nmol/min and 200 dpm/min) than portal vein FFA uptake (33%, i.e., 400 nmol/min and 200 dpm/min). In this case, the model outlined in APPENDIX A will overestimate the proportion of FFAs delivered to the liver from visceral adipose tissue. In this numeric example, total hepatic FFA delivery is 1,400 nmol/min, i.e., 800 from arterial sources and 600 (42.9%) from visceral lipolysis. The appearance of total FFAs in the hepatic vein is 800 nmol/min, and the appearance of radioactive FFAs in the hepatic vein is 400 dpm/min. The model would predict that 50% of hepatic FFA delivery is of visceral adipose tissue origin and would thus be incorrect.

This study was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grants DK-40484 and DK-14507 and by the Mayo Foundation.

**REFERENCES**