Regional glycerol and free fatty acid metabolism before and after meal ingestion

MICHAEL D. JENSEN
Endocrine Research Unit, Mayo Clinic, Rochester, Minnesota 55905

Jensen, Michael D. Regional glycerol and free fatty acid metabolism before and after meal ingestion. Am. J. Physiol. 276 (Endocrinol. Metab. 39): E863–E869, 1999.—We measured splanchnic and leg glycerol [and free fatty acid (FFA)] uptake and release in 11 healthy volunteers before and after meal ingestion to assess whether regional FFA-to-glycerol release ratios mirror systemic release ratios. Basal splanchnic triglyceride release was also assessed. Although basal splanchnic glycerol uptake (111 ± 18 µmol/min) accounted for most of systemic glycerol rate of appearance (156 ± 20 µmol/min), leg glycerol uptake was also noted. The basal, systemic FFA-to-glycerol release ratio was less (2.6 ± 0.2, P < 0.05) than the splanchnic ratio of 6.1 ± 1.3, and the leg FFA-to-glycerol release ratio under fed conditions was less than the systemic ratio (0.9 ± 0.1 vs. 1.6 ± 0.2, respectively, P < 0.05). Basal splanchnic triglyceride production rates were 74 ± 20 µmol/min, which could produce equimolar amounts of glycerol in the peripheral circulation via lipoprotein lipase action. In summary, 1) regional FFA-to-glycerol release ratios do not mirror systemic ratios, 2) leg glycerol uptake occurs in humans, and 3) splanchnic triglyceride production rates are substantial relative to systemic glycerol appearance. Glycerol appearance rates may not be a quantitative index of whole body lipolysis.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

GLYCEROL RATE of appearance (Rₐ) measured using isotopic tracers is widely used as a quantitative measure of whole body lipolysis (1, 4, 7, 16, 17, 24, 29, 31, 32). The ratio of free fatty acid (FFA) to glycerol Rₐ has been used as a quantitative index of intra-adipocyte fatty acid recycling, whereas the difference between fatty acid oxidation (measured using indirect calorimetry) and three times glycerol Rₐ has been taken as a measure of total fatty acid recycling (1, 4, 7, 31, 32). Although fatty acid recycling is considered to be minimal under basal conditions, hyperinsulinemia substantially reduces the FFA-to-glycerol release ratio (4), which is interpreted as reflecting increased intra-adipocyte recycling. These calculations assume that three FFA molecules become available for every glycerol molecule entering the circulation. Several lines of evidence are used to support these assumptions, including 1) glycerol is only released by lipolysis; 2) there is insufficient glycerol kinase in muscle to prevent release of any glycerol generated from intracellular lipolysis; 3) splanchnic release of glycerol is minimal and thus underestimation of glycerol Rₐ because of first-pass hepatic clearance is not a problem; and 4) underestimation of lipolysis because of partial hydrolysis of triacylglycerols is unlikely. The second and third assumptions may not be entirely correct, however.

Glycerol kinase activity has been reported in muscle of vertebrate animals (25), and recent studies have documented uptake of glycerol across the upper extremity of humans (6, 18, 19). These observations suggest that muscle is capable of taking up and metabolizing glycerol from the circulation and, by inference, some of the glycerol generated from the hydrolysis of intramuscular triglycerides. These factors could introduce uncertainty in the use of glycerol appearance as a quantitative measure of lipolysis because 1) the glycerol generated from intramuscular “lipolysis” might not quantitatively enter the circulation and 2) glycerol generated from lipoprotein lipase (LPL)-mediated triglyceride hydrolysis at the capillary endothelium in skeletal muscle might be taken up by muscle instead of reentering the bloodstream.

Although the splanchnic release of glycerol appears to be modest in humans, the release of FFA is not (12). Simultaneous measures of splanchnic glycerol and FFA kinetics could be informative; the products of visceral adipose tissue lipolysis enter the systemic circulation only after passing through the liver, which has very high glycerol kinase activity (20). More efficient first-pass clearance of glycerol than FFA by the liver would be expected to produce nonphysiological FFA-to-glycerol release ratios in the hepatic vein. Another relevant aspect of splanchnic lipid metabolism is the hepatic production and release of triglycerides (primarily very low density lipoproteins). At steady state, the net splanchnic triglyceride production rate should be equal to triglyceride clearance via LPL-mediated triglyceride hydrolysis in peripheral tissues, a process that might release glycerol back into the bloodstream. Few investigators appear to have assessed the potential contribution of this process to systemic glycerol Rₐ in humans.

In the process of conducting regional FFA metabolic studies (10) we took the opportunity to examine glycerol kinetics. This allowed us to measure regional FFA-to-glycerol release ratios and to test whether leg uptake of glycerol occurs in vivo. By measuring arteriovenous triglyceride concentration differences we could assess the potential Rₐ of glycerol in the systemic circulation from intravascular triglyceride hydrolysis via LPL. The measures of systemic and regional glycerol and FFA kinetics in the postprandial state allowed us to examine regional vs. systemic FFA-to-glycerol release under conditions of perturbed lipolysis. The results of our studies suggest that several of the assumptions used to justify glycerol Rₐ as a quantitative index of whole body lipolysis may need to be reconsidered.
Subjects. Informed, written consent was obtained from 11 nonobese men and premenopausal women. All volunteers were in good health, taking no medications, and had maintained a stable weight for >2 mo before the studies. The subjects described in this report are a subgroup of the 16 volunteers that participated in a previously published study of regional fatty acid kinetics (10). All volunteers received a glycerol tracer infusion during the course of that study; however, delays in the development of glycerol specific activity (SA) assay prevented the prompt analysis of samples. There was insufficient remaining plasma for measurement of glycerol SA for five of the subjects that were originally reported.

Materials and assays. [9,10-3H]oleate was obtained from NEN Research Products (Wilmington, DE) and bound to human albumin for infusion; [2-3H]glycerol was supplied by Amersham (Arlington Heights, IL), indocyanine green (CardioGreen; Becton-Dickinson, Cockeysville, MD) was used in these studies. Plasma oleate and FFA concentration and oleate SA were determined by a modification (13) of a published HPLC technique (23). Femoral artery, femoral vein, and hepatic vein glycerol and plasma triglyceride concentrations were measured in triplicate two times using a microfluorometric method (9). Plasma free glycerol SA was measured using HPLC (15). Plasma indocyanine green concentrations were measured by spectrophotometry on the day of the study.

Protocol. The details of this study protocol have been previously published (10). In brief, each volunteer consumed all meals in the Mayo Clinic General Clinical Research Center (GCRC) for 2 wk to establish weight maintenance energy intake. They were admitted to the GCRC the night before the study. The next morning, after an overnight fast, the volunteers were transferred to the Vascular Radiology Laboratory where femoral arterial catheters were placed for the infusion of indocyanine green (through a 5-Fr sheath) and the sampling of arterial blood. A femoral venous sheath was placed in the right femoral vein, and a catheter was placed in the right hepatic vein using fluoroscopy. A small amount of contrast dye was used to confirm the position of the hepatic vein catheter. A 0.45% NaCl solution was infused through the sheaths and catheters to maintain patency. The volunteers were transferred back to the GCRC for the completion of the study after the procedure.

Upon return to the GCRC the [3H]oleate (0.3 μCi/min) and [14C]glycerol (0.22 ± 0.2 μCi/min) infusions were started in a forearm peripheral vein, and indocyanine green was infused through the femoral arterial sheath. Sixty minutes were allowed to achieve isotopic steady state, after which arterial, femoral venous, and hepatic venous blood samples were taken at 15-min intervals over the 30 min before meal consumption. The liquid meal (Ensure Plus; Ross Laboratories) contained 6 kcal (25.95 kJ) of fat, 28 kcal (116.50 kJ) of carbohydrate, and 36 kcal (148.80 kJ) of protein. The meal was consumed over a 10- to 20-min interval. The meals contained 755 ± 36 kcal (3,158 ± 150 kJ) with 28 ± 1, 27 ± 1, and 100 ± 5 g of protein, fat, and carbohydrate, respectively. Blood samples were obtained at 30-min intervals for 6 h after meal consumption. After completion of this study all catheters were removed, and hemostasis was obtained. The subject remained in the hospital under observation until the following morning.

Calculations. Leg plasma flow (14) and splanchnic plasma flow (SPF; see Ref. 3) were measured using the arterial, femoral venous, and hepatic venous plasma indocyanine green concentrations. Leg and splanchnic blood flow (SBF) were calculated using each individual’s hematocrit to convert from plasma flow to blood flow.

Systemic FFA and glycerol flux was measured using the mean, steady-state plasma FFA and glycerol SA (dpnmol) and the tracer infusion rates (dpnmol/min) observed during both the basal and meal nadir time intervals (11, 15).

Steady-state conditions are required to perform valid tissue balance calculations (34); therefore, only time intervals that met these requirements were analyzed. Plasma FFA concentration and SA were constant over the baseline sampling interval, as was splanchnic and leg blood flow; therefore, steady-state plasma FFA SA and concentration were used together with measures of leg and hepatic plasma flow to measure regional (leg and splanchnic) FFA Rsa (8, 22). A similar approach was used to measure regional glycerol kinetics, except that, because glycerol is distributed in whole blood (glycerol equilibrates freely between plasma and erythrocytes; see Ref. 2), regional glycerol kinetics were performed using blood flow values.

Some of the formulas used to determine regional glycerol kinetics are provided. To calculate regional substrate kinetics using isotopic tracers, one of the initial values derived is the fractional uptake of the tracer (which reflects the fractional uptake of the substrate).

The fractional extraction of [3H]glycerol by the splanchnic bed equals

\[
\frac{[\text{glycerol}_{a} \times SA_{a} - [\text{glycerol}]_{hv} \times SA_{hv}}{[\text{glycerol}]_{a} \times SA_{a}}
\]

where [glycerol]a is the glycerol concentration in the arterial blood, [glycerol]hv is the glycerol concentration in hepatic venous blood, and SAa and SAhv represent the glycerol specific activities in arterial and hepatic venous blood, respectively.

To calculate the splanchnic uptake of circulating glycerol, splanchnic blood flow must be included

\[
[[\text{glycerol}]_{a} \times SBF] \times [1 - ([\text{glycerol}]_{hv} \times SA_{hv} - ([\text{glycerol}]_{a} \times SA_{a})]
\]

Splanchnic glycerol release can then be calculated

\[
SBF \times ([\text{glycerol}]_{hv} - [\text{glycerol}]_{a}) + \text{splanchnic uptake}
\]

Leg glycerol uptake and release rates were calculated using the same general approach but substituting femoral venous glycerol concentration and SA values and leg blood flow values for hepatic venous and splanchnic blood flow values, respectively.

After meal ingestion a new steady-state nadir (lasting at least 60 min) of FFA and glycerol concentrations and SA was documented in each individual. Together with stable regional blood flow, these conditions permitted the calculation of steady-state meal nadir leg and hepatic FFA and glycerol uptake and release using the mean of three samples. The meal nadir occurred as early as 90–150 min after the meal and as late as 210–270 min postprandially. Because the steady-state nadir values for each individual had been previously identified (10), and because only steady-state conditions can be used to calculate tissue substrate exchange (34), only the nadir postprandial samples were assayed for plasma glycerol SA.

The net basal release of triglycerides from the splanchnic bed was assessed by multiplying hepatic venous-femoral
artery plasma concentration differences by splanchnic plasma flow. Postprandial splanchnic triglyceride kinetics are more complex (26); chylomicron triglycerides enter the circulation via the thoracic duct and are cleared by both splanchnic and peripheral tissues (26). Thus postprandial splanchnic triglyceride balance measures are not meaningful without separate measures of chylomicron and nonchylomicron triglycerides, which were not included as part of this study. Leg triglyceride uptake under both basal and meal conditions was calculated by multiplying the femoral artery-femoral venous plasma concentration differences by leg plasma flow.

Statistics. All results are expressed as means ± SE. Comparisons between basal and meal nadir values, as well as comparisons of the FFA-to-glycerol release ratios between systemic and regional tissue beds, were made using a paired t-test.

RESULTS

Subject characteristics. Samples were available from five men and six women, aged 28 ± 2 yr, weighing 67.4 ± 2.9 kg, and with a body mass index of 22.4 ± 0.5 kg/m². Because of insufficient remaining hepatic venous plasma samples from one volunteer, splanchnic values are available for only 10 subjects in the postprandial state and 9 subjects in the fed state. The individual values for leg and splanchnic blood flow for both study conditions are provided in Tables 1 and 2. The basal leg and splanchnic blood flows were 366 ± 45 and 1,240 ± 138 ml/min, and at the meal nadir the values were 402 ± 63 and 1,433 ± 129 ml/min, respectively.

Glycerol kinetics. The mean arterial, femoral venous, and hepatic venous glycerol concentrations and SA under basal and meal nadir conditions are provided in Table 3, whereas the individual values are provided in Tables 1 and 2. The arterial, femoral venous, and hepatic venous glycerol concentrations were all significantly (P < 0.05) different from each other. The mean systemic and regional glycerol release rates are provided in Table 4, and the individual values are provided in Tables 1 and 2. At the nadir suppression of plasma FFA flux after meal ingestion, systemic and leg glycerol release had decreased significantly, whereas splanchnic glycerol release had not changed from basal values.

Under basal conditions the splanchnic uptake of systemic glycerol was 111 ± 18 µmol/min (74 ± 10% of systemic turnover). The fractional uptake of glycerol across the splanchnic bed was 90 ± 3%. At the meal nadir of FFA flux, splanchnic glycerol uptake had decreased to 52 ± 6 µmol/min (81 ± 11% of systemic turnover). Basal leg glycerol uptake was 12 ± 2 µmol/min and decreased to 7 ± 1 µmol/min under meal nadir conditions (P < 0.01 vs. basal values).

FFA kinetics. Mean systemic and regional FFA release rates are also provided in Table 4, and the individual values are provided in Tables 1 and 2. Systemic FFA appearance was suppressed by an average of 71% at the nadir of meal ingestion, whereas the leg FFA release was suppressed by 83%, and splanchnic FFA release was suppressed by 58%.

The fractional splanchnic FFA uptake was 34 ± 4% under basal conditions and was not substantially differ-

Table 1. Blood flows, concentrations, specific activity and calculated substrate kinetics for subjects in the overnight, postabsorptive state

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leg blood flow, ml/min</td>
<td>284</td>
<td>360</td>
<td>210</td>
<td>209</td>
<td>569</td>
<td>407</td>
<td>577</td>
<td>250</td>
<td>509</td>
<td>468</td>
<td>182</td>
</tr>
<tr>
<td>Splanchnic blood flow, ml/min</td>
<td>647</td>
<td>1,374</td>
<td>839</td>
<td>1,111</td>
<td>1,441</td>
<td>1,470</td>
<td>1,479</td>
<td>768</td>
<td>2,206</td>
<td>1,069</td>
<td></td>
</tr>
<tr>
<td>Glycerol, µM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arterial</td>
<td>75</td>
<td>83</td>
<td>55</td>
<td>75</td>
<td>86</td>
<td>68</td>
<td>126</td>
<td>78</td>
<td>68</td>
<td>136</td>
<td>120</td>
</tr>
<tr>
<td>Femoral vein</td>
<td>152</td>
<td>78</td>
<td>132</td>
<td>58</td>
<td>147</td>
<td>106</td>
<td>181</td>
<td>181</td>
<td>114</td>
<td>260</td>
<td>161</td>
</tr>
<tr>
<td>Hepatic vein</td>
<td>15</td>
<td>9</td>
<td>9</td>
<td>20</td>
<td>22</td>
<td>30</td>
<td>14</td>
<td>17</td>
<td>69</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>Glycerol SA, dpm/nmol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arterial</td>
<td>4.4</td>
<td>2.5</td>
<td>4.0</td>
<td>11.7</td>
<td>1.3</td>
<td>4.3</td>
<td>1.8</td>
<td>3.7</td>
<td>3.4</td>
<td>2.5</td>
<td>3.5</td>
</tr>
<tr>
<td>Femoral vein</td>
<td>1.8</td>
<td>0.9</td>
<td>1.0</td>
<td>3.6</td>
<td>0.9</td>
<td>1.1</td>
<td>0.9</td>
<td>0.8</td>
<td>1.5</td>
<td>0.8</td>
<td>0.8</td>
</tr>
<tr>
<td>Hepatic vein</td>
<td>1.4</td>
<td>0.1</td>
<td>0.8</td>
<td>5.8</td>
<td>0.6</td>
<td>1.0</td>
<td>0.6</td>
<td>1.1</td>
<td>1.6</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td>Glycerol kinetics, µmol/min</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systemic Rₜ</td>
<td>105</td>
<td>154</td>
<td>105</td>
<td>65</td>
<td>306</td>
<td>180</td>
<td>200</td>
<td>109</td>
<td>142</td>
<td>159</td>
<td>190</td>
</tr>
<tr>
<td>Leg uptake</td>
<td>4.0</td>
<td>19.3</td>
<td>4.8</td>
<td>11.8</td>
<td>0.0</td>
<td>16.9</td>
<td>19.3</td>
<td>9.4</td>
<td>9.2</td>
<td>24.7</td>
<td>14.8</td>
</tr>
<tr>
<td>Splanchnic uptake</td>
<td>45.6</td>
<td>113.0</td>
<td>44.4</td>
<td>71.8</td>
<td>189.8</td>
<td>158.5</td>
<td>112.0</td>
<td>47.8</td>
<td>204.5</td>
<td>121.1</td>
<td></td>
</tr>
<tr>
<td>Leg release</td>
<td>26.0</td>
<td>17.6</td>
<td>21.1</td>
<td>8.3</td>
<td>25.9</td>
<td>32.1</td>
<td>51.5</td>
<td>35.2</td>
<td>32.6</td>
<td>83.0</td>
<td>22.3</td>
</tr>
<tr>
<td>Splanchnic release</td>
<td>6.5</td>
<td>11.7</td>
<td>6.1</td>
<td>11.0</td>
<td>9.2</td>
<td>17.9</td>
<td>17.8</td>
<td>8.6</td>
<td>58.2</td>
<td>23.5</td>
<td></td>
</tr>
<tr>
<td>TG balance, µmol/min</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Splanchnic uptake</td>
<td>40</td>
<td>85</td>
<td>38</td>
<td>41</td>
<td>50</td>
<td>39</td>
<td>166</td>
<td>24</td>
<td>40</td>
<td>220</td>
<td></td>
</tr>
<tr>
<td>Leg uptake</td>
<td>3.6</td>
<td>9.3</td>
<td>5.4</td>
<td>8.0</td>
<td>5.5</td>
<td>4.7</td>
<td>4.8</td>
<td>9.9</td>
<td>5.6</td>
<td>9.5</td>
<td>14.1</td>
</tr>
<tr>
<td>Splanchnic release</td>
<td>315</td>
<td>370</td>
<td>323</td>
<td>204</td>
<td>694</td>
<td>457</td>
<td>479</td>
<td>408</td>
<td>226</td>
<td>381</td>
<td>423</td>
</tr>
<tr>
<td>FFA Rₜ</td>
<td>101.4</td>
<td>40.5</td>
<td>78.1</td>
<td>34.9</td>
<td>90.7</td>
<td>64.2</td>
<td>103.0</td>
<td>84.5</td>
<td>52.2</td>
<td>91.3</td>
<td>29.0</td>
</tr>
<tr>
<td>Leg uptake</td>
<td>64</td>
<td>36</td>
<td>36</td>
<td>69</td>
<td>141</td>
<td>89</td>
<td>29</td>
<td>74</td>
<td>99</td>
<td>102</td>
<td></td>
</tr>
</tbody>
</table>

Glycerol concentrations were measured using a microfluorometric method; glycerol specific activity (SA) was measured by HPLC. These values are means of 3 measurements made over a 30-min time interval. [3H]glycerol and [3H]oleate were infused to allow kinetic measurements to be made. Rₜ, rate of appearance; TG, triglyceride; FFA, free fatty acid.
ent at the meal nadir. Basal splanchnic and leg FFA
uptakes were 214 ± 36 and 46 ± 7 µmol/min, respect-
ively. Splanchnic and leg FFA uptakes during the meal
nadir were 21 ± 4 and 9 ± 2 µmol/min.
FFA-to-glycerol release ratios. The FFA-to-glycerol
release ratios in the systemic circulation, from the
splanchnic bed, and from leg tissue under basal and
meal nadir conditions are depicted in Fig. 1. There were
marked discrepancies in the FFA-to-glycerol release
ratios between systemic and regional tissue beds. The
basal splanchnic FFA-to-glycerol Ra ratio was more
than two times as great as the systemic or leg FFA-to-
glycerol Ra ratio. This discrepancy was reduced at meal
nadir such that the splanchnic and systemic ratios
were no longer significantly different; however, the leg
FFA-to-glycerol Ra ratio during the meal sampling
interval was significantly less than the systemic ratio.
Regional triglyceride metabolism. During the basal
period net splanchnic triglyceride release and leg trglyc-
eride uptakes were 74 ± 20 and 7 ± 1 µmol/min,
respectively (individual values are provided in Table 1).
At the meal nadir the net leg triglyceride uptake was
16 ± 3 µmol/min (P < 0.01 vs. basal; see Table 2 for
individual values).
DISCUSSION
These appear to be the first studies to simultaneously
evaluate splanchnic and leg glycerol and FFA kinetics
in humans. Systemic and regional uptake and release
rates of FFA and glycerol were measured before and
after meal ingestion in healthy volunteers to assess the
heterogeneity of the FFA-to-glycerol appearance ratios
under basal and hyperinsulinemic conditions. The frac-
tional splanchnic uptake of systemically delivered glyc-
table 2. Blood flows, concentrations, specific activity and calculated substrate kinetics for subjects in the fed state

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leg blood flow, ml/min</td>
<td>398</td>
<td>431</td>
<td>201</td>
<td>236</td>
<td>899</td>
<td>338</td>
<td>297</td>
<td>292</td>
<td>565</td>
<td>560</td>
<td>206</td>
</tr>
<tr>
<td>Splanchnic blood flow, ml/min</td>
<td>1,069</td>
<td>1,380</td>
<td>983</td>
<td>1,158</td>
<td>1,646</td>
<td>2,388</td>
<td>1,606</td>
<td>1,476</td>
<td>1,194</td>
<td>1,194</td>
<td>1,194</td>
</tr>
<tr>
<td>Glycerol, µM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arterial</td>
<td>54</td>
<td>15</td>
<td>35</td>
<td>44</td>
<td>50</td>
<td>40</td>
<td>37</td>
<td>37</td>
<td>34</td>
<td>34</td>
<td>58</td>
</tr>
<tr>
<td>Femoral vein</td>
<td>66</td>
<td>27</td>
<td>56</td>
<td>58</td>
<td>77</td>
<td>57</td>
<td>38</td>
<td>38</td>
<td>78</td>
<td>78</td>
<td>90</td>
</tr>
<tr>
<td>Hepatic vein</td>
<td>29</td>
<td>9</td>
<td>12</td>
<td>20</td>
<td>31</td>
<td>17</td>
<td>16</td>
<td>16</td>
<td>24</td>
<td>24</td>
<td>46</td>
</tr>
<tr>
<td>Glycerol SA, dpm/mmol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arterial</td>
<td>10</td>
<td>6.2</td>
<td>10.6</td>
<td>24.4</td>
<td>2.03</td>
<td>6.8</td>
<td>5.4</td>
<td>8</td>
<td>5.5</td>
<td>4.5</td>
<td>5.76</td>
</tr>
<tr>
<td>Femoral vein</td>
<td>3.5</td>
<td>3</td>
<td>2</td>
<td>4.5</td>
<td>8.1</td>
<td>0.8</td>
<td>2.5</td>
<td>2.5</td>
<td>3.7</td>
<td>2.3</td>
<td>2.1</td>
</tr>
<tr>
<td>Hepatic vein</td>
<td>1.48</td>
<td>1.6</td>
<td>0.2</td>
<td>7.8</td>
<td>0.33</td>
<td>2.6</td>
<td>0.6</td>
<td>0.9</td>
<td>0.41</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Glycerol concentrations were measured using a microfluorometric method; glycerol SA was measured by HPLC. Values are means of 3 measurements made over a 60-min time interval. [3H]glycerol and [3H]oleate were infused to allow kinetic measurements to be made.

Table 3. Glycerol concentration and SA

<table>
<thead>
<tr>
<th>Glycerol</th>
<th>Concentration, µmol/L</th>
<th>SA, dpm/mmol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterial</td>
<td>Basal 88 ± 8</td>
<td>3.9 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>Meal 43 ± 4*</td>
<td>8.1 ± 1.8*</td>
</tr>
<tr>
<td>Femoral vein</td>
<td>Basal 143 ± 17</td>
<td>1.3 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>Meal 60 ± 5*</td>
<td>2.8 ± 0.5*</td>
</tr>
<tr>
<td>Hepatic vein</td>
<td>Basal 28 ± 7</td>
<td>1.4 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>Meal 21 ± 2</td>
<td>1.8 ± 0.7</td>
</tr>
</tbody>
</table>

Values are means ± SE. Systemic and leg values refer to n = 11 subjects; splanchnic values refer to n = 10 subjects for basal and n = 9 subjects for meal. Arterial, femoral vein, and hepatic vein concentrations are all significantly (P < 0.05) different for each interval. *P < 0.05 vs. basal.

Table 4. Glycerol and FFA kinetics

<table>
<thead>
<tr>
<th>Glycerol</th>
<th>FFA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systemic appearance, µmol/min</td>
<td>Basal 156 ± 20</td>
</tr>
<tr>
<td></td>
<td>Meal 389 ± 40</td>
</tr>
<tr>
<td></td>
<td>Leg release, µmol/min</td>
</tr>
<tr>
<td></td>
<td>Splanchnic release, µmol/min</td>
</tr>
<tr>
<td></td>
<td>17 ± 5</td>
</tr>
</tbody>
</table>

Values are means ± SE. Systemic and leg values refer to n = 11 subjects; splanchnic values refer to n = 10 subjects. *P < 0.05 vs. basal.
GLYCEROL AND FFA KINETICS

Fig. 1. Ratio of free fatty acid (FFA) rate of appearance (Ra) to glycerol Ra in the systemic circulation and across the leg and splanchnic beds plotted for both basal and meal conditions; n = 11 subjects, except for splanchnic values, where n = 10 for basal and n = 9 for meal. *P < 0.05 vs. systemic values. ‡P < 0.05 vs. basal values.

esterol and FFA was quite different, suggesting that hepatic fractional uptake of the two substrates also differs. If this is the case, the greater fractional hepatic extraction of glycerol relative to FFA could account for the basal splanchnic FFA-to-glycerol release ratio of >6:1 (compared with the average systemic ratio of 2.6:1), by allowing more of the FFA than glycerol derived from visceral adipose tissue lipolysis to reach the systemic circulation. The net basal splanchnic triglyceride production rate was substantial relative to systemic glycerol Ra. Thus, if the hydrolysis of circulating lipoprotein triglycerides by peripheral LPL releases glycerol back into the bloodstream, this could contribute substantially to glycerol appearance. Leg glycerol uptake was documented, suggesting that significant peripheral (skeletal muscle) glycerol kinase activity is present in humans. Taken together, these findings imply that glycerol metabolism is more complex than is commonly appreciated and that glycerol Ra cannot be used as a single, quantitative measure of whole body lipolysis.

The leg glycerol uptake we observed is consistent with the finding of glycerol uptake across human forearm (6, 18, 19) by use of a combination of tracer and arteriovenous balance techniques. Although it has been postulated that isotope disequilibrium accounts for the apparent uptake of glycerol across the extremities (18), this seems unlikely considering the duration of the tracer infusions used in this study and by Landau et al. (19). We conclude that peripheral tissues clearly participate in glycerol clearance. The presence of small but significant amounts of glycerol kinase in skeletal muscle (25) suggests that muscle may use glycerol as a fuel or as a direct precursor for acylglycerol synthesis in humans. If skeletal muscle can take up and use glycerol, then it should be capable of reutilizing glycerol generated from intramuscular triglyceride hydrolysis if that process occurs at relatively low rates, such as would be expected in the resting state.

Three significant, potential sources of glycerol Ra are apparent. The first, and almost certainly the major, source is adipose tissue lipolysis. Adipocytes lack glycerol kinase (21); therefore, lipolysis should result in the quantitative release of glycerol into the circulation. A second possible source of glycerol is the hydrolysis of circulating lipoprotein triglycerides via capillary endothelial LPL. If all glycerol produced from the hydrolysis of circulating triglycerides reenters the plasma space, however, is that adipose tissue lipolysis is markedly inhibited while LPL-mediated triglyceride hydrolysis is not. In the present study, the clearance of triglycerides by leg tissue could potentially account for all of the glycerol appearing in femoral venous blood during meal ingestion. If the glycerol
generated from lipoprotein triglyceride hydrolysis enters the circulation, its contribution to systemic glycerol $R_a$ would be exaggerated relative to the adipose tissue component after meal ingestion or during hyperinsulinemia.

Some of our findings are quite similar to those of Landau et al. (19), who studied regional glycerol kinetics in 60-h-fasted men. Fractional extraction of glycerol by the splanchnic bed was at least 90% in both studies and similar to that observed in overnight-fasted dogs (27). Not unexpectedly, glycerol flux in our overnight postabsorptive volunteers was less than one-half that found in fasting subjects (19), although splanchnic glycerol uptake was roughly similar. Splanchnic glycerol uptake was a lesser fraction of total glycerol uptake in 60-h-fasted men (~30%; see Ref. 19) than in our subjects. This likely reflects the fact that, because fractional extraction is already nearly complete, the ability of the splanchnic bed to increase uptake is limited by splanchnic blood flow and glycerol concentration. Thus, as glycerol $R_a$ increases, extrasplanchnic sites may become more important sources of glycerol removal.

In summary, the fractional extraction of FFA and glycerol by the splanchnic bed is markedly different, strongly suggesting that hepatic glycerol uptake is more efficient than hepatic FFA uptake. This likely accounts for the splanchnic FFA-to-glycerol release ratios of >6:1 that we observed in humans under postabsorptive conditions. In addition, we confirmed that glycerol is taken up by lower-extremity tissue (most likely muscle), implying that there is a functional amount of glycerol kinase present in skeletal muscle. Finally, the net splanchnic production rates of lipoprotein triglycerides are significant, and the fate of the glycerol released from LPL-mediated hydrolysis is uncer-tain. Each of these factors introduces significant uncertainty into the assumptions used to claim that glycerol $R_a$ is a quantitative measure of whole body lipolysis. We recommend that systemic glycerol $R_a$ be interpreted judiciously in regard to questions of fatty acid cycling.

We acknowledge the technical assistance of M. Leanne Barry, Rita Nelson, Jan Aikens, the staff of the Mayo Clinic General Clinical Research Center, and the Mayo Clinic Vascular Radiology Department. Susan Leachman provided valuable editorial assistance.

This work was supported by National Institutes of Health Grants DK-45343 and RR-00585, by the Minnesota Obesity Center (DK-55905), and by the Mayo Foundation.

Address for correspondence and reprint requests: M. D. Jensen, Endocrine Research Unit, 5–194 Joseph, Mayo Clinic, Rochester, MN 55905 (E-mail: jensen.michael@mayo.edu).

Received 13 October 1998; accepted in final form 20 January 1999.

REFERENCES


