Glycerol and nonesterified fatty acid metabolism in human muscle and adipose tissue in vivo

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Coppack, Simon W., Mai Persson, Robert L. Judd, and John M. Miles. Glycerol and nonesterified fatty acid metabolism in human muscle and adipose tissue in vivo. Am. J. Physiol. 276 (Endocrinol. Metab. 39): E233–E240, 1999.—To determine the relationship between glycerol and nonesterified fatty acid (NEFA) release from adipose tissue, and to test whether forearm muscle and abdominal adipose tissue are capable of extracting these two lipolytic products from the circulation, 13 male subjects were studied after an overnight fast during combined infusion of radiolabeled palmitate and glycerol. Blood samples were taken from a radial artery, a deep forearm vein, and a superficial abdominal vein before and during a 2-h infusion of glucose at −7 mg·kg−1·min−1. The ratio of the appearance rates of total NEFA to glycerol was ~3/1 during the baseline period but decreased to 1.3/1 during glucose infusion. There was significant extraction of both glycerol and NEFA by forearm muscle. In contrast, there was no apparent uptake of glycerol by adipose tissue. Adipose tissue NEFA uptake was undetectable during the baseline period but became significant during glucose infusion. These data indicate that there is very little to no in situ reesterification of NEFA in adipose tissue after an overnight fast. During glucose infusion, there was apparently a relative increase in the fraction of glycerol derived from the action of lipoprotein lipase and an increase in reesterification in situ.

METHODS

Subjects. Thirty-six male volunteers gave written, informed consent to partake in a protocol approved by the Mayo Institutional Review Board. All volunteer subjects were healthy nonsmokers and took no medications. The subjects were 26.8 ± 1.3 (SE) yr old and had a body mass index of 23.4 ± 0.5 kg·m−2, body fat content of 16.4 ± 0.9%, and total body fat mass of 11.9 ± 0.8 kg. Biochemistry screen, hematol. screen, and urinalysis were normal in each subject.

Protocol. The subjects came to the Mayo General Clinical Research Center at 1700 and on arrival ate a standard meal of 20 kcal/kg lean body mass, with 50% of calories as carbohydrate, 30% as fat, and 20% as protein. Thereafter, the subjects ate nothing and consumed only caffeine- and calorie-free liquids. Body composition was determined by dual-energy X-ray absorptiometry (Lunar Instruments, Madison, WI).

At 0700 the subjects were awakened. By use of local anesthesia, blood sampling cannulas were inserted into a superficial abdominal vein (20), an antecubital vein (usually the median cubital vein) draining deep forearm tissues (39, 40), and the contralateral radial artery. The superficial abdominal vein cannula was positioned so that its tip was just superior to the inguinal ligament (7, 20). In two subjects, no anatomically suitable antecubital deep vein could be cannulated. Samples from forearm veins were always taken after exclusion of the hand circulation for 2 min by inflating a sphygmomanometer cuff at the wrist to 60–100 mmHg above arterial pressure. Oxygen saturation was measured in each vein, and if initial deep venous O2 was >60% (39), the cannula was moved in an attempt to achieve acceptable values.
Antecubital samples from two additional subjects were discarded because deep venous O₂ saturations were not consistently below 60%.

An infusion cannula was inserted in a forearm vein ipsilateral to the cannulated artery. All cannulas were kept clear with constant infusions of sterile 150 mM NaCl.

After the cannulas were in place, at time −120 min, 150 µCi of 133Xe in sterile saline were injected into the subcutaneous abdominal adipose tissue in the drainage of the abdominal vein being sampled to estimate adipose tissue blood flow (41, 43). Forearm blood flow was measured by mercury strain-gauge plethysmography (20, 48, 49).

A primed (−12 µCi) constant infusion of [2-3H]glycerol (−0.6 µCi/min) and a constant infusion of [9,10-3H]palmitate (0.4 µCi/min) were started at time t = −90 min. Both infusions continued for 210 min. At time 0, an infusion of sterile 50% glucose was infused at 8 mg·kg⁻¹·min⁻¹. In some subjects, this infusion rate was associated with discomfort near the infusion site, and the rate was reduced. Infusion rates actually achieved were between 6 and 8 mg·min⁻¹·kg body weight⁻¹ for all subjects. Blood samples were drawn from all sampling cannulas at −30, −20, −10, and 0 min (usually between 1030 and 1130) and again at 90, 100, 110, and 120 min. Adipose tissue and forearm blood flows were measured between −30 and 0 min and between 90 and 120 min.

Analyses. Blood samples were taken, immediately transferred to precooled tubes containing EDTA, and kept on ice until centrifugation at 4°C, which was done within 30 min of the sample being drawn. Plasma was then stored at −70°C.

For both whole blood glycerol and plasma palmitate, concentration and specific activity (SA) were determined by high-performance liquid chromatography (HPLC) (38). Total NEFA concentrations were measured both by microfluorimetric analysis (20) and by HPLC. Because these two methods of NEFA measurement gave almost identical results, only the HPLC results are presented. Plasma insulin concentration was determined by radioimmunoassay (25) and hematocrit by centrifugation. Plasma triglyceride concentrations were measured enzymatically with correction for glycerol concentration (26); this assay measures mono- and diacylglycerols as TAG (7).

Calculations and statistics. Adipose tissue blood flow was calculated with the assumption of a xenon partition coefficient of 0.6 and a constant infusion rate of 0.4 µCi/min for palmitate and total NEFA (51).

Systemic rates of appearance (Rₐ) of glycerol and palmitate were calculated using steady-state equations (46) in the postabsorptive state

\[ R_a = \frac{2F - ([C_a]_{90} + C_{120})([V_a]_{SA_{a90}} - [SA_{a90}])/(t_{120} - t_{90})]}{[SA_{a90} - [SA_{a120}]} \]

where \( F \) is isotope infusion rate in dpm (kg/min), \( V_a \) is the estimated volumes of distribution (90 ml/kg for palmitate and 250 ml/kg for glycerol) (51), C is arterial plasma concentration, t is time in minutes, and subscripts 90 and 120 indicate values at 90 and 120 min.

The \( R_a \) for total NEFA was calculated from the \( R_a \) of palmitate and ratio of palmitate to total fatty acids (54). The metabolic clearance rate (MCR) of glycerol and that of total NEFA were each calculated as

\[ MCR = \frac{\text{systemic } R_a}{\text{plasma concentration}} \]

Local rates of uptake and release of glycerol and palmitate were calculated as follows (10, 51)

\[ \text{fractional extraction } = FE = \frac{[C_a] - [C_v]}{[C_a]} \]

\[ \text{uptake} = FE \times [C_a] \times \text{blood/plasma flow} \]

\[ \text{release} = \text{uptake} + ([C_v] - [C_a]) \times \text{blood/plasma flow} \]

To examine the relationships between local kinetics and systemic kinetics, we undertook extrapolations from local release or uptake to whole body fat mass (WBFM). The estimated WBFFM release of glycerol, palmitate, and total NEFA was estimated with the assumption that release from subcutaneous abdominal tissue applies to an adipose tissue mass the size of the whole of the WBFFM, as measured by dual photon absorptiometry. Such extrapolation should be interpreted conservatively because of heterogeneity of adipose tissue between different regions (27, 28).

Complete lipolysis of triglyceride produces fatty acids and glycerol in a ratio of 3:1. The ratio of NEFA to glycerol release was calculated for both systemic kinetics and for local release. Previous studies have shown that there is negligible release of mono- or diacylglycerols from adipose tissue (17).

For simplicity of presentation, values are presented as means ± SE or the geometric mean ×/± SE. In most cases, samples from −30, −20, −10, and 0 min were measured as “postabsorptive” values, and samples from 90, 100, 110, and 120 min were measured as “glucose infusion” values. Because we could not be sure that the parameters had a normal distribution, comparisons and correlations have been made using nonparametric statistics (Wilcoxon’s, Mann-Whitney, and Spearman’s tests) throughout.

RESULTS

Adipose tissue blood flow declined significantly (\( P < 0.01 \)) from a geometric mean of 4.60 ×/± 1.17 ml·min⁻¹·100 g tissue⁻¹ in the postabsorptive state to 3.90 ×/± 1.20 ml·min⁻¹·100 g tissue⁻¹ during the glucose infusion. Forearm blood flow was similar before (2.74 ± 0.51 ml·100 g⁻¹·min⁻¹) and during (3.00 ± 0.53 ml·100 g⁻¹·min⁻¹) glucose infusion. Arterial plasma insulin and glucose concentrations are shown in Fig. 1. There were significant increases (\( P < 0.001 \)) in both glucose and insulin concentrations during infusion of glucose.

Figure 2 depicts (A) arterial concentrations of total NEFA and glycerol and (B) SA of palmitate and glycerol.
erol. Total NEFA, palmitate, and glycerol concentrations decreased significantly \( (P < 0.001) \) during infusion of glucose. There were significant increases \( (P < 0.01) \) in both palmitate and glycerol SA in arterial, deep forearm venous, and superficial abdominal venous samples during glucose infusion. Arteriovenous differences across adipose tissue narrowed significantly \( (P < 0.01) \) during glucose infusion.

Baseline palmitate \( R_a \) was \( 111 \pm 10 \) \( \mu \text{mol/min} \) \( (>1.48 \pm 0.13 \mu \text{mol·kg}^{-1}·\text{min}^{-1}) \) and decreased by 70% during glucose infusion (Table 2). In contrast, baseline systemic glycerol \( R_a \) was \( 164 \pm 18 \mu \text{mol/min} \) \( (>2.17 \pm 0.21 \mu \text{mol·kg}^{-1}·\text{min}^{-1}) \) and decreased by only 31% during glucose infusion. The ratio of NEFA to glycerol systemic \( R_a \) was very close to the theoretical 3/1 during the baseline period but decreased to \( \sim 1.3/1 \) during glucose infusion (Table 2).

Systemic MCRs and both forearm and adipose tissue uptakes of glycerol and palmitate/NEFA are shown in Table 3. There was significant uptake of glycerol by forearm. Although the uptake of glycerol by forearm declined in absolute terms during glucose infusion, the FE tended to increase and the systemic glycerol MCR rose \( (P < 0.02) \). The FE of palmitate (and total fatty acids) across forearm tissue increased slightly, but not significantly, and the MCRs increased during glucose infusion. The FE of glycerol across the superficial abdominal tissue was \(-6 \pm 6\%\) in the postabsorptive state and \(7 \pm 13\%\) during glucose infusion, neither significantly different from zero. The FEs of NEFA and palmitate by adipose tissue were not significantly different from zero during the baseline period. During glucose infusion, the FE of glycerol remained negligible, but the FE of NEFA increased to \( 76\% \) \( (P < 0.001) \).

### Table 1. Plasma specific activity

<table>
<thead>
<tr>
<th></th>
<th>Postabsorptive</th>
<th>Glucose Infusion</th>
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<tbody>
<tr>
<td>Glycerol</td>
<td></td>
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<tr>
<td>Arterial</td>
<td>9.49 ± 0.95</td>
<td>13.48 ± 1.8</td>
</tr>
<tr>
<td>Deep forearm vein</td>
<td>4.98 ± 1.11</td>
<td>5.50 ± 1.02</td>
</tr>
<tr>
<td>Subcutaneous abdominal vein</td>
<td>2.70 ± 0.42</td>
<td>7.11 ± 0.69</td>
</tr>
<tr>
<td>Palmitate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arterial</td>
<td>5.13 ± 0.36</td>
<td>20.45 ± 2.68</td>
</tr>
<tr>
<td>Deep forearm vein</td>
<td>3.84 ± 0.28</td>
<td>9.32 ± 1.06</td>
</tr>
<tr>
<td>Subcutaneous abdominal vein</td>
<td>2.31 ± 0.20</td>
<td>6.99 ± 0.16</td>
</tr>
</tbody>
</table>

Values are means ± SE. Units are dpm/nmol. All values during glucose infusion are significantly different from postabsorptive values \( (P < 0.001) \). Values in each blood vessel are significantly different from both other blood vessels at time of measurement \( (P < 0.01) \).
GLYCEROL AND NEFA FLUXES ACROSS ADIPOSE TISSUE

Fig. 3. Arterial, deep forearm venous, and superficial abdominal venous concentrations of glycerol (A) and NEFA (B) during postabsorptive (−30 through 0 min) and glucose infusion (90–120 min) periods. Venous values significantly different from arterial: **P < 0.01; ***P < 0.001. For details of venous cannula placement see METHODS. Values are means ± SE.

Table 2. Systemic indexes of lipolysis

<table>
<thead>
<tr>
<th></th>
<th>Postabsorptive</th>
<th>Glucose Infusion</th>
<th>Percentage Decline</th>
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<tbody>
<tr>
<td>Arterial concentration,</td>
<td></td>
<td></td>
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<tr>
<td>µmol/l</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Palmitate</td>
<td>136.8 ± 9.1</td>
<td>22.7 ± 3.4</td>
<td>82.3 ± 2.8</td>
</tr>
<tr>
<td>Total NEFA</td>
<td>583.8 ± 47.5</td>
<td>94.3 ± 13.9</td>
<td>82.6 ± 2.7</td>
</tr>
<tr>
<td>Glycerol</td>
<td>53.8 ± 4.4</td>
<td>26.8 ± 3.6</td>
<td>48.0 ± 5.8</td>
</tr>
<tr>
<td>Systemic Ra, µmol/min</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Palmitate</td>
<td>111.0 ± 10.0</td>
<td>29.4 ± 2.5</td>
<td>69.8 ± 3.6</td>
</tr>
<tr>
<td>Total NEFA</td>
<td>469.5 ± 43.5</td>
<td>121.8 ± 9.7</td>
<td>70.0 ± 4.0</td>
</tr>
<tr>
<td>Glycerol</td>
<td>163.6 ± 18.0</td>
<td>115.9 ± 17.6</td>
<td>31.3 ± 3.9</td>
</tr>
<tr>
<td>Ratio of total NEFA Ra to glycerol Ra</td>
<td>2.97 ± 0.19</td>
<td>1.29 ± 0.17</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE. NEFA, nonesterified fatty acids; Ra, appearance rates. All glucose infusion values were significantly different from postabsorptive values, P < 0.001. Total NEFA systemic Ra was calculated from palmitate Ra.

Table 4 shows the release of NEFA and glycerol by forearm and adipose tissue. As expected, adipose tissue was releasing relatively large amounts of glycerol and fatty acids in the postabsorptive state, and this release was reduced by glucose infusion. The ratio of NEFA to glycerol release was close to 3/1 during the baseline period in both tissues; it did not change significantly in the forearm during glucose infusion but decreased to 1.07/1 (P < 0.001) in adipose tissue.

In the postabsorptive state, arterial TAG concentrations (780 ± 56 µmol/l) were higher (P < 0.05) than both deep forearm and adipose tissue venous concentrations (757 ± 63 and 742 ± 52 µmol/l, respectively). During glucose infusion, TAG concentrations declined significantly (P < 0.05) in all sites, but arterial concentrations (710 ± 60 µmol/l) remained higher than deep forearm and adipose tissue venous concentrations (681 ± 72 and 680 ± 55 µmol/l, respectively). Circulating lipoprotein-TAG clearance by adipose tissue before and during glucose infusion was 130 ± 24 and 100 ± 30 nmol·100 g tissue⁻¹·min⁻¹, respectively (Table 2). The ratios of local clearance of circulating TAG to local glycerol release were 94 ± 43 and 18.6 ± 8% (for deep forearm and subcutaneous abdominal tissues, respectively) in the baseline period and 140 ± 72 and 170 ± 100%, respectively, during glucose infusion.

There was a significant interindividual correlation (r₈ = 0.63, P < 0.05) between the systemic NEFA Ra and the estimated WBFM release of NEFA (calculated as described in METHODS) in the postabsorptive state. The systemic NEFA Ra and the estimated WBFM release of palmitate each correlated significantly with postabsorptive arterial NEFA concentration (r₈ = 0.73 for each, P < 0.01).

Table 3. Systemic clearance and local uptake of fatty acids, glycerol, and triglyceride

<table>
<thead>
<tr>
<th>Metabolic clearance rate, µmol·100 ml tissue⁻¹·min⁻¹</th>
<th>Postabsorptive</th>
<th>Glucose Infusion</th>
<th>Percentage Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>NEFA</td>
<td>318 ± 56</td>
<td>62 ± 11</td>
<td>77 ± 44</td>
</tr>
<tr>
<td>Glycerol</td>
<td>55 ± 15</td>
<td>35 ± 3</td>
<td>40 ± 20</td>
</tr>
<tr>
<td>CirculatingTAG</td>
<td>52 ± 20</td>
<td>48 ± 24</td>
<td>NS</td>
</tr>
<tr>
<td>Adipose tissue local uptake, µmol·100 g tissue⁻¹·min⁻¹</td>
<td>36 ± 151</td>
<td>82 ± 26</td>
<td>$§$</td>
</tr>
</tbody>
</table>

Values are means ± SEM. TAG, triglyceride or triacylglycerol. For calculation of fractional extraction and local uptake see METHODS. Percentage change different from zero: *P < 0.05; †P < 0.01; ‡P < 0.001. § See text. NS, not significant.
Table 4. Local release of fatty acids and glycerol

<table>
<thead>
<tr>
<th></th>
<th>Postabsorptive</th>
<th>Glucose Infusion</th>
<th>Percentage Decline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forearm local release, nmol·100 ml tissue⁻¹·min⁻¹</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Total NEFA</td>
<td>225 ± 46.4</td>
<td>94.2 ± 12.3</td>
<td>49.6 ± 6.8*</td>
</tr>
<tr>
<td>Glycerol</td>
<td>95.0 ± 19.9</td>
<td>50.4 ± 11.2</td>
<td>32.0 ± 13.7*</td>
</tr>
<tr>
<td>Ratio of total NEFA to glycerol</td>
<td>2.84 ± 0.59</td>
<td>2.50 ± 0.62</td>
<td>NS</td>
</tr>
<tr>
<td>Adipose tissue local release, nmol·100 g tissue⁻¹·min⁻¹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total NEFA</td>
<td>2,285 ± 274</td>
<td>77.0 ± 12.5</td>
<td>96.4 ± 0.4†</td>
</tr>
<tr>
<td>Glycerol</td>
<td>797 ± 108</td>
<td>50.7 ± 10.8</td>
<td>92.3 ± 1.3†</td>
</tr>
<tr>
<td>Ratio of total NEFA to glycerol</td>
<td>2.94 ± 0.17</td>
<td>1.07 ± 0.43</td>
<td>†</td>
</tr>
</tbody>
</table>

Values are means ± SE. For calculation of local release please see METHODS. Percentage decline greater than zero: *P < 0.01; †P < 0.001.

In each individual, the estimated WBFM release of NEFA and of glycerol, extrapolated from the abdominal adipose tissue depot, was less than systemic R_a, being 57 ± 6% for NEFA and 59 ± 6% for glycerol in the postabsorptive state, and <10% for each during glucose infusion.

**DISCUSSION**

These studies were conducted to determine the relative rates of release of NEFA and glycerol from adipose tissue and also to determine whether skeletal muscle and adipose tissue extract NEFA and glycerol from the circulation. Tracer infusion was combined with arteriovenous balance measurements in forearm and superficial abdominal tissues, which served as paradigms for muscle and adipose tissue, respectively. The combination of isotope dilution and arteriovenous sampling has value for partition uptake and release of substrates in tissue beds where the two processes occur simultaneously (6, 11, 22). Previous work indicates that the superficial abdominal vein provides a nearly pure venous effluent from adipose tissue, with minimal to no contribution from underlying fascia and muscle and with concentrations of glycerol similar to those found in adipose tissue interstitial fluid (1, 44). Our results show that the ratio of NEFA to glycerol release was very close to the theoretical 3/1 during the postabsorptive period, when calculated from either the systemic kinetic data or from adipose tissue balance data. This value is similar to that of Wolfe and Peters (54), suggesting that previous values may have been erroneous because of analytic errors. Our local glycerol and NEFA release measurements were done using both enzymatic and HPLC-based methods, and our HPLC methods have been well validated (29, 37). Our findings do not support the concept of partial hydrolysis of TAG in adipose tissue in the postabsorptive state (16, 24); they accord with Samra et al. (43), whose net balance data suggested that once fatty acids have been generated by the action of HSL, reesterification of the fatty acids before their release into the circulation is a quantitatively minor process.

During glucose infusion, the ratio of NEFA to glycerol release, both systemic and in adipose tissue, decreased dramatically. This observation could be interpreted to indicate significant in situ reesterification of NEFA in adipocytes, as previously proposed (54). The concept of intra-adipocyte reesterification of fatty acids was first suggested by in vitro studies (47); however, the elegant work of Edens et al. (14) indicates that the apparent reesterification found in earlier studies is likely an artifact of in vitro conditions related to albumin content of the incubation medium. An additional or alternative explanation for our results is that free glycerol generated by the action of LPL on TAG-rich lipoproteins is released into the systemic circulation (14, 52), whereas LPL-generated NEFA are preferentially taken up by the adipocyte. This hypothesis is supported by the results in this study, which showed that the relative contribution of LPL to glycerol release by adipose tissue increased when HSL was suppressed during glucose infusion (and as also occurs after meals), whereas the relative contribution of LPL to NEFA release did not. Thus only a small proportion (19 ± 8%) of adipose tissue glycerol release comes from LPL action in the postabsorptive state, but during glucose infusion LPL action could account for all of the glycerol released locally. Previous work (8) suggests that local TAG clearance is associated with release of glycerol, whereas LPL output is abolished, in accordance with the view that LPL is a mediator of fat storage (13). Our current results indicate that uptake of circulating glycerol by adipose tissue is minimal to negligible.

During glucose infusion, lipolysis was suppressed. Between 90 and 120 min of infusion, there had been substantial inhibition of both glycerol and NEFA release as judged by systemic and local indexes. Glycerol concentration and SA showed no change with time between 90 and 120 min. NEFA concentration and SA changed slightly, so we used non-steady-state equations to estimate systemic NEFA R_a (29, 37, 51). Very similar values (i.e., within 2%) were obtained using steady-state equations. Non-steady-state conditions can potentially confound calculations of kinetics for substances with slow fractional turnover [e.g., glucose (18), but are quantitatively less important for NEFA, where the use of steady-state equations under non-steady-state conditions introduces only small (<5%) errors (29, 37)]. Our data demonstrate that different indexes of lipolysis were suppressed to varying extent during glucose infusion (Tables 2 and 4), e.g., systemic glycerol R_a was 32% suppressed, whereas adipose tissue local NEFA release was 96% suppressed. Because of a concern about fatty acid reesterification in fat cells, it has been suggested that measurement of glycerol R_a provides a more quantitative reflection of lipolytic activity in adipose tissue than does measurement of NEFA R_a (31, 51). However, LPL activity may be responsible for a major proportion of systemic glycerol (but not NEFA) appearance when HSL is suppressed (see previous paragraph), implying that glycerol R_a is a less specific
and less accurate measure of HSL activity than NEFA \( R_a \). The relative nonspecificity (for HSL) of the measurement of systemic glycerol appearance raises questions about the value of using combined NEFA-glycerol tracer infusion to determine rates of triglyceride-fatty acid cycling as an indication of excess mobilization of fatty acids from adipocytes for transport to other tissues (32, 53, 54). The choice of tracer for measuring systemic lipolysis thus depends on whether a specific measurement of HSL is desired (in which case, a NEFA tracer might be preferable) or whether a composite measurement of HSL and LPL activity is preferred (here a glycerol tracer would probably be best). Our data show that local indexes can vary from systemic indexes, reiterating the heterogeneity of response between different lipolytic organs and different adipose tissue depots (27, 28).

In the postabsorptive state there was net uptake of NEFA in forearm muscle. This net uptake, which has been reported previously in forearm studies (19), obscures the fact that both uptake and release of fatty acids are taking place, documented by the simultaneous occurrence of extraction and dilution of tracer by the tissue bed. Similarly, there was significant uptake (FE \( \sim 40\% \)) of glycerol by the forearm. This observation is in agreement with a previous report (15) that showed glycerol tracer uptake in the human forearm. Such uptake indicates that muscle contains the enzymatic machinery, presumably glycerol kinase, to metabolize glycerol. A recent microdialysis study (36) reported surprisingly high concentrations of glycerol in muscle interstitial fluid; although this observation was of interest, contamination of microdialysis tubing with glycerol might account for those findings. Our findings of avid uptake of glycerol by muscle would suggest that there is no significant blood-to-interstitium concentration gradient. The documented extraction of glycerol by skeletal muscle, together with evidence that there is avid uptake of glycerol by the kidney (6), shows that substantial extrahepatic glycerol utilization is taking place, in contrast with previous dogma (35). In fact, it is clear from our data and previous studies of glycerol kinetics (32, 55) that the postabsorptive liver is responsible for less than one-half of systemic glycerol utilization, because glycerol clearance (42–55 \( \text{ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \) in our study) exceeds hepatic blood flow in humans (\(-20 \text{ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}\)) by more than twofold (23). Prolonged fasting increases the amount of glycerol metabolized in the liver (2).

Subcutaneous abdominal adipose tissue behaved differently from forearm muscle. There was no significant uptake of glycerol during the baseline period or during glucose infusion. Previous work by Kurpad et al. (33) has shown an apparent uptake of glycerol by adipose tissue, but it is not clear whether steady-state conditions were achieved in those studies, because a relatively short (60-min) tracer infusion was used. The longer time between the start of isotope infusion and blood sampling, or perhaps the use of subjects with different body composition and hence possibly smaller intra-adipose glycerol pools, may have led to different results in our study.

There was also no detectable uptake of NEFA by adipose tissue during the postabsorptive period. This observation is compatible with the concept that fatty acid trafficking in adipocytes is partitioned; uptake is mediated by LPL and release by HSL. However, there was avid uptake of NEFA during glucose infusion. This is consistent with a model of NEFA flow dependent on the affinity gradient of unbound fatty acids (42) across the tissue endothelium. Thus fatty acid flow will be predominantly efflux from lipolytic tissues but at least partially influx to nonlipolytic tissue, such as forearm muscle or adipose tissue, when HSL is inhibited by insulin. In support of such a model, we have found differences in NEFA saturation of albumin in interstitial fluid when compared with plasma (30). The direction of flow in this model would depend on the direction of an affinity gradient related to occupancy of NEFA-binding sites on albumin. The quantitative importance of adipose tissue fatty acid uptake from the circulating NEFA pool is minor. Thus it can be estimated from local lipoprotein-TAG clearance across this tissue bed in both the current study and previous work (8) that LPL-mediated uptake of lipoprotein-fatty acids may be 250–350 \( \text{nmol} \cdot 100 \text{g tissue}^{-1} \cdot \text{min}^{-1} \) during glucose infusion and 450–900 \( \text{nmol} \cdot 100 \text{g tissue}^{-1} \cdot \text{min}^{-1} \) in the postprandial state, both far exceeding the uptake from the NEFA pool observed here.

Although mean forearm blood flow increased during our protocol by \( \sim 9\% \) in this study, this was not statistically significant. Although the study was not designed to have statistical power to examine changes in forearm blood flow, it is also probable that the relatively short-term and gradual insulinemia induced by this protocol did not provide as strong a vasodilator stimulus as a hyperinsulinemic clamp (3, 48). Conversely, the adipose tissue blood flow declined slightly. This decline is of interest because within 90 min of a mixed meal, which also causes hyperinsulinemia and hyperglycemia, lean healthy subjects will increase adipose tissue blood flow (7). Adipose tissue blood flow remains unchanged by hyperinsulinemic clamp (45). Thus there are apparently different differences in adipose tissue blood flow depending on whether insulinemia is induced by intravenous glucose or a meal and whether it is accompanied by hyperglycemia. The mechanisms of adipose tissue blood flow regulation are not known, although autonomic nerves are believed to play some role.

In summary, our study confirms previous reports indicating significant glycerol uptake in muscle tissue while demonstrating minimal to no uptake of glycerol by adipose tissue. There was no measurable uptake of NEFA by adipose tissue under postabsorptive conditions but significant uptake during glucose infusion. The ratio of NEFA to glycerol appearance was \( \sim 3/1 \) under basal conditions but decreased markedly during glucose infusion. In the basal state LPL action contributed only to a minor extent to lipolysis but became the major contributor to adipose tissue lipolysis during glucose infusion. Additional studies of LPL-mediated
fatty acid uptake are needed to put this latter finding into perspective.

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