The in vivo metabolism of triglycerides (TG) has received renewed interest in the past few years. This was motivated by the demonstration that high TG levels contribute to the development of insulin resistance (24, 30) as well as atherosclerosis (12). Therefore, TG metabolism as free fatty acid (FFA) metabolism has implications for hyperlipidemias, obesity, and diabetes. In the postabsorptive state TG are secreted by the liver. These TG are synthesized by use of fatty acids either produced by hepatic lipogenesis [de novo lipogenesis (DNL)] or provided by the uptake of plasma FFA (reesterification) or the degradation of lipoproteins taken up by the liver. The fractional contribution of DNL to TG secretion can be estimated by use of [1-13C]palmitate (1,2-13C2]acetate infusion. However, these approaches rely on several assumptions that have to be tested. First, they assume that the liver is the only tissue responsible for the reesterification of plasma FFA, which is questionable because splanchic FFA uptake was measured at 25–30% of FFA appearance rate (36), far less than current estimates of plasma FFA reesterification. Moreover, there is evidence that other tissues, such as muscles, can also reesterify FFA (7). Second, the contribution of lipoprotein breakdown by liver has never been estimated to our knowledge. Third, the calculation of FFA reesterification (difference between FFA Rt and Lox) assumes that plasma FFA are the only source of fatty acids oxidized. This last assumption received recent experimental support. Sidossis et al. (33) found that, when labeled carbon exchanges in the citric acid cycle are taken into account, the classic discrepancy (14, 24) between plasma FFA oxidation (measured by the excretion rate of labeled CO2 during infusion of a labeled fatty acid) and total lipid oxidation is no longer observed in postabsorptive subjects, i.e., plasma FFA and total lipid oxidation values are nearly equal. However, there is also evidence that fatty acids from both tissues (8) and plasma TG (38) are also oxidized. Aarsland et al. (1) recently described another method based on the kinetics of the appearance of labeled fatty acids in plasma TG during [1,2-13C2]acetate infusion. However, the only directly measured value is DNL; calculation of total TG secretion rate requires extrapolation for plateau value, and the contribution of the reesterification of plasma FFA was not measured.

In the present study, the simultaneous use of [13C]palmitate and deuterated glycerol infusions, deuterated water ingestion, and indirect calorimetry allowed us to measure more directly most parameters of plasma FFA and TG metabolism in postabsorptive subjects. Lipogenesis was calculated from deuterium incorporation in the palmitate of TG (10). The kinetics of the appearance of [13C]palmitate in TG during the infusion of the tracer and of its disappearance after interruption of the infusion allowed the calculation of, respectively, plasma FFA reesterification by liver and the Rt of plasma TG. The comparison of the Rt of glycerol and FFA gave the “intracellular” reesterification rate (4, 22) (i.e., the reesterification of fatty acids released by breakdown of tissue TG directly within tissue, without any appear-
ance in the circulating plasma pool), and the comparison of FFA $R_t$ and either FFA or total $L_{ox}$ gave upper and lower limits for "extracellular" reesterification rate (i.e., the reesterification of fatty acids released in plasma by one tissue and taken up by another). Comparison of this last value with liver reesterification gave the contribution of hepatic and extrahepatic tissues to total "extracellular" reesterification. The results confirm that lipogenesis is a minor contributor to TG secretion in postabsorptive subjects; however, reesterification of plasma FFA contributes only about one-half to TG secretion. Last, most extracellular reesterification takes place in extrahepatic tissues.

METHODS

Subjects. Informed written consent was obtained from five normal volunteers (three men and two women, aged 22–30 yr). All had a normal body weight (body mass index: 22.7 ± 0.9) and normal plasma glucose, triglyceride, and cholesterol values. None was taking any medication. They consumed a weight-maintaining diet with ≥200 g carbohydrate and abstained from heavy physical activity or alcohol consumption for the week before the studies. The last meal was consumed between 7:00 and 8:00 PM on the day before the tests.

Protocols. The protocol of the study was approved by the Ethical Committee of Lyon and by the Institut National de la Santé et de la Recherche Médicale, and the study was conducted according to the Hurriet law. All tests were performed in the Centre de Recherche en Nutrition Humaine de Lyon. On the day before the tests, the subjects drank a loading dose of deuterated water (3 g/kg body water, one-half after the evening meal and one-half at 10:00 PM; Eurisotop, Saint Aubin, France). Then until the end of the study, they drank only water enriched with 2H$_2$O (4.5 g·H$_2$O/L of drinking water). All tests were initiated in the postabsorptive state. At 7:30 AM an indwelling catheter was inserted in a forearm vein for the infusion of tracers. Another catheter was placed in a dorsal vein of the opposite hand, which was kept at 55°C, to obtain arterialized blood. After an initial blood sampling and collection of expired gas in the initial state, a bolus of NaH$_2$CO$_3$ (1.0 µmol/kg) was infused intravenously and primed-continuous infusions of [1-13C]palmitate (Mass Trace Woburn, MA; 0.03 µmol·kg$^{-1}$·min$^{-1}$) and [1,2,3-2H$_2$]glycerol (Mass Trace; 0.05 µmol·kg$^{-1}$·min$^{-1}$) after a bolus of 0.5 µmol/kg were initiated and continued for 4 h. Blood samples were collected at 60, 120, 180, 200, 220, and 240 min and during the 6 h after the interruption of tracer infusion. Expired gas samples were collected every hour, except for the period of 180–300 min, when samples were collected every 15 min. Respiratory gas exchanges were measured from 120 to 240 min (Deltatrak metabolic monitor, Datex, Helsinki, Finland). Urine samples were collected from 0 to 240 min for determination of nitrogen excretion.

Analytic procedures. Metabolites were assayed with enzymatic methods (4, 5) on neutralized perchloric extracts of plasma (glucose, glycerol) or on plasma (FFA, TG). Plasma insulin and glucagon levels were determined by radioimmunoassay (4). Deuterium enrichment in plasma water was measured by isotope ratio mass spectrometry (optima 18, Fisons Instruments, Middlewich, UK) (40) and expressed as molar percent excess. Glycerol was purified by sequential ion-exchange chromatography, and its enrichment was determined by gas chromatography-mass spectrometry by use of the triacetate derivative (3); samples were injected into a gas chromatograph (HP5890, Hewlett-Packard, Palo Alto, CA) equipped with a 25-m fused silica capillary column (OV1701, Chrompack, Bridgewater, N J) interfaced with a mass spectrometer (HP5871A, Hewlett-Packard) working in the electron impact mode. Ions of mass-to-charge ratio (m/z) 145–148 were selectively monitored; standard curves containing known amounts of natural and labeled glycerol were run with the biological samples. After addition of heptadecanoic acid as internal standard for the determination of plasma palmitate concentration, plasma lipids were extracted by the method of Folch et al. (11). TG and FFA were separated by thin-layer chromatography, scraped off the silica plates, and eluted from silica with ether (10). The methylated (FFA) and transmethylated (TG) derivatives of palmitate were prepared according to Morrison and Smith (28). Total enrichment (i.e., 13C and deuterium) of the palmitate of FFA and of TG was measured by gas chromatography-mass spectrometry, monitoring ions of m/z 270–273 (10). 13C enrichment was selectively determined by gas chromatography isotope ratio mass spectrometry (6). Deuterium enrichment was calculated by subtracting 13C enrichment from the total enrichment. Palmitate concentrations were calculated from the area ratio of the peaks corresponding to palmitate and to heptadecanoate; standard curves containing known concentration ratios of these two fatty acids were run with the samples. 13C enrichment in the CO$_2$ of expired gas was measured by gas chromatography isotope ratio mass spectrometry (17).

Calculations. Glycerol and palmitate turnover rates were calculated from their respective enrichments in deuterium and 13C measured during the 180- to 240-min period with steady-state equations. Plasma FFA $R_t$ was calculated from palmitate $R_t$ by use of the ratio of palmitate to FFA concentration. The fractional contribution of lipogenesis to TG-palmitate was calculated from the deuterium enrichments in plasma water and in the palmitate of plasma TG, as previously described (10). In short, the deuterium enrichment that would be obtained if lipogenesis were the only source of TG-palmitate was calculated from plasma water enrichment; the comparison of the actual enrichment observed with that theoretical value gives the contribution of lipogenesis to the circulating pool of TG-palmitate. TG kinetics were calculated from the decay of 13C enrichment in TG-palmitate after the end of the infusion of [1-13C]palmitate; this approach is comparable to the one used with radioactively labeled tracers (35). A single exponential was fitted to the data: $IE = IE_0 e^{-kt}$, where $IE$ is the isotopic enrichment and $k$ is the fractional turnover rate ($FR_t$). The half-life time of the plasma TG pool is then calculated as $t_{1/2} = 0.693/FR_t$. The absolute TG $R_t$ is calculated as $TG = FR_t M$, where $M$ is the plasma pool of TG obtained by multiplying the TG concentration by the plasma volume estimated to 37 ml/kg (9). Absolute de novo lipogenesis is obtained as $DNL = DNL\%\cdot TG\cdot R_t$ (3), where the factor 3 acknowledges the fact that there are three fatty acids per molecule of TG. The fractional contribution of the hepatic reesterification ($FRE_h$) of FFA to the plasma TG pool is calculated from the 13C enrichment of plasma palmitate and the increase of 13C enrichment in TG palmitate during the infusion of [1-13C]palmitate by use of the formula $FRE_h = (IE_{Pal} - IE_{Pal})/IE_{Pal}$, where $t_1$ and $t_2$ are the times when samples are taken, $IE$ is the corresponding IE of TG-palmitate, and $IE_{Pal}$ is the enrichment at plateau of plasma palmitate. The comparison of FRE$_h$ with FR$_t$ gives the percent contribution of reesterification to TG turnover rate: $RE_h = FRE_h/FR_t$. The absolute contribution of reesterification, $RE$, to the TG secretion is then $RE = RE_h \cdot TG\% \cdot R_t$. Three times $RE$ gives the rate of reesterification of plasma FFA by the liver ($RE_{hep}$).
Table 1. Hormone and metabolite concentrations

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Concentration (μmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>4.54 ± 0.26</td>
</tr>
<tr>
<td>FFA</td>
<td>427 ± 51</td>
</tr>
<tr>
<td>Glycol</td>
<td>59 ± 6</td>
</tr>
<tr>
<td>TG</td>
<td>0.95 ± 0.09</td>
</tr>
<tr>
<td>Insulin</td>
<td>8.4 ± 0.7</td>
</tr>
<tr>
<td>Glucagon</td>
<td>103 ± 15</td>
</tr>
</tbody>
</table>

Values are means ± SE. FFA, free fatty acids; TG, triglycerides.

Table 2. Plasma FFA and glycerol turnover rates

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Turnover Rate (μmol·kg⁻¹·min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycerol Rt</td>
<td>2.05 ± 0.24</td>
</tr>
<tr>
<td>FFA Rt</td>
<td>6.04 ± 1.04</td>
</tr>
<tr>
<td>FFA Rt/glycerol Rt</td>
<td>2.90 ± 0.23</td>
</tr>
</tbody>
</table>

Values are means ± SE. Rt, turnover rate.

Plateau levels of enrichment and concentration were obtained during the 120- to 240-min period, and kinetic parameters were calculated with equations for steady-state conditions. In addition, when measuring glycerol enrichment, we found no evidence for a partial loss of one or two deuterium atoms from the labeled glycerol infused, contrary to what was observed by Previs et al. (29) in the perfused rat liver system (i.e., in the plasma glycerol samples there were increases only in the 148/145 ion ratios and no increases in the 146 or 147/145 ratios). Therefore, there is no evidence that glycerol R, was artifactually measured. The FFA over glycerol R ratio is shown in Table 2. This ratio was near to three, and therefore there was little RE inc (0.02 ± 0.42 μmol·kg⁻¹·min⁻¹).

The evolution of CO₂ enrichments is shown in Fig. 2. FFA oxidation rate, calculated from labeled CO₂ excretion rate by use of the acetate correction factor or the acetate correction factor of Sidossis et al. (33), is compared in Table 3 with L ox. This L ox calculated from the respiratory gaseous exchange data, was corrected for the measured lipogenesis to convert the net L ox to the total L ox rate. This correction was almost negligible given the low lipogenic rate of normal postabsorptive subjects (see next paragraph).

Use of the acetate correction factor raised the contribution of FFA oxidation to the FFA disappearance rate from 32 to 45% and to total L ox from 62 to 91% (Table 3). Therefore the “non-FFA” oxidation rate (considered to be the oxidation of fatty acids from plasma or tissue TG) was very low (0.29 ± 0.30 μmol·kg⁻¹·min⁻¹) with the use of the acetate correction factor.

Table 4 shows the kinetics of plasma TG and the contribution of lipogenesis and plasma FFA reesterification to TG secretion. Reesterification was calculated from the increase of 13C enrichment in TG-palmitate.
deuterated glycerol to measure glycerol kinetics. Previs et al. (29) showed recently in perfused rat livers the presence of a substrate cycling between the infused glycerol and liver triose phosphates. When [1,2,3-2H3]glycerol is infused, this cycling results in the release of glycerol molecules having lost one or more deuterium atoms. If this loss of deuterium occurs to a significant extent in vivo, it would induce an artifactual decrease of the enrichment of glycerol measured during infusion of deuterated tracer and an overestimation of glycerol turnover rate. This possibility seemed unlikely, because we previously found comparable glycerol turnover rates in rats infused with [2-13C]glycerol or [1,2,3,-2H3]glycerol (4). Our present finding that there was no detectable appearance of glycerol molecules having lost one or more deuterium in human subjects infused with deuterated glycerol confirms the validity of this tracer for determination of glycerol kinetics in humans.

Extracellular reesterification of FFA is calculated as the difference between FFA disappearance rate and oxidation (1,19). It relies thus on the way this oxidation is estimated. One can use either lipid oxidation measured by indirect calorimetry or the oxidation rate calculated from the excretion in expired gas of labeled CO2 during the infusion of a 13C- or 14C-labeled fatty acid. Several authors repeatedly found a large discrep-

### Table 3. Plasma FFA and total lipid oxidation rates

<table>
<thead>
<tr>
<th></th>
<th>Bicarbonate correction</th>
<th>Acetate correction</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FFA oxidation</strong></td>
<td>µmol · kg⁻¹ · min⁻¹</td>
<td>µmol · kg⁻¹ · min⁻¹</td>
</tr>
<tr>
<td>% of FFA Rₜ</td>
<td>1.90 ± 0.45</td>
<td>2.76 ± 0.65</td>
</tr>
<tr>
<td><strong>non-FFA oxidation</strong></td>
<td>µmol · kg⁻¹ · min⁻¹</td>
<td></td>
</tr>
<tr>
<td>% of FFA Rₜ</td>
<td>31.7 ± 2.9</td>
<td>45.3 ± 4.2</td>
</tr>
<tr>
<td><strong>Lₜox</strong></td>
<td>µmol · kg⁻¹ · min⁻¹</td>
<td></td>
</tr>
<tr>
<td>% of FFA Rₜ</td>
<td>1.05 ± 0.25</td>
<td>0.29 ± 0.30</td>
</tr>
<tr>
<td><strong>FRt,h</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.05 ± 0.25</td>
<td>0.29 ± 0.30</td>
</tr>
</tbody>
</table>

FFA oxidation was calculated from labeled CO₂ excretion rate by use of either bicarbonate or acetate correction factor. Total lipid oxidation (Lₜox) was calculated from gaseous exchanges and corrected for lipogenesis.

during the infusion of labeled palmitate. This increase was linear with time (Fig. 1), and the intercept of the regression line with the time axis shows a delay of ~30 min for the appearance of 13C in TG-palmitate. There was a similar delay between the end of labeled palmitate infusion and the beginning of the decline of enrichment in TG-palmitate. The FRₜ of plasma TG was 0.21 ± 0.02/h, and their turnover rate was 0.11 ± 0.05 µmol · kg⁻¹ · min⁻¹. The fractional contribution of reesterification (FRE) was 0.10 ± 0.05/h. Thus RE contributed only one-half to TG secretion. Deuterium enrichment in plasma water was stable throughout the study at 0.35 ± 0.08% (Fig. 3). The mean deuterium enrichment of palmitate in plasma TG during the 0- to 240-min period of the study was 0.29 ± 0.08%. The calculated contribution of lipogenesis was low (3.9 ± 0.9%, Table 4), representing an absolute synthetic rate of fatty acids of 0.013 µmol · kg⁻¹ · min⁻¹. Therefore, nearly 50% of the TG secretion rate was not accounted for by lipogenesis and RE. The contribution of hepatic reesterification to plasma FFA Rₜ was calculated to represent 0.16 ± 0.02 µmol · kg⁻¹ · min⁻¹ (Table 5). This hepatic reesterification was compared with the total REₜox calculated by subtracting from FFA Rₜ the Lₜox rate obtained either from the excretion rate of 13CO₂ or gaseous exchanges; whatever the calculation, liver was a minor contributor to REₜox.

### DISCUSSION

Using a combination of stable isotope-labeled tracer and indirect calorimetry, we measured in normal subjects most aspects of plasma FFA (turnover rate, oxidation, and reesterification) and TG (turnover rate, contribution of reesterification and lipogenesis to secretion) kinetics. In agreement with previous reports (1, 3), we found that the “intracellular reesterification” of fatty acids was almost negligible in normal postabsorptive subjects. This result is based on the comparison of plasma glycerol and fatty acid turnover rates. We used deuterated glycerol to measure glycerol kinetics. Previs et al. (29) showed recently in perfused rat livers the presence of a substrate cycling between the infused glycerol and liver triose phosphates. When [1,2,3-2H₃]glycerol is infused, this cycling results in the release of glycerol molecules having lost one or more deuterium atoms. If this loss of deuterium occurs to a significant extent in vivo, it would induce an artifactual decrease of the enrichment of glycerol measured during infusion of deuterated tracer and an overestimation of glycerol turnover rate. This possibility seemed unlikely, because we previously found comparable glycerol turnover rates in rats infused with [2-13C]glycerol or [1,2,3,-2H₃]glycerol (4). Our present finding that there was no detectable appearance of glycerol molecules having lost one or more deuterium in human subjects infused with deuterated glycerol confirms the validity of this tracer for determination of glycerol kinetics in humans.

Extracellular reesterification of FFA is calculated as the difference between FFA disappearance rate and oxidation (1,19). It relies thus on the way this oxidation is estimated. One can use either lipid oxidation measured by indirect calorimetry or the oxidation rate calculated from the excretion in expired gas of labeled CO₂ during the infusion of a 13C- or 14C-labeled fatty acid. Several authors repeatedly found a large discrep-

### Table 4. Kinetic parameters of plasma TG

<table>
<thead>
<tr>
<th></th>
<th>FRₜ, h⁻¹</th>
<th>t₁/₂, h</th>
<th>% of FFA Rₜ</th>
<th>% of TG Rₜ</th>
<th>tₚ/₂, h</th>
<th>tₚ/₆, h</th>
<th>% of TG Rₜ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.21 ± 0.02</td>
<td>3.44 ± 0.33</td>
<td>0.11 ± 0.05</td>
<td>49.4 ± 5.7</td>
<td>3.9 ± 0.9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE. FRₜ, fractional turnover rate. t₁/₂, half-life time.

### Table 5. Total extracellular reesterification rate and contribution of liver and extrahepatic tissues to reesterification

<table>
<thead>
<tr>
<th></th>
<th>Extracellular</th>
<th>Hepatic</th>
<th>Extrahepatic</th>
</tr>
</thead>
<tbody>
<tr>
<td>FFA Rₜ - FFAox</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bicarbonate</td>
<td>4.11 ± 0.66</td>
<td>0.16 ± 0.02</td>
<td>3.95 ± 0.65</td>
</tr>
<tr>
<td>Acetate</td>
<td>3.27 ± 0.54</td>
<td>0.16 ± 0.02</td>
<td>3.11 ± 0.53</td>
</tr>
<tr>
<td>FFA Rₜ - Lₜox</td>
<td>3.02 ± 0.61</td>
<td>0.16 ± 0.02</td>
<td>2.86 ± 0.60</td>
</tr>
</tbody>
</table>

Values are means ± SE. Oxidation of FFA (FFAox) was calculated from labeled CO₂ excretion rate by use of bicarbonate or acetate correction factor. Lₜox, total lipid oxidation rate measured by indirect calorimetry and corrected for lipogenesis.
ancy between these two measurements (14, 24, 33). This was generally interpreted as showing that FFA are only a part of the lipid oxidized and that fatty acids from plasma or tissue TG are also oxidized. If this interpretation is correct, to calculate the extracellular reesterification one should use the oxidation rate obtained from the excretion of labeled CO₂. However, Sidossis et al. (32, 33) provided evidence that plasma FFA oxidation measured with tracer was underestimated because of the loss of labeled carbon through exchanges in the citric acid cycle during the oxidation process before the release of the labeled carbon as CO₂. This was based on the comparison of the labeled CO₂ kinetics in expired gas after infusion of labeled acetate and palmitate was stopped and on the demonstration of the appearance, with both tracers, of labeled carbons in plasma compounds such as lactate or glutamate (reflecting exchanges at the oxaloacetate and α-keto-glutarate levels in the citric acid cycle) (32). They proposed to use, instead of the classic bicarbonate recovery factor, an acetate recovery factor that would also take into account these isotopic exchanges (33). With this factor they reported that FFA oxidation accounted in postabsorptive subjects for all (33) or 80% (34) of lipid oxidation measured by indirect calorimetry, instead of 50–60%. Our present finding that FFA oxidation represented 90% of total lipid oxidation when the acetate factor was used (instead of 62% with the bicarbonate factor) would agree with their results. Therefore, if Sidossis et al. are correct, one could use for calculating extracellular fatty acid recycling either tracer-determined plasma FFA oxidation or lipid oxidation measured by indirect calorimetry [although the latter has to be corrected for lipogenesis when significant lipogenesis occurs (19)]. On the other hand, the results from Sidossis et al. are difficult to reconcile with the previous demonstration that, at least in animals, fatty acids from very low density lipoprotein (VLDL)-TG contribute to lipid oxidation (8, 38, 39): when fatty acid-labeled VLDL-TG were infused, labeled CO₂ was excreted without any significant appearance of labeled fatty acid in the plasma FFA pool. This contribution of VLDL-TG was found to be as important as that of FFA in rats (38). In the present experiments, labeled fatty acids were incorporated in plasma TG with an enrichment reaching at the end of the 4-h infusion of [1-13C]palmitate one-third to one-half of that in plasma FFA. If these fatty acids from plasma TG were oxidized, their contribution to lipid oxidation could not be distinguished from that of FFA (unless dual tracer experiments or short-term infusion of labeled fatty acid were performed). Therefore, although one could argue that, if plasma TG significantly contributes to lipid oxidation, the decay of CO₂ enrichment in expired gas after labeled palmitate and acetate infusions are stopped should be different, contrary to what was observed (33), additional studies are needed to clarify these points. Whatever the correct value to be used for its calculation, extracellular reesterification is an important process, because its upper and lower estimates were 70 and 55% of FFA turnover rate (Table 3).

Plasma TG kinetics were calculated from the decay of label in palmitate of plasma TG after the [1-13C]palmitate infusion was stopped. The values obtained agree with those reported previously for VLDL-TG using radioactive tracers or other approaches with stable isotope-labeled tracers (1, 15, 18, 21, 31). DNJ, expressed as a relative or an absolute value, was a minor contributor to TG secretion, in agreement also with previous reports (10, 18, 26). Plasma FFA reesterification is considered to be the main or nearly exclusive source (2, 27) of liver TG synthesis and secretion. Actually we found that hepatic reesterification of plasma FFA was a large contributor but accounted for only one-half of the turnover rate of plasma TG. This value is lower than the one (80–100%) reported by Barter and Nestel (2) in normal subjects when these investigators compared the plateau values of the enrichment of palmitate in plasma FFA and TG during infusion of tritiated palmitate. However, they measured enrichments in plasma drawn from a superficial vein draining mainly subcutaneous adipose tissue. FFA enrichment is already decreased in mixed venous blood compared with arterial samples (20); this dilution is probably greater in a superficial vein. Therefore, Barter and Nestel may have overestimated the contribution of plasma FFA reesterification. If lipogenesis and plasma FFA reesterification do not account for all liver TG secretion, the remaining fatty acids could be provided by the breakdown of plasma lipoprotein taken up by liver (presumably VLDL remnants) or by the mobilization of fatty acids of previously stored TG and phospholipids. Our data do not allow us to determine which of these two potential sources is the more important, but there is in vitro evidence in support of the latter (37).

Last, we compared the total extracellular and the hepatic reesterification rates. Whatever the calculation chosen for determining the total extracellular reesterification, the contribution of liver appeared minor, showing that most of this reesterification takes place in extrahepatic tissues (presumably muscles and adipose tissue). It should be stressed that, had liver reesterification accounted for the totality of plasma TG secretion, this contribution to extracellular reesterification would anyway have remained minor. This conclusion agrees with the recent estimate from FFA splanchnic balance and glycerol kinetics data that splanchnic FFA uptake represents only 25% of the amount of plasma FFA to be reesterified (23). Therefore, the assumption (19) that all extracellular reesterification occurs in liver is not verified.

In conclusion, using simple and noninvasive methods we were able to measure most aspects of plasma FFA and TG kinetics, especially with regard to liver lipid metabolism. We show that liver reesterification is only a minor part of the metabolic fate of plasma FFA and, although a major contributor to it, does not account for the totality of TG secretion. This approach will be
useful for investigating abnormalities of lipid metabolism in situations such as obesity, diabetes, and stress.

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