Regulation of free fatty acid metabolism by insulin in humans: role of lipolysis and reesterification

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Diabetes Research and Training Center, Departments of Medicine and of Pediatrics, Vanderbilt University School of Medicine, Nashville, Tennessee 37232-2230, and Diabetes Research Laboratories, Department of Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania 15261

Campbell, Peter J., Michael G. Carlson, James O. Hill, and Nurjahan Nurjhan. Regulation of free fatty acid metabolism by insulin in humans: role of lipolysis and reesterification. Am. J. Physiol. 263 (Endocrinol. Metab. 26): E1063–E1069, 1992.—The regulation of lipolysis, free fatty acid appearance into plasma (FFA R, and FFA reesterification and oxidation were examined in seven healthy humans infused intravenously with insulin at rates of 4, 8, 25, and 400 mU·m⁻²·min⁻¹. Glycerol and FFA R, were determined by isotope dilution methods, and FFA oxidation was calculated by indirect calorimetry or by measurement of expired ¹⁴CO₂ from infused [¹⁴C]palmitate. These measurements were used to calculate total FFA reesterification, primary FFA reesterification occurring within the adipocyte, and secondary reesterification of circulating FFA molecules. Lipolysis, FFA R, and secondary FFA reesterification were exquisitely insulin sensitive [the insulin concentrations that produced half-maximal suppression (EC₅₀), 106 ± 26, 91 ± 20 vs. 80 ± 16 pM, P = not significant] in contrast to insulin suppression of FFA oxidation (EC₅₀, 324 ± 60, all P < 0.01). The absolute rate of primary FFA reesterification was not affected by the increase in insulin concentration, but the proportion of FFA molecules undergoing primary reesterification doubled over the physiological portion of the insulin dose-response curve (from 0.23 ± 0.06 to 0.44 ± 0.07, P < 0.05). This served to magnify insulin suppression of FFA R, twofold. In conclusion, insulin regulates FFA R, by inhibition of lipolysis while maintaining a constant rate of primary FFA reesterification.

Glycerol; adipose tissue

FAT OXIDATION supplies much of the body's energy needs, particularly during situations such as fasting. Therefore, regulation of fat deposition and mobilization and free fatty acid appearance into plasma (FFA R, and FFA reesterification and oxidation must play an important role in energy homeostasis. FFA R, represents the difference between the rates of lipolysis and primary FFA reesterification occurring within adipocytes. Circulating FFA molecules are either oxidized in tissues such as muscle or undergo secondary reesterification in the liver. Insulin is a potent inhibitor of lipolysis (27), but it is unknown whether it also stimulates primary FFA reesterification.

Currently, several isotopic methods are used to evaluate primary and secondary FFA reesterification in vivo. Primary FFA reesterification can be measured as the difference between lipolysis, calculated from glycerol turnover, and FFA R, (37, 38). Secondary FFA reesterification is calculated as FFA R, minus FFA oxidation (6, 16–18, 24, 31). FFA R, is measured by the isotope dilution technique ([¹⁴C]palmitate) and FFA oxidation by indirect calorimetry (24, 31) or from collection of expired ¹⁴CO₂ (6, 16 18). Whole body FFA reesterification would be equivalent to the sum of primary and secondary FFA reesterification calculated in the above manner. The alternative approach is to calculate whole body FFA reesterification as lipolysis minus FFA oxidation, measured by indirect calorimetry (37, 38). Thus secondary FFA reesterification is estimated as whole body FFA reesterification minus primary FFA reesterification.

The first aim of the present study was to determine the role of suppression of lipolysis, and stimulation of primary FFA reesterification, in the regulation of FFA R, by insulin in healthy human volunteers. Because there is no agreement as to the best approach to the evaluation of FFA reesterification in vivo, the second aim was to compare the different methods used to measure FFA reesterification in human volunteers (6, 16–18, 24, 31, 37, 38). Therefore, we determined the response of primary, secondary, and whole body FFA reesterification to sequential insulin infusions using a combination of the isotopic methods and indirect calorimetry described previously. If the different approaches to measurement of FFA reesterification outlined above were valid, they should give similar estimates of FFA reesterification during different plasma insulin concentrations. If not, the differences would be analyzed to determine our preferred approach for the evaluation of FFA metabolism in vivo.

METHODS

Subjects. We studied five male and two female lean healthy volunteers (34 ± 3 yr, 24 ± 1 kg/m² body mass index, 24 ± 3% body fat, and 102 ± 2% ideal body wt). All volunteers had normal glucose tolerance, as judged from a fasting plasma glucose <6 mmol/l and a normal oral glucose tolerance test result. Body composition was determined from body density measured by underwater weighing (15). Subjects consumed a weight-maintenance diet containing at least 200 g carbohydrate for 3 days before study. The study was approved by the Vanderbilt University Committee for the Protection of Human Subjects, and informed written consent was obtained from all volunteers before their participation in the study.

Experimental design. Volunteers were admitted to the Clinical Research Center at 5:00 P.M. on the day preceding the study, where they received a standard meal. After a 12-h overnight fast, an antecubital vein was cannulated with an 18-gauge catheter for infusion of insulin and isotopes. A hand vein from the contralateral arm was cannulated retrogradely with a 20-gauge cannula, and the hand was placed in a thermoregulated Plexiglass box maintained at a minimum of 55°C to permit sampling of arterialized blood. At 6:00 a.m. prilmed continuous infusions of [³H]glycerol (Tracer Technology, Newton, MA; 1 µmol/kg, 0.11 µmol/min), and [¹⁴C]palmitate (0.25 µCi/min) were begun, and a 5 µCi intravenous bolus of [¹⁴C]sodium bicarbonate was given to prime the bicarbonate pool. Beginning at 9:00 A.M. subjects received sequential insulin infusions at rates of 4, 8, 25, and 400 mU·m⁻²·min⁻¹ for 2 h each rate, respectively. Sequential insulin infusions have been used previously to

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Eli Lilly, Indianapolis, IN) was dissolved in 0.9% saline con- 

infusions given on separate days. The insulin (Humulin regular, 

have been shown to produce similar results as individual insulin 

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necessary for measurement of plasma glucose concentration, 

ley, CA). Blood was withdrawn at 5- to 10-min intervals as 

along with a carrier infusion of 0.9% saline (100 ml/h) contain-

pending on the changes in the plasma glucose concentration.

the Harvard pump (Harvard Instruments, Boston, MA), de-

glucose infusion rates were made by altering the percent dial of 

molecules by thin-layer chromatography. [2H5]glycerol enrich-

P-scintillation counter. The ratio of palmitate to total FFA 

concentration was determined by gas chromatography of the 

spectrometer (Delsi/Nermag, Argenteuil, France) using electron 

radioactivity in the hyamine hydroxide solution was measured 

in a P-scintillation counter and, the CO2 trapping capacity of 

analyzer, and CO2 concentration by an infrared analyzer. 14C02 

2900 Energy Measurement Module). Ventilation was measured 

the hyamine hydroxide solution was determined by titration 


glycerol specific activity (25) to reach a steady state. The deuterated glyc-

erol infusion rate was subtracted from the total glycerol turn-

were determined using the steady-state calculation (infusion 

Calculations and statistics. Palmitate and glycerol turnover 

were determined using the steady-state calculation (infusion 

rate divided by specific activity or enrichment) in view of the 

relatively short time for glycerol enrichment (4) and FFA spe-

fic activity (25) to reach a steady state. The deuterated glyce-

rol infusion rate was subtracted from the total glycerol turn-

over to obtain the endogenous turnover rate. FFA turnover was 

calculated as the product of palmitate turnover and the ratio of 

FFA to palmitate concentration.

Fat oxidation was calculated by indirect calorimetry after 

correction for protein oxidation (13). Molar triglyceride oxid-
Fractional FFA reesterification, the proportion of FFA molecules reesterified without leaving adipose tissue, was calculated as the ratio of primary FFA reesterification to lipolysis

\[
\text{fractional FFA RE} = \frac{\text{FFA RE}}{\text{lipolysis}} \quad (9)
\]

Insulin sensitivity was assessed as the insulin concentration that effectively suppressed FFA metabolism by fifty percent (EC50), determined from the appropriate insulin dose-response curve. The final insulin infusion was assumed to produce maximal suppression of FFA kinetics.

All values are expressed as means ± SE. Because we assume that adipose tissue is the predominant source of fat and that lipid oxidation occurs mainly in muscle, FFA and glycerol Ra were corrected for fat mass, whereas fat oxidation was corrected for lean body mass. Statistical analyses were performed using analysis of variance for repeated measures, Tukey's test for post hoc comparisons, and Student's t tests corrected for multiple comparisons by the Bonferroni procedure.

RESULTS

Glucose, insulin, and FFA concentrations. The volunteers had fasting glucose and insulin concentrations of 4.9 ± 0.1 mM and 54 ± 12 pM, respectively. The plasma glucose coefficient of variation during the euglycemic clamps was 6%. The fasting plasma FFA concentration was 638 ± 52 μM and decreased significantly during every insulin infusion rate except the last, reaching a nadir of 113 ± 22 μM during the final insulin infusion (Table 1).

Glycerol and FFA Ra. Glycerol Ra declined with the first two insulin infusions, from a fasting rate of 12.6 ± 1.3 to 5.6 ± 1.1 μmol·kg fat mass⁻¹·min⁻¹ (overall P < 0.001) but did not change significantly thereafter. FFA Ra fell progressively with every insulin infusion, from 28.9 ± 3.5 to 4.5 ± 0.9 μmol·kg fat mass⁻¹·min⁻¹ (P < 0.001).

The insulin concentrations that produced half-maximal inhibition of glycerol and FFA Ra were similar (106 ± 26 vs. 91 ± 20 pM, P = not significant (NS); Fig. 1).

FFA oxidation. RQ FFA oxidation decreased progressively from 5.5 ± 0.4 at baseline to 1.5 ± 0.4 μmol·kg lean body mass (LBM)⁻¹·min⁻¹ during the last insulin infusion, and 14CO₂ FFA oxidation decreased from 2.7 ± 0.4 to 0.9 ± 0.1 μmol·kg LBM⁻¹·min⁻¹ (both P < 0.001). The ratio of 14CO₂ FFA oxidation to RQ FFA oxidation remained ~50% throughout the insulin dose-response curve (0.52 ± 0.10 vs. 0.47 ± 0.08 vs. 0.49 ± 0.07 vs. 0.63 ± 0.11 vs. 0.49 ± 0.10, P = NS). The EC50 values for suppression of RQ FFA oxidation and 14CO₂ FFA oxidation were the same (324 ± 60 vs. 264 ± 84 pM, P = NS; Fig. 2).

Total FFA reesterification. The dose-response curves for total FFA reesterification, calculated as either the sum of primary and secondary FFA reesterification, or the difference between lipolysis and RQ FFA oxidation, are shown in Fig. 3. The two curves decreased in parallel during the first two insulin infusions (from 28.1 ± 3.4 to 10.9 ± 2.6 μmol·kg fat mass⁻¹·min⁻¹, P < 0.001, and

Table 1. Plasma concentrations of insulin, glucose, and FFA molecules

<table>
<thead>
<tr>
<th>Insulin Infusion Rate, mU·min⁻¹</th>
<th>Insulin, pM</th>
<th>Glucose, mM</th>
<th>FFA, μM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>54±12</td>
<td>4.9±0.1</td>
<td>638±52</td>
</tr>
<tr>
<td>4</td>
<td>84±6</td>
<td>4.7±0.1</td>
<td>397±82</td>
</tr>
<tr>
<td>8</td>
<td>192±14</td>
<td>5.1±0.2</td>
<td>273±58</td>
</tr>
<tr>
<td>25</td>
<td>468±22</td>
<td>5.3±0.1</td>
<td>137±23</td>
</tr>
<tr>
<td>400</td>
<td>13,450±1,400</td>
<td>5.1±0.1</td>
<td>113±22</td>
</tr>
</tbody>
</table>

Values are means ± SE. FFA, free fatty acid.
from 19.7 ± 3.0 to 3.2 ± 2.7 μmol·kg fat mass⁻¹·min⁻¹, \( P < 0.01 \), with the sum of primary and secondary FFA reesterification being greater both after the overnight fast (28.1 ± 3.4 vs. 19.7 ± 3.0 μmol·kg fat mass⁻¹·min⁻¹, \( P < 0.01 \)) and at their nadir during the second insulin infusion (10.9 ± 2.6 vs. 3.2 ± 2.7 μmol·kg fat mass⁻¹·min⁻¹, \( P < 0.01 \)). Thereafter the curves for FFA reesterification appeared to diverge; however, the apparent rebound of the lower curve (the difference between lipolysis and RQ FFA oxidation) was not significant.

**Lipolysis and primary and secondary FFA reesterification.** Lipolysis and primary FFA reesterification are shown in Fig. 4. The difference between the two curves represents FFA Ra. The absolute rate of primary FFA reesterification did not change throughout the insulin infusions, with the rate after the overnight fast similar to that during the maximal insulin infusion (8.9 ± 2.2 vs. 9.3 ± 1.5 μmol·kg fat mass⁻¹·min⁻¹, \( P = \text{NS} \)). However, fractional FFA reesterification, the proportion of FFA reesterified without entering the plasma pool, doubled over the portion of the insulin dose-response curve that remained within the physiological range (0.23 ± 0.06 vs. 0.44 ± 0.07, \( P < 0.05 \); Fig. 5). Secondary FFA reesterification (or reesterification of FFA molecules from the plasma pool) was suppressed by insulin regardless of the calculation used (Fig. 6). However, the rates calculated as the difference between FFA Ra and 14C0₂ FFA oxidation were twofold greater than the rates obtained from the difference between whole body FFA reesterification and primary FFA reesterification. Using the former calculation, secondary FFA reesterification decreased from an overnight fasted value of 19.2 ± 2.3 to 1.4 ± 0.4 μmol·kg fat mass⁻¹·min⁻¹ \( (P < 0.001) \), whereas using the latter calculation, secondary FFA reesterification decreased from 10.8 ± 2.0 to 0.7 ± 1.7 μmol·kg fat mass⁻¹·min⁻¹ \( (P < 0.001) \) (Fig. 6).

**Insulin sensitivity of FFA Ra, secondary reesterification, and RQ oxidation.** The EC₅₀ values for suppression of FFA Ra, secondary reesterification, and RQ oxidation were different (91 ± 20 vs. 80 ± 16 pM, \( P < 0.001 \)). Post hoc comparisons indicated that this difference was the result of the increased EC₅₀ of FFA oxidation, which was greater than that for FFA Ra \( (P < 0.01) \) and secondary reesterification \( (P < 0.001) \), whereas the EC₅₀ values for FFA Ra and secondary reesterification were not different (91 ± 20 vs. 80 ± 16 pM, \( P = \text{NS} \)).

**DISCUSSION**

The aim of these experiments was to determine the roles of lipolysis and FFA reesterification in the regulation of FFA metabolism in healthy human volunteers and to distinguish between primary (occurring within the adipocyte) and secondary (occurring distant to the originating adipocyte after passage through the vascular compartment) FFA reesterification. Only changes in primary FFA reesterification would affect release of FFA from adipose tissue and the measurement of FFA Ra. The discovery that whole body and secondary FFA reesterification differed according to the experimental approach used for its measurement (Figs. 3 and 6) indicated the need to reevaluate the assumptions underlying these measurements.

The insulin dose-response curves for suppression of total FFA reesterification, calculated either as the rate of lipolysis minus that of RQ FFA oxidation, or as the sum of primary and secondary FFA reesterification, were clearly different (Fig. 3). FFA reesterification measured as the sum of primary and secondary FFA reesterification was 50% greater at baseline and was suppressed by the increasing insulin concentrations until maximum suppression occurred at a plasma insulin concentration of ~180 pM. FFA reesterification, calculated as lipolysis...
minus RQ FFA oxidation, declined in parallel for the first two insulin infusions; the apparent rebound during the final two insulin infusions was not significant. Similar differences existed between the two insulin dose-response curves for suppression of secondary FFA reesterification (Fig. 6). Secondary FFA reesterification, calculated as FFA Rₚ minus ¹⁴CO₂ FFA oxidation, was twofold greater than the value obtained by the alternative calculation, the difference between total and primary FFA reesterification. The main difference between these alternative approaches to the evaluation of FFA reesterification in vivo was the use of either indirect calorimetry or ¹⁴CO₂ collection to measure fat oxidation and the twofold greater value for fat oxidation given by indirect calorimetry (Fig. 2).

Some investigators have speculated that the lower value for fat oxidation obtained by measurement of expired ¹⁴CO₂ during a [¹⁻¹⁴C]palmitate infusion represents the oxidation of intravascular FFA molecules, whereas the larger value obtained by indirect calorimetry represents oxidation of both intracellular and intravascular FFA molecules (6, 16–18). The more likely explanation for the different values would appear to be the incomplete collection of CO₂ from carbon-labeled FFA molecules. It is customary to compensate for the exchange of carbon label in the bicarbonate pool using a correction factor of 0.8 (22), but no correction has been made for the exchange of carbons in the oxaloacetate pool of the tricarboxylic acid cycle. Labeled carbon from FFA enters the tricarboxylic acid cycle as acetyl-coenzyme A, where it retains its labeled carbon until it enters the oxaloacetate pool. Here it is in equilibrium with oxaloacetate derived from pyruvate, and it could either enter the gluconeogenic pathway or continue around the tricarboxylic acid cycle and be oxidized to CO₂. Wolfe and Jahoor (30) have shown that only 80% of [¹⁻¹³C]acetate infused into human volunteers was recovered as ¹³CO₂. Other assumptions in the measurement of ¹³CO₂ FFA oxidation are that the plasma palmitate specific activity reflects that of the mitochondrial FFA pool where oxidation occurs and that CO₂ expiration is in equilibrium with intracellular FFA oxidation. However, after the abrupt cessation of [¹⁻¹⁴C]FFA infusions in human volunteers, the half-life of plasma [¹⁴C]FFA was only a few minutes, whereas expiration of ¹⁴CO₂ continued for several hours (11, 19), indicating that these critical assumptions in the measurement of ¹³CO₂ FFA oxidation were incorrect. Thus ¹⁴CO₂ FFA oxidation would underestimate FFA oxidation, resulting in an overestimate of secondary (Eq. 3) and total (Eq. 5) FFA reesterification (Figs. 3 and 6).

Indirect calorimetry measures net FFA oxidation, that is, the difference between FFA oxidation and lipogenesis (13). After the overnight fast and during the initial low-dose insulin infusions, lipogenesis would have been minimal and would have had little effect on the calculation of lipid oxidation. This assumption is supported by the minimal stimulation of glucose disposal that would have occurred at these low plasma insulin concentrations (27). It is also interesting to speculate that the step-down in the insulin dose-response curve for suppression of lipid oxidation that occurs between the low- and high-rate insulin infusions might represent the switching on of lipogenesis, causing the apparent sudden reduction in lipid oxidation at that point (Fig. 2). Thus, when total FFA reesterification is measured as lipolysis minus RQ FFA oxidation, the reversal of the insulin inhibition of total and secondary FFA reesterification at plasma insulin concentrations >180 pM, although not significant, could represent the stimulation of lipogenesis and underestimation of FFA oxidation by indirect calorimetry (Figs. 3 and 6). Fortunately, this problem does not influence the evaluation of the physiological regulation of FFA reesterification, since suppression of FFA metabolism in vivo appears to be complete below a plasma insulin concentration of 180 pM (Figs. 1, 3, 4, and 6), before one would expect appreciable insulin stimulation of lipogenesis. However, the final two points of the insulin dose-response curves, which represent maximal physiological and pharmacological plasma insulin concentrations, should be interpreted with caution because significant lipogenesis would be expected with that degree of hyperinsulinemia.

Wolfe et al. (37) have considered the impact of mesenteric and intramuscular lipolysis on these isotopic estimates of FFA metabolism. Less than ten percent of whole body FFA flux comes from mesenteric lipolysis, and only one-quarter of that is cleared in the first pass through the liver (3, 33). Therefore measurement of FFA Rₚ using [¹⁻¹⁴C]palmitate would miss <3% of released FFA molecules. Furthermore, there is no net release of glycerol from the gut in resting dogs (33) or humans (5), consistent with minimal mesenteric lipolysis in the resting state. Because muscle, like adipose tissue, has minimal glycerol kinase activity (26) and subsequently little glycerol is metabolized within muscle, lipolysis of intramuscular triglyceride would increase the isotopic estimate of primary FFA reesterification to the extent that FFA molecules were oxidized by the muscle before entering the plasma pool. If intramuscular lipolysis and oxidation of the released FFA molecules were significant, it would reduce the ratio of FFA Rₚ to glycerol Rₚ below the usual value of 3:1. However, in this and other studies, this ratio has remained close to 3:1 under a wide range of physiological conditions (37, 38). Measurement of triglyceride in muscle biopsy specimens from healthy human volunteers indicates that intramuscular triglyceride would account for <2% of the total body fat of our volunteers (9, 21). Furthermore, the arteriovenous difference of plasma FFA molecules across resting and exercising muscle could supply all of the energy from fat without invoking the need for intramuscular fat stores (1). Only under extreme conditions, such as exercise to exhaustion, has acute depletion of intramuscular lipid stores been demonstrated (9, 21). Finally, there was no significant arteriovenous difference of glycerol across forearm muscle in humans fasted overnight (14), and the arteriovenous balance of glycerol was unaffected by an insulin infusion of 0.1 mU·kg⁻¹·min⁻¹ in healthy humans (34), comparable to the intermediate insulin infusions in the present experiments. For these reasons, we concur with Wolfe et al. (37) that these isotopic measurements of lipid metabolism are reasonably accurate.

Our results provide sufficient information to evaluate...
the current methods for measurement of FFA reesterification in vivo. As discussed previously, estimates based on the measurement of $^{14}$CO$_2$ FFA oxidation are probably invalid. The alternative approach is to calculate total FFA reesterification as lipolysis, estimated from glycerol turnover, minus RQ FFA oxidation (Fig. 3). This calculation remains valid as long as it is restricted to the lower plasma insulin concentrations most relevant to lipid metabolism in vivo, when lipogenesis is negligible. Primary FFA reesterification is calculated as lipolysis minus FFA $R_a$ (Fig. 4), and secondary FFA reesterification is calculated indirectly as total minus primary FFA reesterification (Fig. 6). With the use of these calculations, our results confirm that lipolysis and FFA $R_a$ are equally sensitive to suppression by insulin, as shown by their parallel insulin dose-response curves and equivalent insulin concentrations that produced half-maximal suppression, an index of insulin sensitivity ($EC_{50}$ values, 106 ± 26 vs. 91 ± 20 pM, $P = \text{NS}$). The new finding is that the absolute rate of primary FFA reesterification was unaffected by the insulin infusions, despite the large fall in lipolytic rate and supply of FFA substrate and did not change from its basal value, even during the final supraphysiological insulin infusion (8.9 ± 2.2 vs. 9.3 ± 1.5 $\mu$mol kg fat mass$^{-1}$ min$^{-1}$, $P = \text{NS}$). Although the absolute rate of FFA reesterification was unchanged, the fraction of FFA molecules reesterified after lipolysis increased twofold in response to insulin over the physiological range of the insulin dose-response curve (0.23 ± 0.06 to 0.44 ± 0.07, $P < 0.05$; Fig. 5). The maintenance of a constant rate of primary FFA reesterification during the decline in the rate of lipolysis magnified the insulin suppression of FFA $R_a$. This is illustrated in Fig. 7. The bottom curve represents the actual dose-response curve for suppression of FFA $R_a$. The top curve represents what would happen if primary FFA reesterification was suppressed by insulin, proportional to the insulin suppression of lipolysis. This has little effect in the early portion of the dose-response curve, but, by the third insulin infusion (within the physiological portion of the curve), this adjustment would almost halve the insulin suppression of FFA $R_a$ (8.8 ± 1.5 vs. 14.2 ± 2.0 $\mu$mol kg fat mass$^{-1}$ min$^{-1}$, $P < 0.01$). Thus minor changes in the absolute rate of primary FFA reesterification, in conditions such as obesity or non-insulin-dependent diabetes mellitus (NIDDM), could have a major impact on FFA $R_a$.

In contrast to the response of primary FFA reesterification, reesterification of intravascular FFA molecules, or secondary FFA reesterification, was exquisitely sensitive to insulin, similar to FFA $R_a$ (Figs. 1 and 6). The greater insulin sensitivity of FFA $R_a$ and secondary reesterification compared with oxidation ($EC_{50}$ values, 91 ± 20 and 80 ± 16 vs. 324 ± 60 pM, $P < 0.01$ and 0.001, respectively) indicated that RQ FFA oxidation was regulated by factors other than simple FFA availability. After the overnight fast, hepatic VLDL secretion would account for approximately one-third of secondary FFA reesterification (20), and the remainder could replete hepatic triglyceride stores. In the fed state, insulin secretion would halt secondary FFA reesterification, whereas VLDL production would continue using stored triglyceride (12) and dietary fat. Other major sites for secondary FFA reesterification appear unlikely in view of the minimal uptake of FFA molecules by adipose tissue (7) and the relatively small amount of intramuscular triglyceride.

In summary, we compare the current methods for the measurement of FFA reesterification in vivo. With the use of the suggested approach, it was possible to determine whole body FFA reesterification as well as to distinguish between the individual contributions from primary and secondary FFA reesterification. Our results confirm that insulin is a potent suppressor of lipolysis and indicate that total and secondary FFA reesterification are also exquisitely sensitive to suppression by insulin in healthy human volunteers. Although the absolute rate of primary FFA reesterification was unaffected by the increase in the plasma insulin concentration, fractional primary FFA reesterification doubled, and this magnified insulin suppression of FFA $R_a$. We conclude that primary FFA reesterification is an important component in the regulation of FFA $R_a$ by insulin in lean young healthy humans. Further studies are needed to determine the importance of primary FFA reesterification in other conditions such as obesity or NIDDM.

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