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

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Campbell, Peter J., Michael G. Carlson, James O. Hill, and Nurjahan Nurjan. Regulation of free fatty acid metabolism 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, 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. primed continuous infusions of [3H]glycerol (Tracer Technology, Newton, MA; 1 μmol/kg, 0.11 μmol/min), and [1-14C]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...
assess lipolysis and FFA metabolism in vivo (16, 23, 27) and have been shown to produce similar results as individual insulin infusions given on separate days. The insulin (Humulin regular, Eli Lilly, Indianapolis, IN) was dissolved in 0.9% saline containing 2% human serum albumin (Cutter Laboratories, Berkeley, CA). Blood was withdrawn at 5- to 10-min intervals as necessary for measurement of plasma glucose concentration, and variable amounts of glucose were infused as a 50% solution, along with a carrier infusion of 0.9% saline (100 ml/h) containing 0.26 mmol/ml potassium and 0.18 mmol/ml phosphorus as monophosphates and dibasic phosphates to prevent hypokalemia and hypophosphatemia and to clamp arterial glucose concentrations at 5 mmol/l. The necessary adjustments in the glucose infusion rates were made by altering the percent dial of the Harvard pump (Harvard Instruments, Boston, MA), depending on the changes in the plasma glucose concentration.

Volunteers were fitted with a ventilated hood during the last 30 min of the baseline period and each insulin infusion rate for collection of expired air and measurement of oxygen consumption and CO₂ production. Expired CO₂ was trapped in a hyamine hydroxide solution containing phenolphthalein to indicate the point of neutralization. Arterialized blood samples were collected at -20, -10, and 0 min before the start of the insulin infusions and at 100, 110, and 120 min of each 2-h insulin infusion for measurement of hormones and isotopic specific activities and enrichments. Urine was collected throughout the 11-h study for measurement of total nitrogen and estimation of protein oxidation.

**Analytical techniques.** Plasma glucose concentration was measured by a glucose oxidase method (Beckman glucose analyzer, Fullerton, CA). Insulin concentrations were measured by radioimmunoassay. Plasma palmitate concentration was determined by high-performance liquid chromatography (25) with [1-¹³C]palmitate as the internal standard, and radioactivity was determined by measurement of the palmitate fraction on the β-scintillation counter. The ratio of palmitate to total FFA concentration was determined by gas chromatography of the methyl esters after extraction and isolation of the plasma FFA molecules by thin-layer chromatography. [3-²H₃]glycerol enrichment was determined by gas chromatography-mass spectrometry. The isotopic enrichment of the glycerol tributyldimethylsilyl derivative was determined on a Nermag R10-10C mass spectrometer (Delta/Nermag, Argenteuil, France) using electron impact ionization. Ions were monitored at mass-to-charge ratios 377 and 382 to measure the isotope enrichment of the [²H₃]glycerol infused into the plasma.

Respiratory gas exchange measurements were performed by computerized open-circuit indirect calorimetry (Sensor Medics 2900 Energy Measurement Module). Ventilation was measured by a mass flowmeter, oxygen concentration by a paramagnetic analyzer, and CO₂ concentration by an infrared analyzer. ¹⁴CO₂ radioactivity in the hyamine hydroxide solution was measured in a β-scintillation counter and, the CO₂ trapping capacity of the hyamine hydroxide solution was determined by titration with 0.1 N hydrochloric acid. Urinary nitrogen was measured by the Kjeldahl procedure.

**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 specific activity (25) to reach a steady state. The deuterated glycerol infusion rate was subtracted from the total glycerol turnover 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 oxidation was calculated from the fat oxidation, assuming a molecular weight of 861 for a typical triglyceride (palmitoyl-stearoyl-oleoyl-glycerol, C₅₆H₁₀₆O₆). Because each triglyceride molecule contains three FFA molecules, this was multiplied by three to obtain molar FFA oxidation. Negative values of FFA oxidation were obtained for two of the seven volunteers during the final insulin infusion, presumably the result of lipogenesis exceeding oxidation. These values were assumed to be zero in subsequent analyses. Oxidation of intravascular palmitate was calculated by dividing the ¹⁴CO₂ production rate [disintegrations·min⁻¹ (dpm·min⁻¹)] by palmitate specific activity (dpm/µmol). FFA oxidation was calculated as the product of palmitate oxidation to the FFA-to-palmitate ratio. An important assumption in this calculation is that plasma palmitate specific activity reflects that of the intracellular pool where oxidation occurs. The ¹⁴CO₂ production rate was corrected by a factor of 0.81 for incomplete collection of ¹⁴CO₂ from the bicarbonate pool (22). To distinguish between these two measurements of FFA oxidation, the isotopic measurement will be referred to as ¹⁴CO₂ FFA oxidation and the measurement obtained by indirect calorimetry as respiratory quotient (RQ) FFA oxidation.

**Lipolysis was calculated as 3 × glycerol turnover (see Eq. 1).** Glycolysis did not contribute to glycerol turnover in humans (28), and very-low-density lipoproteins (VLDL) accounted for <4% of plasma glycerol turnover in rats (35). Results from in vitro experiments indicate that triglycerides undergo complete hydrolysis in adipose tissue (8, 32), and mono- and diacylglyceride concentrations in human adipose tissue are very low (2). Significant glycerol metabolism within the adipocyte is impossible because human adipose tissue lacks the glycerol kinase enzyme necessary for phosphorylation of glycerol before its entry into the glycolytic or gluconeogenic pathways (29). Therefore, lipolysis results in the release of one glycerol molecule and three FFA molecules.

FFA oxidation within adipocytes is negligible (30), so the only possible fates for FFA molecules within adipocytes are either release from the cell or reesterification (RE). Thus primary or intra adipocyte FFA reesterification was determined from the difference between total lipolysis and FFA turnover (see Eq. 2). Secondary FFA reesterification, that is FFA reesterification after release from the adipose tissue into the circulation, was calculated as the difference between FFA RE₂ (which during the steady-state condition of these experiments also represents FFA disposal) and ¹⁴CO₂ FFA oxidation (see Eq. 3).

Total FFA reesterification was calculated as the sum of primary and secondary FFA reesterification (see Eq. 4).

\[
\text{lipolysis} = 3 \times \text{glycerol RE}_3 \tag{1}
\]

\[
1^* \text{FFA RE} = \text{lipolysis} - \text{primary FFA RE} \tag{2}
\]

\[
2^* \text{FFA RE} = \text{secondary FFA RE} - 1^* \text{CO}_2 \text{FFA oxidation} \tag{3}
\]

\[
\text{total FFA RE} = 1^* \text{RE} + 2^* \text{RE} \tag{4}
\]

By substituting Eqs. 2 and 3, Eq. 4 can be solved

\[
\text{total FFA RE} = \text{lipolysis} - 1^* \text{CO}_2 \text{FFA oxidation} \tag{5}
\]

In an alternative approach to the estimation of FFA reesterification, total FFA reesterification was calculated as the difference between lipolysis and RQ FFA oxidation (see Eq. 6). Secondary FFA reesterification was then obtained as the difference between this value for total FFA reesterification and primary FFA reesterification (Eq. 7).

\[
\text{total FFA RE} = \text{lipolysis} - \text{RQ FFA oxidation} \tag{6}
\]

\[
2^* \text{FFA RE} = \text{total FFA RE} - 1^* \text{FFA RE} \tag{7}
\]

Substituting Eqs. 6 and 2, Eq. 7 can be solved

\[
2^* \text{FFA RE} = \text{total FFA RE} - \text{lipolysis} \tag{8}
\]
Fractional FFA reesterification, the proportion of FFA molecules reesterified without leaving adipose tissue, was calculated as the ratio of primary FFA reesterification to lipolysis:

\[
\text{fractional FFA RE} = \frac{\text{FFA RE}}{\text{lipolysis}}
\]

(9)

Insulin sensitivity was assessed as the insulin concentration that effectively suppressed FFA metabolism by fifty percent (EC\text{50}), 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\(^{-1}·\text{min}^{-1}\) (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\(^{-1}·\text{min}^{-1}\) (P < 0.001). The insulin concentrations that produced half-maximal inhibition of glycerol and FFA Ra were similar (91 ± 20 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\(^{-1}·\text{min}^{-1}\) during the last insulin infusion, and \(^{14}\text{CO}_2\) FFA oxidation decreased from 2.7 ± 0.4 to 0.9 ± 0.1 μmol·kg LBMI\(^{-1}·\text{min}^{-1}\) (both P < 0.001). The ratio of \(^{14}\text{CO}_2\) 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 EC\text{50} values for suppression of RQ FFA oxidation and \(^{14}\text{CO}_2\) 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\(^{-1}·\text{min}^{-1}\), P < 0.001, and 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\(^{-1}·\text{min}^{-1}\), P < 0.001, and

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

<table>
<thead>
<tr>
<th>Insulin Infusion Rate, mU·m(^{-2}·\text{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±26</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 = 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 ¹⁴CO₂ 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 = 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 \( R_F \) minus \(^{14}\)CO\(_2\) 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 \(^{14}\)CO\(_2\) 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 \(^{14}\)CO\(_2\) during a \([1-^{14}\text{C}]\)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 CO\(_2\) 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 CO\(_2\). Wolfe and Jahoor (36) have shown that only 80% of \([1-^{13}\text{C}]\)acetate infused into human volunteers was recovered as \(^{13}\text{CO}_2\). Other assumptions in the measurement of \(^{14}\text{CO}_2\) FFA oxidation are that the plasma palmitate specific activity reflects that of the mitochondrial FFA pool where oxidation occurs and that CO\(_2\) expiration is in equilibrium with intracellular FFA oxidation. However, after the abrupt cessation of \([1-^{14}\text{C}]\)FFA infusions in human volunteers, the half-life of plasma \([^{14}\text{C}]\)FFA was only a few minutes, whereas expiration of \(^{14}\text{CO}_2\) continued for several hours (11, 19), indicating that these critical assumptions in the measurement of \(^{14}\text{CO}_2\) FFA oxidation were incorrect. Thus \(^{14}\text{CO}_2\) 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 \( R_F \) using \([1-^{14}\text{C}]\)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 \( R_F \) to glycerol \( R_G \) 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 = 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 μmol·kg·fat mass$^{-1}$·min$^{-1}$, P = 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 curves, 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 μmol·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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