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. Glycero- l 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.

gycerol; 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_) 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 glycerone 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 ([¹⁻¹⁴C]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). Then 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. primed continuous infusions of [⁵⁷⁷H]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 con-
fusions given on separate days. The insulin (Humulin regular, El064 FREE FATTY ACID METABOLISM IN HUMANS

necessary for measurement of plasma glucose concentration,

monophosphates and dibasic phosphates to prevent hypokale-
mia and hypophosphatemia and to clamp arterial glucose con-
centrations at ~5 mmol/l. The necessary adjustments in the

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 consump-
tion and CO₂ production. Expired CO₂ was trapped in a
hyamine hydroxide solution containing phenolphthalein to in-
dicate 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 isotope spe-
cific 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 ana-
lizer, Fullerton, CA). Insulin concentrations were measured by
radioimmunoassay. Plasma palmitate concentration was deter-
mined by high-performance liquid chromatography (25) with
[¹⁴H₅]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. [¹⁴H₃]glycerol enrich-
ment was determined by gas chromatography–mass spectrome-
try. The isotopic enrichment of the glycerol tributyldimethyls-
ilyl derivative was determined on a Nermag R10-10C mass
spectrometer (Delsi/Nermag, Argenteuil, France) using electron
impact ionization. Ions were monitored at mass-to-charge ra-
tios 377 and 382 to measure the isotope enrichment of the
[¹⁴H₃]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 spe-
cific activity (25) to reach a steady state. The deuterated gly-
cerol 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 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 distin-
guish 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 diacylgly-
ceride concentrations in human adipose tissue are very low (2).
Significant glycerol metabolism within the adipocyte is impos-
sible because human adipose tissue lacks the glycerol kinase
enzyme necessary for phosphorylation of glycerol before its en-
try into the glycolytic or gluconeogenic pathways (29). There-
fore, 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 pri-
mary 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 rees-
terification after release from the adipose tissue into the circu-
lation, was calculated as the difference between FFA Rₜ (which
during the steady-state condition of these experiments also rep-
resents 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).

lipolysis = 3 × glycerol Rₜ
(1)

1° FFA RE = lipolysis − FFA Rₜ
(2)

2° FFA RE = FFA Rₜ − ¹⁴CO₂ FFA oxidation
(3)

total FFA RE = 1° RE + 2° RE
(4)

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

total FFA RE = lipolysis − ¹⁴CO₂ FFA oxidation
(5)

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

total FFA RE = lipolysis − RQ FFA oxidation
(6)

2° FFA RE = total FFA RE − 1° FFA RE
(7)

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

2° FFA RE = FFA Rₜ − RQ FFA oxidation
(8)
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Fractional FFA reesterification, the proportion of FFA molecules reesterified without leaving adipose tissue, was calculated as the ratio of primary FFA reesterification to lipolysis

\[
\text{fractional FFA RE} = \frac{1^o \text{FFA RE}}{\text{lipolysis}}
\]

Insulin sensitivity was assessed as the insulin concentration that effectively suppressed FFA metabolism by fifty percent (EC\(_{50}\)), 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 \( R_a \) 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 nM, respectively. The plasma glucose coefficient of variation during the euglycemic clamps was 6%. The fasting plasma FFA concentration was 638 ± 52 nM and decreased significantly during every insulin infusion rate except the last, reaching a nadir of 113 ± 22 nM during the final insulin infusion (Table 1).

Glycerol and FFA \( R_a \). Glycerol \( R_a \) declined with the first two insulin infusions, from a fasting rate of 12.6 ± 1.3 to 5.6 ± 1.1 \( \mu \)mol·kg fat mass\(^{-1}\)·min\(^{-1}\) (overall \( P < 0.001 \)) but did not change significantly thereafter. FFA \( R_a \) fell progressively with every insulin infusion, from 28.9 ± 3.5 to 4.5 ± 0.9 \( \mu \)mol·kg fat mass\(^{-1}\)·min\(^{-1}\) (\( P < 0.001 \)). The insulin concentrations that produced half-maximal inhibition of glycerol and FFA \( R_a \) were similar (91 ± 20 nM, \( P = \text{not significant} \) (NS); Fig. 1).

FFA oxidation. RQ FFA oxidation decreased progressively from 5.5 ± 0.4 at baseline to 1.5 ± 0.4 \( \mu \)mol·kg lean body mass (LBM)\(^{-1}\)·min\(^{-1}\) during the last insulin infusion, and \( ^{14} \)CO\(_2\) FFA oxidation decreased from 2.7 ± 0.4 to 0.9 ± 0.1 \( \mu \)mol·kg LBＭ\(^{-1}\)·min\(^{-1}\) (both \( P < 0.001 \)). The ratio of \( ^{14} \)CO\(_2\) 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, \( P = \text{NS} \)). The EC\(_{50}\) values for suppression of RQ FFA oxidation and \( ^{14} \)CO\(_2\) FFA oxidation were the same (324 ± 60 vs. 264 ± 84 nM, \( P = \text{NS} \); 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 \( \mu \)mol·kg fat mass\(^{-1}\)·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 \( \mu \)mol·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·m(^{-2})·min(^{-1})</th>
<th>Insulin, nM</th>
<th>Glucose, mM</th>
<th>FFA, nM</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>5.7±0.1</td>
<td>397±87</td>
</tr>
<tr>
<td>8</td>
<td>102±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.

Fig. 1. Insulin dose-response curves for suppression of free fatty acid (FFA) and glycerol rates of appearance (\( R_a \)).

Fig. 2. Insulin dose-response curves for suppression of FFA oxidation, measured using indirect calorimetry [respiratory quotient (RQ)] or expired \( ^{14} \)CO\(_2\).

Fig. 3. Insulin dose-response curves for suppression of total FFA reesterification. Total FFA reesterification was calculated as sum of primary and secondary FFA reesterification and as difference between lipolysis and RQ FFA oxidation.
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 Rₗ. 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 Rₗ 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 Rₗ, secondary reesterification, and RQ oxidation.** The EC₅₀ values for suppression of FFA Rₗ, 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 Rₗ (P < 0.01) and secondary reesterification (P < 0.001), whereas the

**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 Rₗ. 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 $R_m$ 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 [1-14C]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 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 [1-13C]acetate infused into human volunteers was recovered as $^{13}$CO$_2$. Other assumptions in the measurement of $^{14}$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 [1-14C]FFA infusions in human volunteers, the half-life of plasma [14C]FFA was only a few minutes, whereas expiration of $^{14}$CO$_2$ continued for several hours (11, 19), indicating that these critical assumptions in the measurement of $^{14}$CO$_2$ FFA oxidation were incorrect. This $^{14}$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 $R_m$ 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 $R_m$ 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 physiologic 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


