What Does the Measurement of Whole-Body Fatty Acid Rate of Appearance in Plasma by Using a Fatty Acid Tracer Really Mean?

Bettina Mittendorfer,[1] Olivia Liem,[1] Bruce W. Patterson,[1] John M. Miles,[2] and Samuel Klein[1]

We evaluated the validity of using a single fatty acid tracer to assess total plasma long-chain free fatty acid (FFA) kinetics and the relationship between the rate of appearance (R_a) of fatty acids in plasma and the fatty acid composition of adipose tissue triglyceride (TG). A mixture of [^{13}C]-labeled myristate, palmitate, stearate, oleate, and linoleate was infused in healthy men during basal conditions and during conditions that stimulate (epinephrine infusion) and inhibit (insulin infusion) lipolysis of adipose tissue TGs. Calculated total FFA, R_a based on palmitate, oleate, or linoleate tracers, was within 15% of the measured sum of the individual fatty acid R_a under all conditions, whereas stearate and myristate tracers consistently underestimated and overestimated total FFA R_a, respectively. The fatty acid R_a profile closely matched the fatty acid profile of subcutaneous adipose tissue TGs during epinephrine infusion, but not during basal conditions and insulin infusion. Our data support the common practice of using labeled palmitate or oleate as fatty acid tracers for assessing total plasma FFA kinetics and suggest that a source of lipids other than adipose tissue TG release fatty acids into the systemic circulation. *Diabetes* 52: 1641–1648, 2003

Adipose tissue triglycerides (TGs) are the major endogenous source of fuel in humans. The mobilization of this fuel store involves the hydrolysis of TGs and the release of free fatty acids (FFAs) into the bloodstream. Although adequate availability of plasma FFA is critical for normal function and survival, excessive FFA release into plasma can have adverse medical effects by increasing hepatic glucose production (1,2), decreasing insulin-mediated glucose disposal (1,2), and stimulating VLDL-TG production (3). Therefore, understanding FFA kinetics has important physiological and clinical implications.

Fatty acid kinetics are usually determined by assessing the dilution of a labeled fatty acid tracer that is infused into the bloodstream. It is assumed that the dilution of the fatty acid tracer in plasma is predominantly caused by entry of unlabelled fatty acids released by hydrolysis of adipose tissue TG. The rate of appearance (R_a) of a single fatty acid in plasma is often used to estimate total FFA kinetics, by dividing the individual fatty acid R_a by the relative contribution of this fatty acid to total plasma FFA concentration (4–6). This extrapolation assumes that the R_a of the fatty acid being traced is representative of the release of other fatty acids from adipose tissue and that the relative concentration of this fatty acid in plasma represents its relative release rate from adipose tissue. Data from studies conducted in isolated adipocytes and in regional subcutaneous adipose tissue suggest that the relative rate of release of specific fatty acids from adipose tissue into plasma is influenced by fatty acid structure (7–14). However, the relationship between whole-body systemic fatty acid kinetics and adipose tissue fatty acid composition is not known.

The purpose of the present study was to evaluate whether a single fatty acid tracer provides a reliable estimate of total plasma FFA kinetics and whether the systemic release of individual fatty acids into plasma reflects fatty acid composition in adipose tissue TG in human subjects during different physiological conditions. We measured the R_a of five fatty acids (myristate, palmitate, stearate, oleate, and linoleate) into plasma, using stable isotope–labeled tracer methods, in the basal postabsorptive state and during conditions that stimulate (epinephrine infusion) and inhibit (insulin infusion) lipolysis of adipose tissue TG. Adipose tissue TG fatty acid composition was determined in biopsy samples from upper (abdominal) and lower (gluteal) subcutaneous fat depots. We hypothesized that the accuracy of estimating total FFA R_a with a single fatty acid tracer and the relationship between the R_a of individual fatty acids and adipose tissue TG fatty acid composition depends on the choice of tracer and the physiological condition.

RESEARCH DESIGN AND METHODS

Subjects. Seven men (age 28 ± 2 years, weight 78 ± 3 kg, BMI 25 ± 1 kg/m^2) participated in this study. All subjects were considered healthy after completing a comprehensive medical examination that included a history and physical examination, a 12-lead electrocardiogram, and standard blood tests. No subject was taking regular medications or smoked tobacco. Written informed consent was obtained from all subjects before their participation in the study, which was approved by the Human Studies Committee and the General Clinical Research Center (GCRC) Scientific Advisory Committee of Washington University School of Medicine (St. Louis, MO).

TABLE 1

Relative fatty acid content in subcutaneous abdominal and gluteal adipose tissue TGs

	Abdominal fat (% total)	Gluteal fat (% total)	Absolute difference between sites (%)
Myristate	4.0 ± 0.2	3.6 ± 0.2	0.4 ± 0.1*
Palmitate	24.4 ± 0.5	22.7 ± 0.2	1.7 ± 0.6
Stearate	5.3 ± 0.2	4.2 ± 0.2	1.1 ± 0.3*
Oleate	44.3 ± 0.5	45.9 ± 0.8	1.4 ± 0.5
Linoleate	16.3 ± 0.9	15.8 ± 0.7	0.5 ± 0.2
Other fatty acids	5.6 ± 0.3	7.8 ± 0.3	2.2 ± 0.4*

Data are means ± SE. *$P < 0.05$, abdominal vs. gluteal TG fatty acid composition.

Isotope infusion protocol. Subjects were admitted to the GCRC on the evening before the isotope infusion study; at 2000, they consumed a meal containing 12 kcal/kg body wt (55% of total energy carbohydrates, 30% fat, and 15% protein) and a liquid formula (Ensure; Ross Laboratories, Columbus, OH) containing 240 kcal (64% of total energy carbohydrates, 22% fat, and 14% protein). Subjects then fasted until completion of the study the following day.

At 0700, a catheter was inserted into an antecubital vein to infuse stable isotope–labeled tracers, hormones, and glucose. A second catheter was placed into a radial artery for blood sampling. In one of the subjects, the second catheter was placed into a contralateral hand vein and the hand was heated to 55°C by using a thermostatically controlled box to obtain arterialized blood samples (15). At 0800, ~100 mg of abdominal and gluteal subcutaneous adipose tissues were obtained by using a modified mini-liposuction technique (16). The biopsy samples were rinsed with ice-cold saline, transferred into liquid nitrogen, and stored at −70°C for further analysis.

At 0900, a three-stage isotope infusion study was performed while subjects remained lying in bed. A constant infusion (12.8 ± 0.5 μmol fatty acids/min) of a mixture of ^{13}C-labeled fatty acids ([1-^{13}C]myristate, [1-^{13}C]palmitate, [1-^{13}C]stearate, [1-^{13}C]oleate, and [1-^{13}C]linoleate) bound to human albumin was started and maintained for 230 min by using a calibrated peristaltic pump (Gemini PC 2; IMED, San Diego, CA). Eighty minutes after starting the tracer infusion, epinephrine (Abbott Laboratories, North Chicago, IL) was infused for 30 min (0.015 μg · kg^{-1} · min^{-1}) by using a calibrated syringe pump (Harvard Apparatus, South Natick, MA), and subjects' heart rate and rhythm were monitored with a cardiac monitor. Immediately after the epinephrine infusion was completed, insulin (Novo Nordisk Pharmaceuticals, Princeton, NJ) was infused for 120 min (30 mU · m^2 body surface area^{-1} · min^{-1}; initiated with a two-step priming dose: 120 mU · m^{-2} min^{-1} for 5 min, followed by 60 mU · m^{-2} min^{-1} for 5 min) by using a calibrated peristaltic pump (Gemini PC 2). During insulin infusion, plasma glucose concentration was measured every 10 min, 20% dextrose was infused as necessary to maintain euglycemia (5.5 ± 0.2 mmol/l), and the tracer infusion rate was decreased by 60% to minimize changes in isotopic enrichment caused by the insulin-induced suppression of lipolysis.

Blood sampling. Blood samples were obtained before the start of the tracer infusion to determine background plasma fatty acid tracer-to-tracee ratios (TTRs), every 5 min during the last 25 min of the basal period, every 5 min during epinephrine infusion, and every 10 min during the last 30 min of insulin infusion to determine plasma fatty acid, epinephrine, and insulin concentrations and fatty acid TTR.

Blood samples were collected in chilled tubes containing 1) EDTA to determine fatty acid TTR and concentrations, 2) reduced glutathione and EGTA to determine plasma catecholamine concentration, and 3) EDTA and trasylol to determine plasma insulin concentrations. Blood samples were placed in ice, and plasma was separated by centrifugation within 30 min of collection. Plasma samples were stored at −70°C until final analyses were performed. Blood samples used to determine glucose concentration were collected in tubes containing heparin and immediately analyzed.

Materials. K$^+$ salts of [1-^{13}C]palmitic acid, [1-^{13}C]oleic acid, [1-^{13}C]stearic acid, and [1-^{13}C]myristic acid and the Na$^+$ salt of [1-^{13}C]linoleic acid were purchased from Cambridge Isotope Laboratories (Andover, MA), bound to human albumin (4), and mixed together to produce a solution containing the following molar ratio of labeled fatty acids: 7.5% myristate (C14:0), 19.5% palmitate (C16:0), 16% stearate (C18:0), 39% oleate (C18:1Δ9), and 18% linoleate (C18:2Δ9,12). This composition was specifically chosen to produce similar TTRs among individual fatty acids in plasma based on their estimated R_a. The final fatty acid mixture was divided into 500-ml aliquots for use in each tracer infusion study. This mixture of fatty acid tracers was used to reduce the variability in tracer delivery that could result if each fatty acid tracer were infused via a separate pump. An aliquot of the tracer infusate was collected at the end of each study. The measured concentration and TTR of each fatty acid tracer in the infusate was used to calculate fatty acid kinetics.

Sample analyses. Plasma glucose concentration was determined by using a glucose analyzer (Yellow Springs Instrument, Yellow Springs, OH). Plasma insulin and epinephrine concentrations were measured as previously described (5,17). Plasma fatty acid concentrations were quantified by gas chromatography (5890-II; Hewlett-Packard, Palo Alto, CA) after adding heptadecanoic acid to plasma as an internal standard (18,19). Plasma fatty acid TTRs were determined by gas chromatography–mass spectrometry (MSD 5973 system with capillary column; Hewlett-Packard) as previously described (19). Ions of the fatty acid methyl esters were produced by electron impact ionization, and ions of mass-to-charge ratios 242 and 243 (myristate), 270 and 271 (palmitate), 298 and 299 (stearate), 296 and 297 (oleate), and 294 and 295 (linoleate) were monitored. A fivefold range of volumes (0.5, 1.0, 1.5, 2.0, and 2.5 μl) was injected for each sample to account for the concentration dependency of measuring TTRs for fatty acid methyl esters.

Adipose tissue TG fatty acid composition was determined by extracting lipids (20) and separating TGs by using thin-layer chromatography. An internal standard of heptadecanoic acid was added to the recovered TG fraction, which was then hydrolyzed, the resulting fatty acids were esterified, and the percent molar distribution of individual fatty acids in TG was determined by gas chromatography (Hewlett-Packard 5890-II).

Calculations. Isotopic steady state was achieved during the last 30 min of the basal and insulin infusion periods, so the R_a of individual fatty acids was calculated by using Steele's equation for steady-state conditions (21). During epinephrine infusion, there was a progressive increase in plasma fatty acid concentration and decrease in fatty acid TTR, so fatty acid R_a was calculated by using Steele's equation for non–steady-state conditions (21,22). The volume of distribution (plasma volume) was assumed to be 55 ml/kg fat-free mass (23); fat-free mass was measured by dual-energy X-ray absorptiometry. The total release of each fatty acid during 30 min of epinephrine infusion was calculated as the area under the R_a curve above baseline by using the trapezoid method.

Total fatty acid R_a was determined in two ways: 1) calculated total R_a was obtained by dividing the R_a of each individual fatty acid by the proportional contribution of each individual fatty acid to the total concentration of the five

TABLE 2

Plasma fatty acid concentrations during basal conditions and during epinephrine and insulin infusions

	μmol/l			Total FFA concentration (%)		
	Basal	Epinephrine*	Insulin	Basal	Epinephrine	Insulin
Myristate	8 ± 1	25 ± 3†	1 ± 0.1†	2 ± 0.1	2 ± 0.1	2 ± 0.1
Palmitate	97 ± 10	279 ± 27†	13 ± 1†	26 ± 1	27 ± 1†	28 ± 1†
Stearate	49 ± 4.3	109 ± 10†	6 ± 1†	13 ± 1	11 ± 1†	14 ± 1
Oleate	142 ± 12	412 ± 37†	15 ± 2†	38 ± 1	39 ± 1	34 ± 1†
Linoleate	63 ± 7	186 ± 24†	8 ± 1†	17 ± 1	18 ± 1	17 ± 1
Other FFA	12 ± 1	37 ± 5†	2 ± 0.4†	3 ± 0.1	4 ± 0.2	5 ± 1†
Total FFA	371 ± 34	1048 ± 96†	45 ± 4†	100	100	100

Data are means ± SE. *Peak concentrations during epinephrine infusion; †$P < 0.05$ vs. corresponding basal value.

TABLE 3
Individual measured fatty acid rate of appearance (R_a) in plasma during basal conditions and during epinephrine and insulin infusions

| | R_a (μmol $\cdot$ kg^{-1} $\cdot$ min^{-1}) | | | Basal (%) | |
	Basal	Epinephrine*	Insulin	Epinephrine	Insulin
Myristate	0.09 ± 0.01	0.40 ± 0.02†	0.02 ± 0.00†	373 ± 54[b]	20 ± 3[ac]
Palmitate	0.99 ± 0.11	3.47 ± 0.44†	0.20 ± 0.02†	368 ± 52[b]	21 ± 2[c]
Stearate	0.32 ± 0.03	0.80 ± 0.06†	0.08 ± 0.01†	266 ± 30	25 ± 1
Oleate	1.36 ± 0.19	6.13 ± 0.52†	0.20 ± 0.02†	492 ± 67[a]	16 ± 1[b]
Linoleate	0.68 ± 0.10	2.53 ± 0.36†	0.11 ± 0.01†	402 ± 60[ab]	17 ± 1[ab]
Sum	3.44 ± 0.45	13.30 ± 1.30†	0.61 ± 0.06†	413 ± 54[ab]	19 ± 1[c]

Data are means $\pm$ SE. *Peak value during epinephrine infusion; †$P < 0.01$ vs. corresponding basal value. Values representing the percent change from basal during either epinephrine or insulin infusion that share the same letter as other values in that column are not significantly different; values that do not share the same letter in a column are significantly different ($P < 0.05$).

fatty acids in plasma, and *2*) measured total R_a was obtained by summing the five measured individual fatty acid R_a values.

The relative contribution of each individual fatty acid R_a to total plasma fatty acid R_a was calculated as the individual fatty acid R_a divided by the sum of the five measured fatty acid R_a values. The clearance rate of individual fatty acids from plasma was calculated by dividing the fatty acid rate of disappearance (R_d) by the fatty acid concentration in plasma. R_d was assumed to be equal to R_a during steady state conditions. During epinephrine infusion, average fatty acid R_d and plasma fatty acid concentration during the last 15 min of epinephrine infusion were used to calculate clearance.

Statistical analyses. A one-way ANOVA with repeated measures was used to test the significance of differences in kinetics between individual fatty acids and fatty acid composition in adipose tissue TG and plasma FFA during each study period and for the relative change in fatty acid kinetics from basal
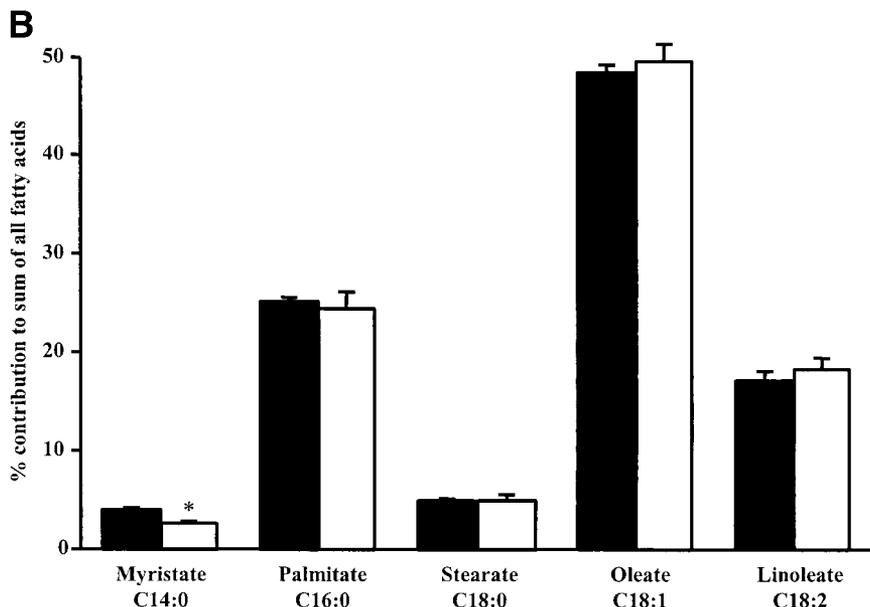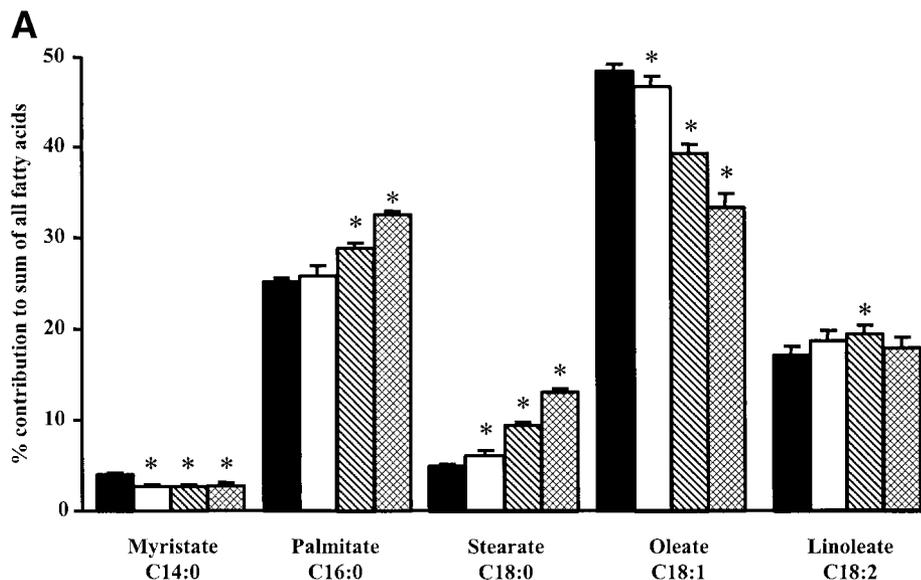
FIG. 1. *A*: Fatty acid distribution in adipose tissue TG (■; average value for abdominal and gluteal adipose tissue TG) and relative fatty acid R_a during basal conditions (▧), epinephrine infusion (□), and insulin infusion (▨). *B*: Adipose tissue fatty acid distribution (■; average value for abdominal and gluteal adipose tissue TG) and the increase in fatty acid R_a above baseline during epinephrine infusion (□). *$P < 0.05$ vs. adipose tissue value. Values are means $\pm$ SE.
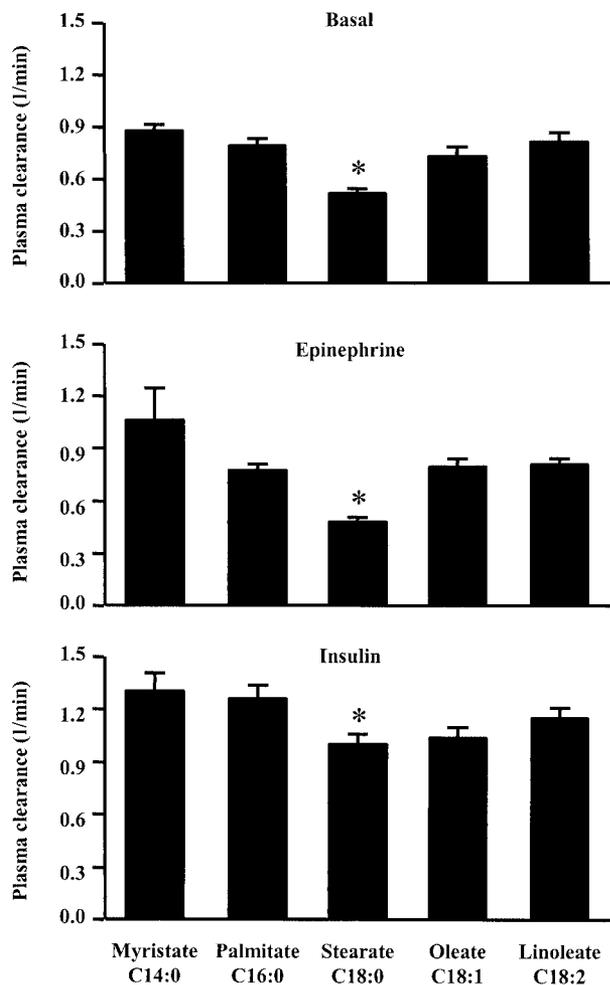
FIG. 2. Fatty acid clearance rates from plasma during basal conditions, epinephrine infusion, and insulin infusion. *P < 0.05 vs. other fatty acids. Values are means ± SE.

values in response to hormone infusions. Significant F ratios were followed by Tukey's analysis. A P value ≤0.05 was considered statistically significant. All values are expressed as means ± SE.

RESULTS

Plasma hormone concentrations. Plasma epinephrine concentration increased from basal values of 61 ± 15 to 418 ± 23 pg/ml during epinephrine infusion ($P < 0.05$) and returned to baseline (44 ± 8 pg/ml) during insulin infusion. Plasma insulin concentration was similar during basal conditions (4.2 ± 0.3 μU/ml) and epinephrine infusion (5.4 ± 0.4) and increased to 44 ± 5 during insulin infusion ($P < 0.05$).

Adipose tissue and plasma fatty acid concentrations. Fatty acid composition of subcutaneous abdominal and gluteal adipose tissue TG was similar; the relative concentration of a fatty acid at one site was within 2% of its relative concentration at the other site (Table 1).

The concentrations of individual fatty acids in plasma during basal conditions, epinephrine infusion, and insulin infusion are shown in Table 2. The five fatty acids for which we assessed kinetics in this study accounted for >95% of total plasma FFA concentration.

Epinephrine infusion caused a two- to threefold increase in the plasma concentrations of all FFA ($P < 0.05$)

(Table 2). However, the relative increase in plasma stearate ($126 \pm 17\%$) was less ($P < 0.05$) than the relative increase in the other FFAs (myristate $203 \pm 36\%$, palmitate $196 \pm 33\%$, oleate $199 \pm 32\%$, and linoleate $204 \pm 33\%$). Epinephrine infusion had either a small or insignificant effect on the relative contribution of individual fatty acids to total plasma FFA concentration.

Insulin infusion caused a marked and similar relative decrease in the concentrations of all plasma FFAs (myristate $88 \pm 2\%$, palmitate $87 \pm 1\%$, stearate $87 \pm 1\%$, oleate $89 \pm 1\%$, and linoleate $88 \pm 1\%$; all $P < 0.05$) (Table 2). Insulin infusion had either a small or insignificant effect on the relative contribution of individual fatty acids to total plasma FFA concentration.

The proportional contribution of myristate, stearate, and oleate to total FFA concentration in plasma were different from that in adipose tissue TG (all $P < 0.05$) (Tables 1 and 2).

Individual fatty acid kinetics. The R_a of individual fatty acids during basal conditions, as well as epinephrine and insulin infusions, is shown in Table 3. The order of fatty acid R_a during basal conditions was oleate→palmitate→linoleate→stearate→myristate. The relative mobilization rate of each fatty acid with respect to the fatty acid composition of adipose tissue TG is shown in Fig. 1A. As lipolytic rate progressively decreased from epinephrine to basal to insulin conditions, the relative contribution from palmitate and stearate progressively increased, whereas the relative contribution from oleate progressively decreased with respect to their distribution in adipose tissue TG. Stearate demonstrated the greatest variability in relative R_a during different physiological conditions; the relative release of myristate into plasma was consistently lower than its relative distribution in adipose tissue TG.

Epinephrine infusion increased the R_a of all fatty acids ($P < 0.05$), but the relative increase in the R_a of individual FFAs varied (Table 3). The relative increase in individual fatty acid R_a into plasma above baseline closely matched the relative fatty acid content in adipose tissue TG (Fig. 1B). Insulin infusion decreased the R_a of all fatty acids ($P < 0.05$), but the relative change from basal values differed among individual fatty acids (Table 3).

The plasma clearance rates of palmitate, oleate, and linoleate were not significantly different from each other. However, the clearance rate of stearate from plasma was ~30% lower ($P < 0.05$) and the plasma clearance rate of myristate tended to be higher than that of palmitate, oleate, and linoleate during all experimental conditions (Fig. 2).

Total FFA kinetics. The accuracy of calculating the measured sum of total FFA R_a from a single fatty acid tracer during each study stage is shown in Fig. 3. The calculated total FFA R_a value, based on palmitate, oleate, or linoleate tracers, was within 15% of the measured total FFA R_a value (i.e., the sum of individual fatty acid R_a values) during all conditions. In contrast, the error in calculating total FFA R_a from either stearate or myristate tracers was much greater; stearate consistently underestimated and myristate consistently overestimated the measured values. The magnitude of the error in estimating total FFA R_a based on stearate kinetics increased with increasing lipolytic rate, whereas the magnitude of the error based on myristate
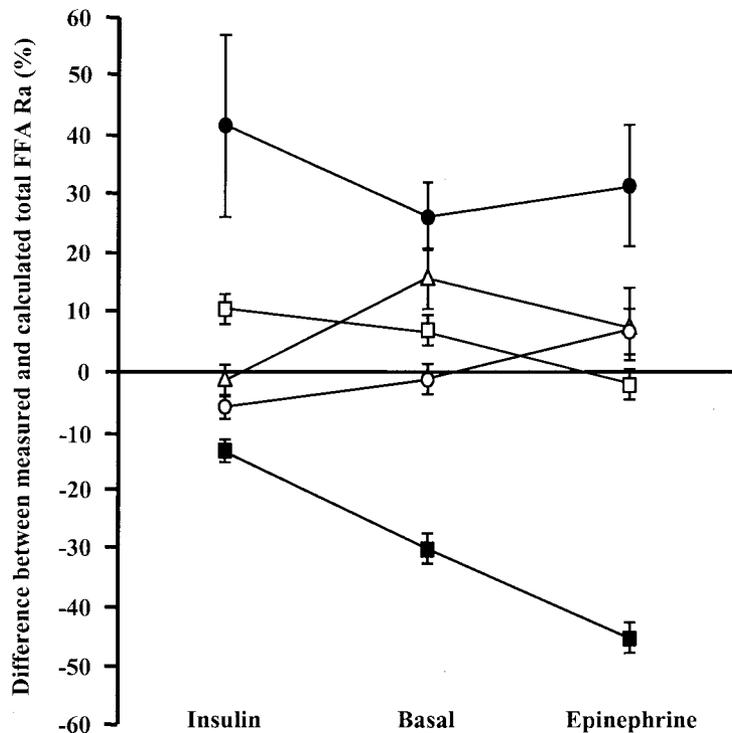
FIG. 3. Relative difference between calculated total FFA R_a, based on the use of a single fatty acid tracer, and measured total FFA R_a, determined as the sum of the R_a of five individual fatty acids ([calculated total FFA R_a − measured total FFA R_a]/measured total FFA R_a × 100), during basal conditions, epinephrine infusion, and insulin infusion. ●, myristate (C14:0); □, palmitate (C16:0); ■, stearate (C18:0); ○, oleate (C18:1); △, linoleate (C18:2). Values are means ± SE.

kinetics remained the same across the range of lipolytic rates. There was a direct relationship between fatty acid plasma clearance rate and the magnitude of the error in calculated total FFA R_a (Fig. 4).

The effects of epinephrine and insulin infusions on calculated total FFA R_a are shown in Fig. 5. The relative increase in calculated total FFA R_a above baseline during epinephrine infusion based on stearate tracer was lower than the other fatty acid tracers or the measured total FFA R_a value ($P < 0.05$). The relative decrease in calculated total FFA R_a based on stearate tracer kinetics was less than the other fatty acid tracers or the measured total FFA R_a value ($P < 0.05$).

DISCUSSION

The major aim of the present study was to evaluate the validity of using a single fatty acid tracer as an index of total plasma FFA kinetics. Our data demonstrate that calculating total FFA R_a by using palmitate, oleate, or linoleate tracers provides a reasonable estimate (within 15%) of the measured sum of individual FFA R_a values across a physiological range of adipose tissue TG lipolytic rates. In contrast, stearate and myristate tracers consistently underestimated and overestimated total FFA R_a, respectively. The magnitude of the error in tracing true total FFA kinetics was similar at all rates of lipolysis (~30%) when myristate was used as the tracer, whereas the magnitude of the error increased progressively (from ~15 to 45%) with increasing lipolytic rates when stearate was the tracer. These data support the common practice of using labeled palmitate or oleate as fatty acid tracers for assessing total plasma fatty acid kinetics.

The second aim of this study was to evaluate the relationship between the R_a of individual fatty acids in plasma and the fatty acid composition of adipose tissue TG. The fatty acid profile in subcutaneous adipose tissue

TG depots in our subjects was similar to values reported previously in human subjects (9,25). During epinephrine infusion, the relative increase in individual fatty acid R_a into plasma closely matched the relative fatty acid content of adipose tissue TG, suggesting that fatty acids released during β-adrenergic–mediated lipolysis are derived almost exclusively from adipose tissue TG. However, the fatty acid R_a profile during basal conditions and when lipolysis was inhibited by insulin infusion did not precisely match the fatty acid profile of adipose TG; relative palmitate and stearate R_a were higher and relative oleate R_a was lower than the proportional contribution of these fatty acids to adipose tissue TG. These data suggest that fatty acids are being released into the systemic circulation from a lipid source that contains palmitate and stearate in higher concentrations and oleate in lower concentrations than abdominal and gluteal subcutaneous adipose tissue TG. In addition, the alterations we observed in the relationship between the fatty acid R_a profile and the distribution of fatty acids in subcutaneous adipose tissue TG during conditions that stimulated and inhibited hormone-sensitive lipase-mediated lipolysis, suggest that the hormones that regulate lipolysis of adipose tissue TG do not regulate lipolysis of lipid present in this other depot. The kinetic data from the present study help explain the metabolic mechanism responsible for the discrepancy between plasma and adipose tissue fatty acid concentrations observed by Spitzer and colleagues (26,27) >35 years ago.

If a lipid pool is the explanation for our findings, it is unlikely that this source is another adipose tissue TG pool, such as visceral fat or other subcutaneous fat depots. Although statistically significant site-specific differences in TG fatty acid composition between various subcutaneous and intra-abdominal fat depots have been reported (28–30), the magnitude of these differences is extremely small (usually <2%) and, therefore, cannot account for the relative
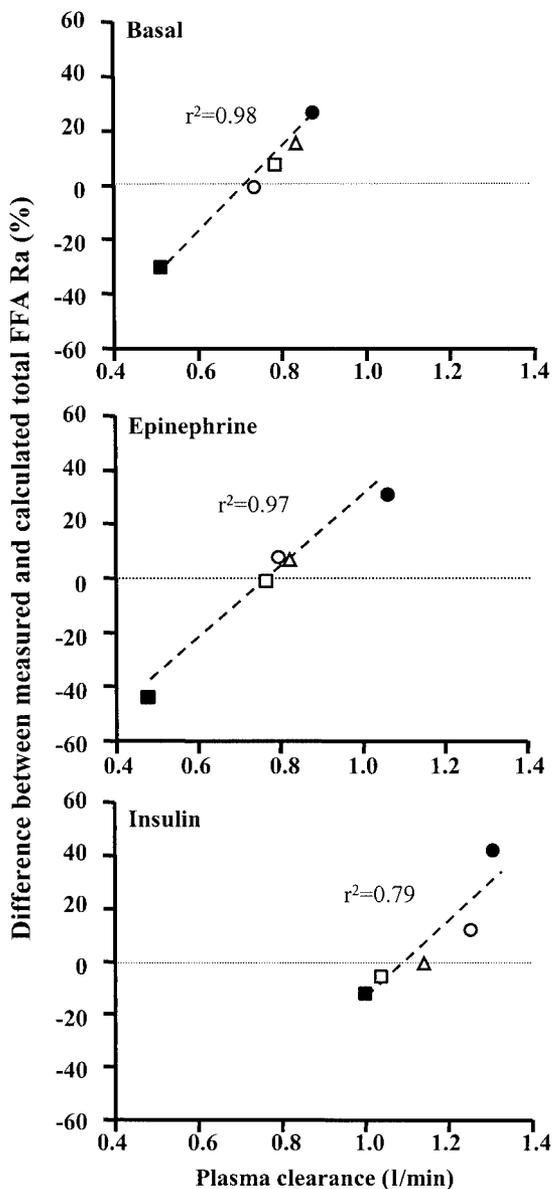
FIG. 4. Relationship between the plasma fatty acid clearance rates and the accuracy of estimating total FFA R_a (relative difference between calculated total FFA R_a, based on a single fatty acid tracer, and measured total FFA R_a, determined as the sum of the R_a of five individual fatty acid R_a values) during basal conditions and during infusions of epinephrine and insulin. ●, myristate (C14:0); □, palmitate (C16:0); ■, stearate (C18:0); ○, oleate (C18:1); △, linoleate (C18:2). Values are means.

mobilization rates we observed for palmitate, stearate, and oleate. It is also unlikely that fatty acids released from lipolysis of circulating TG affected our measured FFA R_a values. Although after a meal, fatty acids can escape into the circulation from lipoprotein lipase–induced hydrolysis of TG in chylomicrons (31), and only a small percent of total fatty acids released into the bloodstream are derived from lipoprotein lipase action on VLDL-TG during postabsorptive conditions (32). Moreover, the composition of fatty acids in plasma TG and in adipose tissue TG was similar in our subjects (data not shown).

We hypothesize that hydrolysis of membrane or circulating phospholipids could account for the FFA R_a profile observed in our subjects, because the relative amount of palmitate and, particularly, stearate is higher and the content of oleate is lower in phospholipids than in adipose tissue TG (33). Release or exchange of only a small portion of fatty acids present in membrane or circulating phospholipids could make a considerable contribution to FFA R_a because of the large body pool of phospholipids (34). However, we are not aware of any studies that have evaluated phospolipase activity in vivo and the contribution of phospholipid-derived fatty acids to total FFA kinetics.

The relative mobilization rates of fatty acids observed in our study appear to contradict the results of studies conducted in isolated adipocytes and in regional subcutaneous adipose tissue (7–14). These previous studies found that the mobilization of fatty acids from adipose tissue is selective, with preferential release of shorter than longer chain fatty acids and unsaturated than saturated fatty acids. This pattern of fatty acid mobilization was not observed in our study, probably because we measured whole-body plasma FFA R_a, which does not completely isolate the release of fatty acids from adipose tissue that is accomplished by in vitro adipocyte or in vivo arteriovenous balance studies.

The relative stimulation and inhibition of stearate release into plasma during epinephrine and insulin infusions differed from that of other fatty acids. The relative increase in stearate R_a during epinephrine infusion was less than the relative increase in the R_a of other fatty acids, which is consistent with data from an earlier study that found that exercise caused a greater increase in the turnover of oleate than stearate (35). We also found the relative decrease in stearate R_a during insulin infusion was less than the relative decrease in other fatty acids but are not aware of previous studies that investigated the effect of inhibiting lipolysis on the release of individual fatty acids. Therefore, in contrast with previous studies that concluded that there is a selective inhibition of adipose tissue stearate release (8,14), our data demonstrate that the release of stearate in response to lipolytic stimuli only appears to be blunted because of increased baseline stearate R_a values.

The ability of a single fatty acid tracer to accurately estimate measured total FFA R_a by dividing the individual fatty acid R_a by the relative contribution of that fatty acid to total plasma FFA concentration varied among individual fatty acids. For this approach to be valid, the relative concentration of a fatty acid in plasma must be directly proportional to its relative rate of release into plasma. Therefore, the reliability of estimating total FFA R_a by a single tracer depends on whether the plasma clearance rate of the "traced" fatty acid is similar to the clearance rate of other fatty acids, because clearance affects plasma FFA concentration. We found a strong correlation between the clearance rate of an individual fatty acid from plasma and the difference between calculated and measured total FFA R_a. Plasma stearate and myristate clearance rates were lower and higher, respectively, than the clearance rate of other fatty acids, and the use of stearate and myristate tracers underestimated and overestimated, respectively, measured total FFA R_a values. Data from earlier studies have shown that the fractional uptake of different fatty acids by forearm tissues was not different,
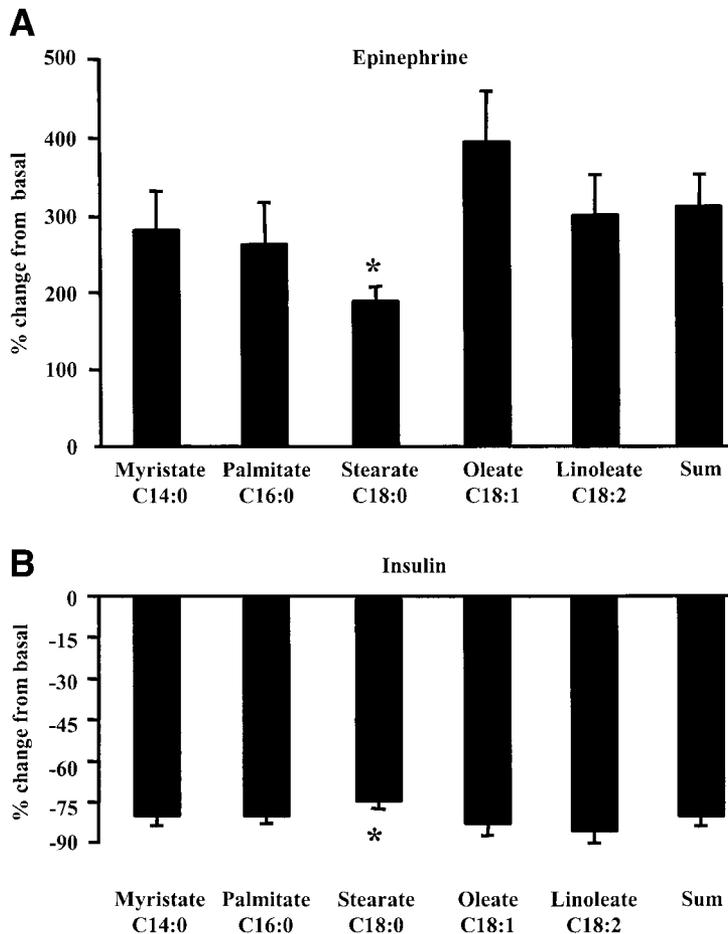
A

B

FIG. 5. Effect of epinephrine (A) and insulin (B) on calculated total FFA R_a, based on individual fatty acid tracers, and the sum of the measured R_a of individual fatty acids. *$P < 0.05$ vs. other fatty acids and the sum of fatty acids. Values are means ± SE.

whereas splanchnic myristate uptake was greater and splanchnic stearate uptake was smaller than the uptake of other fatty acids (35,36). In addition, when injected simultaneously into rats, ^{14}C-stearate disappeared more slowly from plasma than ^{3}H-palmitate (37). Differences in splanchnic clearance of individual fatty acids could explain, at least in part, the differences in whole-body clearance rates between different fatty acids observed in our study.

This is the first study to report measurements of myristate kinetics. Although the use of a myristate tracer did not provide a reliable measure of total FFA kinetics, the relative change in myristate R_a in response to epinephrine or insulin infusion was similar to that of most other long-chain fatty acids. Therefore some fatty acid tracers can be useful as a marker of lipolytic activity during different physiological conditions, even though they may not provide an accurate estimation of total FFA R_a.

In summary, the results of the present study demonstrate that palmitate, oleate, and linoleate tracers provide a reasonable estimate of total FFA R_a across a range of lipolytic rates in humans. However, small differences in the relative R_a of these fatty acids in response to conditions that stimulate or inhibit lipolysis make it important to use the same fatty acid tracer when comparing lipid kinetics during different physiological conditions. Total FFA R_a, determined by using isotope tracers, may not be a measure of lipolysis of adipose tissue TG alone, but could include fatty acids derived from an additional lipid source, possibly membrane phospholipids.

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