Free fatty acid turnover measured using ultralow doses of [U-^{13}C]palmitate

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Abstract Low intravenous infusion rates of [U-^{13}C]palmitate were used to measure systemic palmitate flux at rest (0.5 nmol · kg^{-1} · min^{-1}) and during exercise (2 nmol · kg^{-1} · min^{-1}) in healthy adults. The ^{13}C enrichment of plasma palmitate was determined by gas chromatography/combustion/isotope ratio mass spectrometry (GC/C/IRMS). Palmitate flux was also measured using palmitate radiotracers, which were infused simultaneously to serve as a reference method. Flux values obtained using the two different methods were virtually identical. Overnight postabsorptive palmitate flux was measured on four occasions in three individuals; the flux values were highly reproducible with a coefficient of variation of 11% and 12% for intra-day measurement, and 13% and 8% for inter-day measurement, for [1-^{14}C]palmitate and [U-^{13}C]palmitate, respectively. During exercise, palmitate turnover measured with [U-^{13}C]palmitate and [^{3}H]palmitate were well correlated (r = 0.91). Compared to conventional stable isotope approaches, (e.g., [1-^{13}C]palmitate analyzed by GC/MS), [U-^{13}C]palmitate reduces the tracer dose by two orders of magnitude when the samples are analyzed with GC/C/IRMS. Uniformly labeled [^{13}C]palmitate is suitable for the measurement of free fatty acid flux in humans.—**Guo, ZK., S. Nielsen, B. Burguera, and M. D. Jensen.** Free fatty acid turnover measured using ultralow doses of [U-^{13}C]palmitate. *J. Lipid Res.* 1997. **38:** 1888–1895.

Supplementary key words gas chromatography/combustion/isotope ratio mass spectrometry • [^{14}C]palmitate • [^{3}H]palmitate • exercise

Plasma free fatty acid (FFA) flux, or rate of appearance (R_a), was originally measured using radioactive (^{14}C- or ^{3}H-labeled) fatty acid tracers based on isotope dilution principles (1). The use of stable isotopes, such as [1-^{13}C]palmitate, to measure FFA flux is a more recent development (2). The absence of radiation exposure from stable isotopes has permitted more widespread application of FFA tracer techniques (3–5). However, stable isotopic fatty acid tracers must be used in large amounts in order to achieve sufficient levels of ^{13}C enrichment in plasma FFA for accurate determination by gas chromatography/mass spectrometry (GC/MS). For tracer studies in humans, infusion rates of at least 0.04 μmol · kg^{-1} · min^{-1} of [1-^{13}C]palmitate are needed (4, 5). Two factors explain the need to infuse large amounts of FFA tracers. First, FFA turnover is extremely rapid with a half line of ~3.5 min (6). Therefore, despite a small pool size, the tracer infusion rate must be high. Second, the precision of measuring the tracer mole % enrichment by organic GC/MS is relatively low, requiring tracer concentrations of >0.5% to assure reliable results. Because FFA are not water soluble, the tracers must be bound to albumin for infusion. A 1-h experiment may require as much as 10 g of albumin. The preparation of such tracers is time consuming, and large infusate volumes are required for prolonged experiments. Therefore, a stable FFA tracer that could be used in truly trace amounts would be useful.

The recent availability of GC/C/IRMS made it feasible to use smaller amounts of [1-^{13}C]palmitate because of the instrument's high sensitivity and low detection limit (2 × SD < 0.0005 atom %). Because the compounds are oxidized to CO_2 for the ^{13}C enrichment ($^{13}CO_2/^{12}CO_2$ ratio) measurement, the 1-^{13}C is diluted by the unlabeled carbons, therefore the high sensitivity of isotope ratio mass spectrometry (IRMS) system is not optimally utilized. This problem could be addressed by using a uniformly ^{13}C-labeled fatty acid tracer to eliminate the intramolecular isotope dilution that occurs during the combustion process. This approach should allow the tracer infusion rate to be reduced accordingly.

Background (naturally occurring isotopes) correction is an essential step in the analysis of data obtained from stable isotope tracer studies. The "background" isotopic enrichment present in a pre-study sample is subtracted from that observed in the samples collected during the tracer infusion. This practice assumes that

Abbreviations: GC/C/IRMS, gas chromatography/combustion/isotope ratio mass spectrometry; GC/MS, gas chromatography/mass spectrometry; FFA, free fatty acids; GCRC, General Clinical Research Center; SA, specific activity; HPLC, high performance liquid chromatography; R_a, rate of appearance; APE, atom percent excess; CV, coefficient of variation; S/N, signal/noise.

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all the subsequent samples have the same background enrichment as the baseline sample and works well with GC/MS. With the highly sensitive IRMS, errors may be introduced if the background ^{13}C of the FFA samples collected during the tracer infusion varies significantly from the baseline sample. This is especially a concern with small dose of tracer.

In present studies, we have examined the feasibility of using [U-^{13}C]palmitate as a tracer for the measurement of systemic palmitate flux in humans. We have also assessed the accuracy of this approach by comparing the results with those obtained using radiotracer techniques that are known to be quantitatively accurate (7, 8). The potential problem of significant variations in background ^{13}C in FFA was also evaluated.

MATERIALS

[U-^{13}C]palmitate was purchased from Isotec (99% atom %, Miamisburg, OH), [1-^{14}C]palmitate and [9,10-^{3}H]palmitate were from Amersham Corporation (Arlington Heights, IL); 25% human serum albumin from Bayer Corporation (Elkhart, IN), 0.22 μm membrane filters from Millipore (Bedford, MA), [$^{2}H_{31}$]palmitate and unlabeled palmitate from Sigma Chemical Co. (St. Louis, MO), acetyl chloride/methanol derivatization kit from Alltech (Deerfield, IL).

METHODS

Protocols

Five healthy adults (three males and two females) were included in three protocols (**Table 1**). Informed written consent was obtained from all the subjects. The subjects underwent a general physical examination and laboratory evaluation to exclude illnesses and pregnancy.

Protocol I (natural ^{13}C background enrichment). One woman participated in this protocol. She consumed an isocaloric diet at the Mayo Clinic General Clinical Research Center (GCRC) for 2 days prior to the study. After an overnight fast, an intravenous catheter was inserted into a dorsal hand vein in retrograde mode and the hand was placed in a warming box (70° C) in order to obtain arterialized venous blood samples (9). A second intravenous catheter was placed in a forearm vein and kept patent with an infusion of 0.45% NaCl at 30 ml/h. Four basal blood samples were collected at 10-min intervals. The subject consumed a glucose meal (1 g · kg^{-1} body wt) and six blood samples were collected at 30-min intervals during next 4.5 h. At the end of this period, a constant epinephrine infusion (10 ng · kg^{-1} · min^{-1}) was started via the forearm vein catheter and continued for 90 min and blood samples were collected every 15 min. Plasma was separated by cold centrifugation and stored at −80°C for later analysis.

TABLE 1. Characteristics of the subjects participating in the three protocols

Subject #	Sex	Body Weight	Protocol
		kg	
1	F	59	I
2	M	92	II
3	M	71	II
4	F	64	II
5	M	71	III

Protocol II (steady state palmitate flux). Two men and one woman participated in this protocol. They consumed all of their meals in the GCRC throughout the study and were admitted to the GCRC the night prior to each FFA turnover study. Intravenous catheters for infusions and blood sampling were placed as described in Protocol I. After the collection of a baseline blood sample, non-primed, constant infusions of both [1-^{14}C]palmitate and [U-^{13}C]palmitate were started simultaneously. After 30 min of tracer infusion to assure isotopic equilibration, blood samples were collected at 10-min intervals for 30 min and processed as described in Protocol I. The tracer infusions were stopped immediately after the last blood sample was collected. The same procedures were repeated on 4 consecutive days for each subject.

Protocol III (palmitate flux during exercise). One man participated in this protocol. The evening prior to the study, an intravenous catheter was introduced into a forearm vein for isotope infusions. The next morning a radial artery catheter was placed under local anesthesia for blood sampling during exercise. A blood sample for background purposes and then infusions of [U-^{13}C]palmitate and [9,10-^{3}H]palmitate were started. After 30 min a series of four baseline blood samples were drawn at 10-min intervals. After the baseline period the subject exercised on an bicycle ergometer at a Vo_2 of ~28 ml · kg^{-1} · min^{-1} for 150 min. Blood samples were collected at 30, 60, 90, 120, 130, 140, and 150 min and processed as described above. The tracer infusions were stopped after the last blood sample was collected.

Tracer preparation and infusion

[U-^{13}C]palmitate. The isotopic purity of [U-^{13}C]palmitate was confirmed by GC/MS to be 99%. The tracer was bound to human albumin using the traditional conversion to a potassium salt, but could also be directly bound to albumin.

[U-^{13}C]palmitic acid was converted to potassium salt and then bound to albumin according to the procedures described by Wolfe (10). Briefly, under sterile conditions, the tracer was dissolved in warmed hexane after which 3% mole excess of 0.1 M KOH in 80% methanol was added. The solution was stirred until thoroughly mixed and then evaporated to dryness under a stream of filtered N_2. A small amount of sterile water and albumin (60° C) was slowly added and stirred until the solution was clear (no particulate matter). The tracer/albumin ratio was 5–10 mg of tracer per g of albumin. The tracer–albumin solution was diluted with sterile 0.9% saline to a concentration of 1–2 mg tracer · ml^{-1} and passed through 0.22-μm membrane filters into sterile vials to be used as a stock solution.

The [U-^{13}C]palmitate could also be bound to human albumin without conversion to the potassium salt by dissolving the desired amount in small volume of chloroform and evaporating the solution to dryness under a stream of filtered N_2. Human serum albumin (0.5–1 mg of tracer per g of albumin) was added and stirred on a heated plate set at low for 30 min or until the solution looked clear. An equal volume of sterile 0.9% saline was added, mixed, and filtered into a sterile vial as described above.

Before use, the stock solutions were diluted with sterile saline to appropriate concentrations according to individuals' body weight. The [U-^{13}C]palmitate infusion rates for Protocol II and III were 0.5 nmol · kg^{-1} · min^{-1} and 2 nmol · kg^{-1} · min^{-1}, respectively. The exact infusion rates were confirmed using the volumetric infusion rate and the tracer concentration in the infusate for each subject.

Radiotracers. The radiochemical purity of [1-^{14}C]palmitate (98%) and [9,10-^{3}H]palmitate (96%) was tested by HPLC (7). The radiotracers were bound to albumin directly, diluted with sterile saline, and the solution was filtered through a 0.22-μm filter. The infusion rates were 0.3 μCi · min^{-1} for [1-^{14}C]palmitate and 0.6 μCi · min^{-1} for [9,10-^{3}H]palmitate. Quadruplicate aliquots of the radiotracer infusates were assayed using liquid scintillation counting to determine the exact infusion rates, which were corrected for isotope purity. All infusates were assured to be sterile and pyrogen free before use.

Assays

Plasma palmitate specific activity (SA) determination. Plasma FFA were extracted by Dole's method (11) and then derivatized to bromophenacyl derivatives (7) prior to assay by HPLC. The bromophenacyl palmitate peak was collected, the solvent was air blow-dried, and the palmitate derivative residue was counted on a liquid scintillation counter. The palmitate content of the plasma sample was quantitated using a calibration curve of palmitate standards for both concentration and total palmitate (nmol) (7).

Processing of plasma palmitate for ^{13}C enrichment determination. An aliquot of the collection of the bromophenacyl-palmitate peak from the same HPLC run was air-blow-dried. The bromophenacyl-palmitate residue was then dissolved in 100 μl of anhydrous benzene, to which 0.5 ml of 6% methanolic HCl was added, and set on a heat block at 100°C for 1 h. The resulting palmitate methyl ester was extracted with 2 ml of hexane and 1 ml of water. The palmitate methyl ester was analyzed on GC/C/IRMS. We found that the direct transmethylation process produced a major artifact eluting about 2 min before the palmitate methyl ester, which had the potential to interfere with the analysis of palmitate if large amounts of sample were injected. As an alternative, the extracted plasma total lipids were run on HPLC (12) to separate triglycerides from FFA. The FFA fraction was derivatized to methyl esters using 0.5 ml of 5% methanolic HCl at 70° C for 1 h and extracted as above. No artifact was present in such prepared palmitate methyl ester samples.

GC/C/IRMS operation

The GC/C/IRMS consisted of a Hewlett-Packard 5890 GC and a Finnigan MAT delta S isotope ratio was spectrometer (IRMS) (Finnigan MAT, San Jose, CA). A ceramic microcombustion oxidizer (maintained at 960°C) interfaces between the GC and the IRMS. The GC was equipped with a DB-1 column (60 m × 0.35 mm i.d. × 0.25 μm film, J&W Scientific, Folsom, CA). The oven temperature was programmed as: initial temperature 150°C, increased to 190°C at 5°C · min^{-1}, then increased to 200°C at 1°C · min^{-1} and stayed for 2 min. Finally it was increased to 290°C at 35°C · min^{-1} and stayed for 4 min. The injector and the flame ionization detector were both at 250°C. The injections of palmitate samples (in triplicate) were made by an autosampler controlled by the ISODAT software (Finnigan MAT). Each injection introduced 1 μl of samples (25–50 ng of palmitate in iso-octane) into the column in splitless mode. Helium was the carrier gas at 1 ml · min^{-1} (head pressure 20 psi). For each sample injection, two injections of CO_2 reference gas (Matheson Gas Product, Parsippany, NJ) were made through the inlet system of the IRMS before and after the palmitate peak. The data acquisition was accomplished on-line by the ISODAT software and the data were analyzed off-line. Prior to ^{13}C analysis of plasma palmitate samples, the GC/C/IRMS was calibrated with a series of palmitate standards containing unlabeled palmitate and different concentrations of [U-^{13}C]palmitate ranging from 0.01 to 0.20 mole %. Regression analysis was performed on the observed ^{13}C enrichment versus the actual ^{13}C enrichment

of the palmitate standards. The slope was used to correct the raw ^{13}C enrichment data of plasma palmitate samples.

Calculations

For both rest and exercise data, palmitate R_a was calculated using a steady state formula because when plasma fatty acid R_a is increasing, as seen in Protocol III, the steady state formula is sufficiently accurate (7, 8). The same formula was used for both stable and radioactive tracers. For radiotracer data, following equation was used:

$$\text{Systemic palmitate } R_a\ (\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}) = \text{infusion rate of } [^{14}C]\text{palmitate or } [^{3}H]\text{-palmitate (dpm} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}) / \text{plasma palmitate SA (dpm} \cdot \mu\text{mol}^{-1}). \quad \textit{Eq. 1)}$$

For [U-^{13}C]palmitate data, the observed $\delta^{13}C$ values (‰) were transformed to atom % (AP) using the following equation:

$$AP = (100 \times 0.011237\ (\delta\ ^{13}C/1000 + 1)) / (1 + 0.011237(\delta\ ^{13}C/1000 + 1)). \quad \textit{Eq. 2)}$$

0.011237 is the absolute ^{13}C enrichment of Pee Dee Belemnite (PDB) stone, an international standard for ^{13}C measurement.

Then, atom % excess (APE) is calculated using the following equation:

$$APE = AP - AP_{bl} \quad \textit{Eq. 3)}$$

where AP_{bl} is the AP of baseline plasma palmitate. Palmitate R_a was calculated as following:

$$\text{Palmitate } R_a\ (\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}) = [\text{U-}^{13}C]\text{palmitate infusion rate } (\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}) / \text{plasma palmitate APE.} \quad \textit{Eq. 4)}$$

Because the infusion rates of [U-^{13}C]palmitate were very low (0.5 nmol · kg^{-1} · min^{-1} for Protocol II and 2.0 nmol · kg^{-1} · min^{-1} for Protocol III) they were not included in the equation.

Statistics

Paired *t*-test was used when comparisons were made between the two means of the two tracer methods. When the comparisons were among more than two means, *F*-test (ANOVA) was used.

RESULTS

^{13}C background of human plasma FFA

Figure 1 shows the natural ^{13}C abundance and concentration of plasma palmitate from subject #1. Despite the wide range of palmitate concentrations observed (from 11 to 281 μmol/L), [^{13}C]palmitate background fluctuated only within a narrow range of ~2‰ $\delta\ ^{13}C$ (0.0020 AP) with an average deviation from the mean of 0.6‰ $\delta\ ^{13}C$ (0.0006 AP). This variation occurred primarily when palmitate concentrations were suppressed after glucose ingestion. During the epinephrine infusion, the ^{13}C background was stable.

For comparison purposes the ^{13}C background of oleate and stearate from the same samples are also depicted in the inset of Fig 1. The natural ^{13}C enrichment of palmitate was similar to that of oleate and stearate in the same samples (1.0844 ± 0.0012, 1.0843 ± 0.0006 and 1.0836 ± 0.0023 AP, mean ± SD) with a similar pattern of changes in the enrichment (inset of Fig 1). A strong correlation was noted between the ^{13}C background in palmitate and oleate ($r = 0.70$) and palmitate and stearate ($r = 0.99$).

Steady state palmitate flux (Protocol II)

As expected, relatively stable plasma palmitate SA and enrichment was observed in these subjects at rest after an overnight fast. The systemic palmitate flux values measured by the two methods were comparable within the same day and as the 4-day averages for each subject (**Table 2**). For the 4-day averages, the palmitate flux measured using [U-^{13}C]palmitate was 0.13 and 0.03 μmol · kg^{-1} · min^{-1} greater for subjects #2 and #4, respectively, and 0.08 μmol · kg^{-1} · min^{-1} less for subject #3 than those measured using [1-^{14}C]palmitate. Palmitate flux values not significantly different between the two methods ($P = 0.50$). Both methods provided highly reproducible measures of resting palmitate flux as indicated by the intra-day coefficient of variation (CV) of 11% [1-^{14}C]palmitate and 12% for [U-^{13}C]palmitate and inter-day CV of 13% for [1-^{14}C]palmitate and 8% for [U-^{13}C]palmitate.

To examine whether the small differences in palmitate flux as measured by the two methods were related to the level of systemic palmitate flux, the between-method differences in palmitate flux were plotted against the flux measured using [1-^{14}C]palmitate (**Fig. 2**). It is apparent that the differences are scattered within similar distances from the zero line without a consistent distribution pattern. This suggests that the differences in palmitate flux measured by the two methods were random and independent of palmitate kinetics.

Dynamic palmitate R_a (Protocol III)

Protocol III is very different from Protocol II in that the duration was longer (3.5 h vs. 1 h) and the plasma fatty acid kinetics changed dramatically (several fold rapid increase) during the exercise. Therefore, it is use-

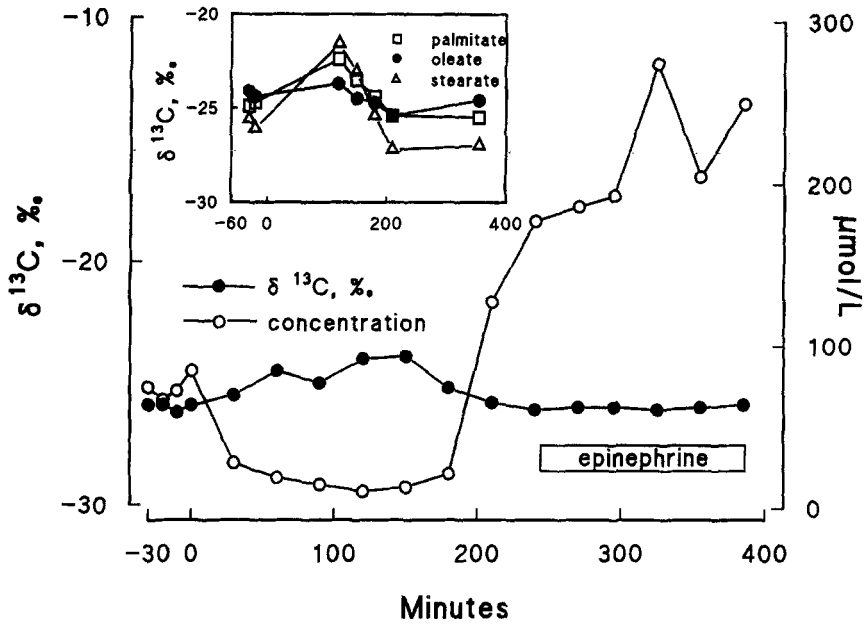

Fig. 1. Plasma palmitate concentrations and δ ^{13}C, ‰ are depicted for subject I, Protocol I. The volunteer consumed glucose at time zero and received an intravenous epinephrine infusion during the interval indicated. The inset displays δ ^{13}C, ‰ values for palmitate, oleate, and stearate measured in the same plasma samples at relevant time points.

ful as a model to evaluate the sensitivity and accuracy of [U-^{13}C]palmitate to measure dynamic changes in FFA R_a. **Figure 3** shows the systemic palmitate R_a in subject #5 before and during 150 min of exercise. Basal palmitate R_a measured by the stable tracer method and [9,10-^{3}H]palmitate were virtually identical. During the exercise, palmitate R_a measured using [U-^{13}C]palmitate reached the plateau in parallel to that measured using [^{3}H]palmitate.

DISCUSSION

These are the first studies to use [U-^{13}C]palmitate to measure plasma FFA turnover. Palmitate flux values obtained using [U-^{13}C]palmitate were closely comparable to those obtained using the [1-^{14}C]palmitate (Table 2) or [^{3}H]palmitate (Fig. 3), indicating that the stable isotopic tracer method is accurate. The difference in flux values between the two methods averaged 1–10%, which, on an individual measurement basis, is as good as the agreement between chemically measured and tracer-predicted fatty acid inflow rates (7,8), and between [^{3}H]- and [1-^{14}C]palmitate-measured FFA flux (6). The intra-day and inter-day variations in palmitate flux values were low and similar for both methods, and likely represent primarily biologic, as opposed to methodologic, variability. The average intra-day measurement variations were almost identical for the two tracer methods, indicating acceptable and similar reproducibility of both methods. The exercise study confirmed that the stable tracer method was sensitive to dynamic changes in plasma FFA R_a and could quantitate these changes accurately. These results suggest that [U-^{13}C]palmitate is a reliable tracer at very low infusion rates and, given the availability of GC/C/IRMS, can provide results comparable to those obtained using radiotracer methods without exposure to ionizing radiation.

Two factors allowed a marked reduction in the tracer infusion rate for these studies. The use of GC/C/IRMS, which lowers the detection limit by an order of magnitude compared with GC/MS (13), is a significant advance in instrumentation for stable isotope studies. The second factor is the elimination of the intramolecular dilution of ^{13}C during the combustion process by administering a uniformly labeled fatty acid tracer in place of a singly labeled tracer.

The precision of ^{13}C analysis was not compromised by the small doses of [U-^{13}C]palmitate used. The plasma ^{13}C palmitate enrichment was typically 0.03–0.04 APE at the infusion rates used in Protocol II (0.5 mg · h^{-1}) and 0.03–0.1 APE with the infusion rate used in Protocol III (2 mg · h^{-1}). Because the IRMS system has a standard deviation of < 0.0003 atom %, the observed APE values ensured a signal/noise (S/N) ratio of 100–300.

TABLE 2. Systemic palmitate flux of subjects in Protocol II

Subject	Day	[1-^{14}C]Palmitate Mean	[1-^{14}C]Palmitate SD	[U-^{13}C]Palmitate Mean	[U-^{13}C]Palmitate SD
2	1	1.13	0.13	1.26	0.13
	2	1.25	0.07	1.32	0.11
	3	0.90	0.13	1.09	0.22
	4	1.08	0.22	1.22	0.24
	Mean	1.09	0.13	1.22	0.17
	SD	0.14		0.10	
3	1	1.63	0.11	1.38	0.06
	2	1.45	0.05	1.61	0.21
	3	1.58	0.25	1.41	0.08
	4	1.25	0.06	1.18	0.22
	Mean	1.48	0.11	1.40	0.14
	SD	0.17		0.17	
4	1	1.47	0.16	1.56	0.03
	2	1.39	0.16	1.58	0.19
	3	1.42	0.22	1.48	0.16
	4	1.78	0.34	1.55	0.28
	Mean	1.51	0.22	1.54	0.16
	SD	0.19		0.05	
Average	Mean	1.36	0.15	1.39	0.16
	SD	0.17		0.11	

Values are μmol · kg^{-1} · min^{-1}. The mean and SD data from the four samples obtained on each study day (intra-day variation) and mean flux values between study days (inter-day variation) are provided. The intra-day variation is indicated by the SD that is in the same row as the mean value, and the inter-day variation is indicated by the SD that is in the same column as the mean value (under the mean value).

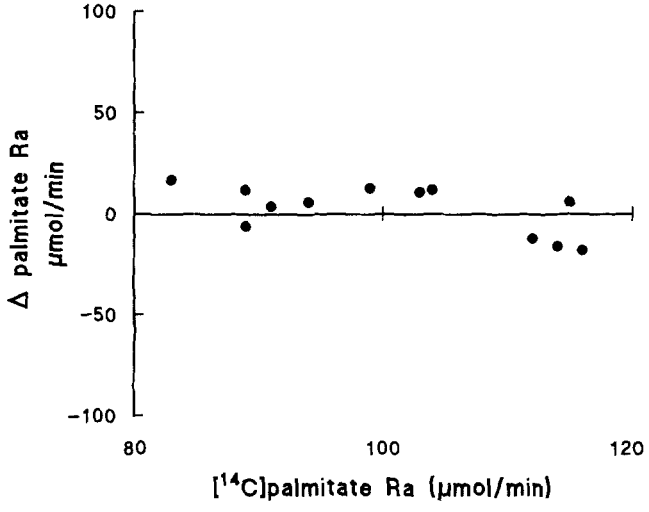

Fig. 2. The difference between palmitate rate of appearance (R_a) measured using [1-^{14}C]palmitate and [U-^{13}C]palmitate is plotted versus palmitate flux measured using [1-^{14}C]palmitate.

Recently, GC/C/IRMS has been recognized for its superior sensitivity and potential for use in human metabolic studies. Metges, Kempe, and Wolfram (14) used GC/C/IRMS to measure the ^{13}C enrichment of fatty acids present in serum lipid fractions after the ingestion of [$^{13}C_1$]oleate-labeled triglycerides. Binnert et al. (15) infused [1-^{13}C]palmitate and used GC/C/IRMS to measure plasma palmitate turnover and the palmitate enrichment in plasma triglycerides. Goodman and Brenna (16) were the first to use uniformly ^{13}C-labeled fatty acids (given by mouth) combined with GC/C/IRMS analysis in a study of lipoprotein triglyceride metabolism. Our studies are the first to compare this approach with methods tested for their quantitative accuracy (7, 8).

The amount of albumin required for FFA tracer studies is proportional to the tracer dose to be infused. For traditional [1-^{13}C]palmitate studies in adult humans, at least 4 g per h of albumin is required based on the maximum fatty acid binding capacity of human albumin (non-fatty acid free) of 10 mg · g^{-1} (3). In our experience, this binding capacity often cannot be realized and substantially greater amounts of albumin are needed for singly labeled FFA tracers. The amount of albumin required for the present [U-^{13}C]palmitate experiments was reduced to 50–100 mg · h^{-1}. The reduced tracer doses made it possible to prepare tracer–albumin complex directly, without converting palmitate to its potassium salt. Thus, the tracer preparation was easy and rapid, even for studies in which highly lipolytic conditions (exercise) were anticipated. Despite the increased expense of [U-^{13}C]palmitate, the reduced doses required and the reduced amount of albumin needed for studies should provide an overall cost and convenience advantage.

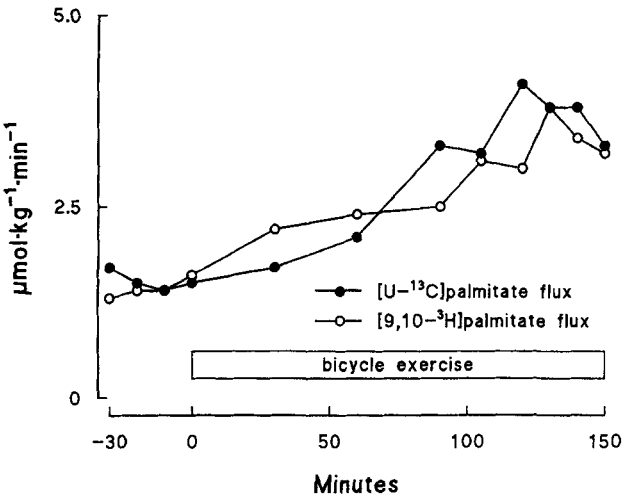

Fig. 3. Palmitate flux measured before and during exercise using both [^{3}H]palmitate and [U-^{13}C]palmitate for the subject participating in Protocol III is depicted.

Isotope discrimination, if present, would cause underestimation of plasma FFA flux as a result of reduced tracer clearance. This is a special concern for [U-^{13}C]palmitate FFA turnover studies because of its greater mass (6%). The lack of consistent and significant differences between [1-^{14}C]palmitate and [U-^{13}C]palmitate measured flux in Protocol II indicates that isotope effects are unlikely during brief experiments. In addition, the similarity of palmitate flux values observed using [U-^{13}C]palmitate and [^{13}H]palmitate during exercise suggests that even with elevated FFA flux rates and during longer experiments uniformly labeled FFA are cleared in a manner similar to traditional radiotracers.

^{13}C background correction. The variation observed in natural ^{13}C abundance of plasma palmitate (Protocol I) indicates that, even with the small tracer dose used in Protocol II, changes in ^{13}C background would contribute little to the variance in the ^{13}C APE measurement of plasma palmitate. A S/N ratio of greater than 50 is easily obtained. With larger doses such as that used in Protocol III, the S/N ratio was nearly 150. The degree of variation in ^{13}C background of plasma palmitate observed in Protocol I is comparable to that reported for human plasma triglyceride fatty acids (14, 16). If, however, the ^{13}C enrichment of plasma FFA samples is low, for example, an APE of 0.01 (S/N ratio < 20), natural variations in ^{13}C background could introduce significant error. One approach to minimize this error is to use non-traced plasma FFA to predict the change in ^{13}C background enrichment of the traced FFA.

We observed that the ^{13}C backgrounds of the different FFA are similar to or parallel to each other over time (see inset of Fig. 1). We have noted similar relationships and parallel patterns in plasma palmitoleate, oleate, and linoleate in the plasma FFA samples from other subjects (Z. K. Guo and M. D. Jensen, unpublished data). Thus, the differences in the natural ^{13}C enrichment of major human plasma FFA are usually small and/or correlated. It may, therefore, be possible to use the non-traced plasma FFA to correct for changes in the ^{13}C background of the traced FFA if very low tracer enrichments are achieved. More studies are needed to test the validity of this approach for use in stable isotopic tracer studies of FFA metabolism.

In summary, GC/C/IRMS has been used to analyze plasma samples obtained during FFA turnover studies using a continuous infusion of [U-^{13}C] palmitate. This approach provides FFA flux values that compare favorably with established radiotracer methods. Substantially reduced amounts of the stable isotopic tracer can be administered without sacrificing the precision of the measurement. The reduced tracer dose greatly simplifies the preparation process for stable tracer studies and allows a reduction in the tracer infusate volume such that studies can be conducted under a greater variety of conditions.

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