

Validation of deuterium-labeled fatty acids for the measurement of dietary fat oxidation during physical activity

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Abstract Measurement of ¹³C-labeled fatty acid oxidation is hindered by the need for acetate correction, measurement of the rate of CO₂ production in a controlled environment, and frequent collection of breath samples. The use of deuterium-labeled fatty acids may overcome these limitations. Herein, d₃₁-palmitate was validated against [1-¹³C]palmitate during exercise. Thirteen subjects with body mass index of 22.9 ± 3 kg/m² and body fat of 19.6 ± 11% were subjected to 2 or 4 h of exercise at 25% maximum volume oxygen consumption (VO_{2max}). The d₃₁-palmitate and [1-¹³C]palmitate were given orally in a liquid meal at breakfast. The d₃-acetate and [1-¹³C]acetate were given during another visit for acetate sequestration correction. Recovery of d₃₁-palmitate in urine at 9 h after dose was compared with [1-¹³C]palmitate recovery in breath. Cumulative recovery of d₃₁-palmitate was 10.6 ± 3% and that of [1-¹³C]palmitate was 5.6 ± 2%. The d₃-acetate and [1-¹³C]acetate recoveries were 85 ± 4% and 54 ± 4%, respectively. When [1-¹³C]acetate recovery was used to correct ¹³C data, the average recovery differences were 0.4 ± 3%. Uncorrected d₃₁-palmitate and acetate-corrected [1-¹³C]palmitate were well correlated ($y = 0.96x + 0$; $P < 0.0001$) when used to measure fatty acid oxidation during exercise. **Thus, d₃₁-palmitate can be used in outpatient settings as it eliminates the need for acetate correction and frequent sampling.**—Raman, A., S. Blanc, A. Adams, and D. A. Schoeller. **Validation of deuterium-labeled fatty acids for the measurement of dietary fat oxidation during physical activity.** *J. Lipid Res.* 2004. 45: 2339–2344.

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Stable isotopes have been used to quantify plasma and dietary fat oxidation in the past (1–3). When a ¹³C-labeled fatty acid is dosed orally or by constant infusion, the ¹³CO₂ in breath can be used to measure the tracer oxidized. The

¹³C liberated during oxidative metabolism, however, is partly sequestered in the intermediates of the tricarboxylic acid (TCA) cycle (~40%) and the bicarbonate pool (10%), causing fat oxidation to be underestimated. To correct for this sequestration, an additional dose of [1-¹³C]acetate is administered. Acetate is converted to acetyl-CoA and is oxidized in the TCA cycle (4). Unfortunately, sequestration is variable and depends on the conditions under which it is measured; hence, estimation of acetate sequestration is essential (5). In addition, the use of ¹³C-labeled fatty acid is further constrained by the need for frequent sampling of breath and the use of a metabolic cart or respiratory chamber to quantify the flux of CO₂ to calculate ¹³C recovery accurately. These factors increase subject burden; thus, an alternative method to quantify fat oxidation in the body would be useful.

One such method for the measurement of fat oxidation is the use of deuterium-labeled fatty acids (3, 6). When oxidized, ²H-labeled fatty acid is metabolized to acetyl-CoA, releasing NADH molecules. The ²H label is released as water, in part, when NADH molecules are oxidized in the respiratory chain. Oxidation of acetyl-CoA in the TCA cycle releases the rest of the deuterium label in the form of ²H-labeled water. This ²H₂O mixes with the body water and can be sampled in the urine (7). Urinary and insensible water losses are minimal (8); hence, the enrichment of label in urine can be used effectively to calculate the cumulative recovery of the label and hence the fat oxidized. Consequently, the need for measurement of CO₂ and flux is also eliminated.

Deuterium-labeled palmitic acid has been validated against acetate-corrected ¹³C-labeled palmitic acid during rest in humans by Votruba, Zeddun, and Schoeller (6), who dosed subjects at rest with ¹³C- and d₃₁-labeled palmitic acid and demonstrated that the cumulative recoveries for

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both tracers were highly correlated ($y = 1.045x - 0.47$; $r^2 = 0.88$; $P < 0.0002$). More importantly, the mean difference in percentage recovery of the labels was $0.5 \pm 2.8\%$ when ^{13}C data were corrected for acetate fixation. This method raises interesting possibilities for use under free-living conditions; however, this method has not been validated under nonresting conditions. Herein, we compared the metabolic fate of orally ingested d_{31} -palmitate and $[1-^{13}\text{C}]$ palmitate during physical exercise of varying durations at moderate intensity. The objective was to ensure the validity of ^2H -labeled fatty acids as an accurate tool to measure dietary fat oxidation under a range of free-living conditions.

METHODS

Materials

Labeled fatty acids were obtained from Cambridge Isotope Laboratories (Andover, MA). $[1-^{13}\text{C}]$ palmitic acid and Na salt of $[1-^{13}\text{C}]$ acetate were 99 atom% ^{13}C . The d_{31} -palmitic acid was 98 atom%, and Na salt of d_3 -acetate was 99 atom% ^2H . The ^{18}O was obtained as water (Isotec, Inc., Miamisburg, OH) and was 10.8 atom%.

Subjects

Healthy volunteers (six males and six females) were recruited, and their characteristics are summarized in **Table 1**. Two additional subjects were recruited for the measurement of natural isotope abundances and daily natural variability of isotopes in the body (body mass index = $22.8 \pm 3.2 \text{ kg/m}^2$; mean \pm SD). The experimental protocol was thoroughly explained to the subjects before their recruitment into the study, and a signed informed consent was obtained from each of the subjects. The protocol was approved by the Institutional Review Board at the University of Wisconsin, Madison. Subjects completed a Physical Activity Readiness Questionnaire (9) to screen out those at risk during exercise. The physical stress test [maximum volume oxygen consumption ($\text{VO}_{2\text{max}}$)] was used to determine their maximal oxygen-consuming capacities, and persons with abnormal cardiac output during this test were excluded from the study. Exclusion criteria included pregnancy; metabolic diseases such as diabetes, thyroid dysfunction, kidney malfunction, and cardiovascular diseases; and subjects who were advised by their physicians to not undertake any physical activity.

Protocol

The study included two visits 3–4 weeks apart. The protocol for both visits was similar for a given subject except for the tracers given. The subject reported to the General Clinical Research Center GCRC at 6:00 PM on the evening before the test. During this day, the subjects were allowed to engage in normal daily ac-

tivities, with the exception of any vigorous activities or structured exercise. At 7:00 PM, they were asked to void, and a urine sample was stored to determine the basal body enrichment in H_2^{18}O . After being weighed, a 0.4 g/kg estimated total body water dose of 10% H_2^{18}O was given to the subjects. Dinner (a standard meal) was provided at 6:00 PM. At 11:00 AM, another urine sample was collected for the determination of the total body water through H_2^{18}O enrichment of bodily fluids at isotopic equilibration, achieved 4 h after dose.

The 24 h energy requirements of the subjects were calculated from the World Health Organization (WHO) equation (10) and were divided into 40% at dinner, 30% at breakfast, and 30% at lunch. An activity factor of 1.5 for the previous day and 1.7 for the test day were added to the WHO calculated energy expenditure. The macronutrient distribution of each standard meal consisted of 50% carbohydrates, 35% fat, and 15% protein.

On the day of the test, subjects were awakened at 6:30 AM, and a urine sample was collected at 6:45 AM. Urine and breath samples were collected at 8:00 AM to measure the natural abundance of ^2H in the body water and the natural $^{13}\text{CO}_2$ abundance in breath, respectively. The first visit consisted of an oral load of $[1-^{13}\text{C}]$ acetate at 2 mg/kg body weight and d_3 -acetate at 10 mg/kg body weight mixed in a liquid replacement meal (Boost high protein; Mead Johnson Nutritionals) during breakfast. The second stay consisted of an oral load of $[1-^{13}\text{C}]$ palmitate at 10 mg/kg body weight and d_{31} -palmitate at 15 mg/kg body weight, mixed in the same liquid replacement meal during breakfast.

Subjects exercised at 10:00 AM for 2 h (2 hr-eEx) or 4 h at a light intensity (25% $\text{VO}_{2\text{max}}$) on a cycle ergometer. Within the 4 h exercise group ($n = 8$), three subjects started exercise at 10:00 AM [45 min after dose; early exercise (4 hr-eEx)] and five subjects started exercise at 1:00 PM [3 h, 45 min after dose; late exercise (4 hr-lEx)].

Measurements of CO_2 flux were taken for 20 min every hour. Respiratory gas exchange (RGE) was measured for 20 min every hour during the rest of the stay including at rest, during exercise, and after exercise using a Deltatrac I metabolic cart (Sensormedics). The O_2 and CO_2 analyzers were calibrated with a standard gas containing a 96% O_2 and 4% CO_2 mixture. The subjects breathed through a mouthpiece with their noses clipped to ensure complete respiratory gas monitoring. The valve is connected to a canopy system, which acts as a mixing chamber, and is exhausted into the Deltatrac system to measure the rate of oxygen consumption and carbon dioxide production. The first 5 min of measurement was excluded, and the hourly RGE was calculated assuming that the subsequent 15 min measurements per hour were representative of the whole hour.

$[1-^{13}\text{C}]$ palmitate oxidation rates were calculated using hourly breath samples collected when subjects blew through straws into 15 ml additive-free VacutainersTM (BD, Franklin Lakes, NJ), and d_{31} -palmitate oxidation rates were calculated from the spot urine samples collected every hour and stored in cryogenically stable tubes (Corning, Inc.). The recovery correction factor for $[1-^{13}\text{C}]$ palmitate and palmitate was determined using the $[1-^{13}\text{C}]$ acetate and d_3 -acetate recovery rates.

Sample analysis

To measure the ratio of $^{13}\text{CO}_2$ to $^{12}\text{CO}_2$ in breath, a sample of breath was injected into a continuous-flow isotope ratio mass spectrometer (IRMS; Delta S; Finnigan MAT). The sample was injected into a continuous helium stream onto a Chromosorb-Q for separation of CO_2 , which is directed into the source of the IRMS. Injections were made in duplicate, and the average standard deviation was 0.3%. Postdose enrichments within a subject were calculated using baseline predose breath samples (7).

TABLE 1. Subject characteristics (mean \pm SD)

| Variable | Women | Men | All |
|-----------------------|---------------|---------------|---------------|
| n | 6 | 7 | 13 |
| Weight (kg) | 58.7 ± 7 | 81.5 ± 11 | 70.1 ± 15 |
| Height (m) | 1.7 ± 0.1 | 1.8 ± 0.1 | 1.8 ± 0.1 |
| Body mass index | 21.3 ± 4 | 24.3 ± 2 | 22.8 ± 3 |
| Total body water (kg) | 32.8 ± 5 | 49.6 ± 10 | 41.2 ± 12 |

To measure the ratio of ^2H to ^1H in urine, a sample (5 ml) of urine was mixed with 200 mg of carbon black to reduce impurities and was passed through a $0.45\ \mu\text{m}$ filter (11). One milliliter of decolorized urine was placed in a 3 ml autosampler vial and analyzed for $^2\text{H}/^1\text{H}$ ratios using the Delta plus IRMS (Finnigan MAT). A $0.8\ \mu\text{l}$ aliquot was injected into a chromium-packed quartz tube held at 850°C to reduce water to hydrogen gas. Each sample was injected three times with independent analysis. Data were corrected for H^{3+} and memory errors. Results were corrected to the standard mean ocean water (SMOW) scale.

Total body water was estimated from ^{18}O enrichments in urine samples collected at baseline and 4 h after dosing with ^{18}O -labeled water. One milliliter of decolorized urine sample was allowed to equilibrate with CO_2 at 25°C for 48 h in a water bath. The ^{18}O enrichment was measured using a continuous-flow IRMS as detailed by Schoeller and Luke (12). Total body water was calculated from the ^{18}O data using the dilution method as described by Schoeller and van Santen (8).

Label calculations

^2H and ^{13}C label recoveries were calculated every hour for 9 h after dose. Baseline enrichment of the individual subject was subtracted from hourly recoveries to derive the increase in label recovery above baseline. Two subjects were made to follow the protocol without dosing, and the average enrichments per hour of these subjects were used to correct for natural variations in abundance of the isotopes attributable to meals and exercise. Thus, the final recovery values ($\Delta\delta$) represent individual recoveries per hour corrected for baseline abundance of individual subjects and for natural variation during the day. Rate of CO_2 production (V_{CO_2}) was obtained from RGE measurements (6).

$$\% \text{ } ^{13}\text{C} \text{ recovery} = 100 \times (V_{\text{CO}_2} \times \Delta\delta \times R_{\text{STD}} / 1,000) / (D \times P \times n / \text{MW} \times 100)$$

where V_{CO_2} is measured in milliliters per minute; $R_{\text{STD}} = ^{13}\text{C}/^{12}\text{C}$ of standard CO_2 ; D = dose in grams; P = ^{13}C isotope atom%; n = number of labeled atoms per molecule of tracer; MW = molecular weight of the tracer ($[1\text{-}^{13}\text{C}]$ acetate Na salt = 83; $[1\text{-}^{13}\text{C}]$ palmitic acid = 257); and isotopic enrichment above the baseline ($\Delta\delta$) per mille (δ) = $(R_U / R_{\text{STD}} - 1) \times 1,000$.

Breath samples were collected for 9 h after dose on both visits to correct for hourly acetate sequestration of acetate label in the TCA cycle. Deuterium recovery was calculated assuming that it is equally distributed across the total body water (TBW).

$$\% \text{ } ^2\text{H} \text{ recovery} = 100 \times (1.035 \times \text{TBW} \times 2 \times \Delta\delta \times R_{\text{STD}} / 1,000) / (D \times P \times n / \text{MW} \times 100)$$

where TBW (moles) is multiplied by a factor of 1.035 to get a ^2H dilution space from the TBW obtained by ^{18}O dilution space; $R_{\text{STD}} = ^2\text{H}/^1\text{H}$ of SMOW; D = dose in grams; P = ^2H isotope atom%; n = number of labeled atoms per molecule of tracer; MW = molecular weight of the tracer (d_3 -acetate Na salt = 83; d_{31} -palmitic acid = 287). The hours after dose represents the midpoint between voids for ^2H recovery calculations.

Because the mean difference in body weight between visits was $0.39 \pm 1.3\ \text{kg}$, the TBW in these subjects was assumed to be constant.

Statistical analysis

A Student's paired *t*-test was used to compare both tracers. Regression analysis was done to correlate the two tracers. ANOVA was used to identify the effects of exercise on the correlation of the tracers. $P \leq 0.05$ was required for statistical significance. All values are presented as means \pm SD. All statistical analyses were performed using STATVIEW version 5.0.1 (SAS Institute, Inc., Cary, NC).

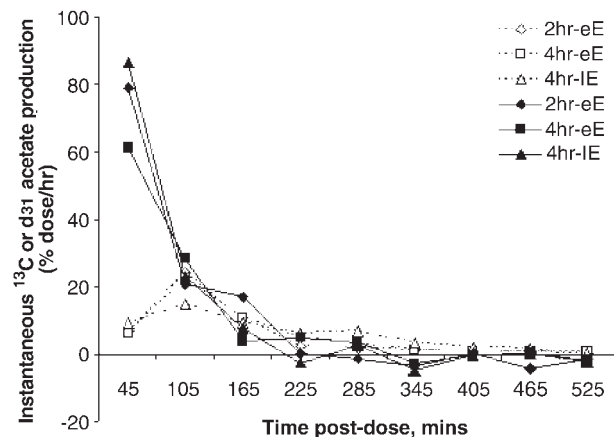


Fig. 1. Recovery of $[1\text{-}^{13}\text{C}]$ acetate and $[^2\text{H}]$ acetate expressed as a percentage of dose per hour ($n = 12$). Hydrogen recoveries were calculated as the cumulative recovery at each time point based on the increment in enrichment above the previous urine sample. The mean $[^2\text{H}]$ acetate recovery was $86 \pm 4\%$ (mean \pm SD), and the mean $[^{13}\text{C}]$ acetate recovery was $57 \pm 8.5\%$ (mean \pm SD). Deuterium recovery was significantly higher than ^{13}C recovery for all exercise groups. 2hr-eE, 2 h early exercise; 4hr-eE, 4 h early exercise; 4hr-lE, 4 h late exercise. Closed symbols represent ^2H acetate recoveries and open symbols represent ^{13}C acetate recoveries.

RESULTS

ANOVAs of the ratios of $[1\text{-}^{13}\text{C}]$ palmitate to d_{31} -palmitate recoveries did not show a significant difference between exercise groups (mean difference = 0.03% ; $P = 0.9$). Hence, exercise groups were combined for this analysis.

Acetate recovery

Cumulative $^{13}\text{CO}_2$ recovery from $[1\text{-}^{13}\text{C}]$ acetate plateaued at ~ 6 h after dose in all exercise groups, as shown in **Fig. 1**. Cumulative $[^{13}\text{C}]$ acetate recoveries 3, 6, and 9 h after dose were $36.3 \pm 9\%$, $52.6 \pm 8\%$, and $56.8 \pm 9\%$, respectively, indicating that most of the label was recovered at 6 h after dose in all exercise groups. $^2\text{H}_2\text{O}$ recovery from d_3 -acetate peaked at 45 min after dose in all three exercise groups, as shown in **Fig. 1**. Cumulative d_3 -acetate recoveries 3, 6, and 9 h after dose were $92.1 \pm 8\%$, $90.6 \pm 4\%$, and $88.4 \pm 4\%$, respectively, indicating that most of the label was recovered at 3 h after dose. Compared with $[1\text{-}^{13}\text{C}]$ acetate, d_3 -acetate showed greater cumulative recovery (mean difference = 31% ; $P < 0.005$) (**Table 2**). Instantaneous d_3 -acetate recovery was significantly higher than ^{13}C at any time during the measurement period.

TABLE 2. Percentage cumulative recovery of label at 9 h after dose (mean \pm SD)

| Exercise | $[^{13}\text{C}]$ acetate | $[^2\text{H}]$ acetate | $[^{13}\text{C}]$ palmitate | $[^2\text{H}]$ palmitate |
|-----------|---------------------------|------------------------|-----------------------------|--------------------------|
| 2 h | 51.2 ± 14 | 89.8 ± 4 | 6.1 ± 2 | 12.0 ± 3 |
| Early 4 h | 53.0 ± 7 | 84.6 ± 3 | 7.5 ± 2 | 13.0 ± 1 |
| Late 4 h | 58.7 ± 5 | 89.9 ± 5 | 4.5 ± 1 | 8.8 ± 2 |

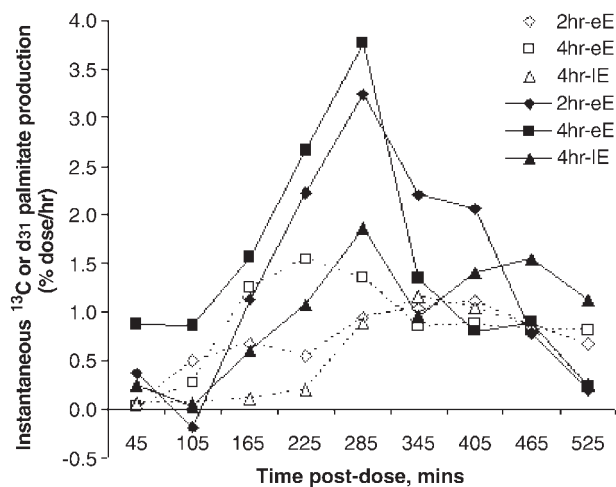


Fig. 2. Recovery of [$1\text{-}^{13}\text{C}$]palmitate and [2H]palmitate expressed as a percentage of dose per hour ($n = 12$) uncorrected for acetate sequestration. Hydrogen recoveries were calculated as the cumulative recovery at each time point based on the increment in enrichment above the previous urine sample. The mean [2H]palmitate recovery was $10.3 \pm 2.8\%$ in all exercise groups together, and that of [^{13}C]palmitate was $5.8 \pm 1.8\%$ in all exercise groups together. Uncorrected [^{13}C]palmitate recovery was significantly different from uncorrected [2H]palmitate recovery (mean difference = 4.7% ; $P < 0.0001$). Closed symbols represent [2H] palmitate recoveries and open symbols represent [^{13}C] palmitate recoveries.

Palmitate recovery

Instantaneous $^{13}\text{CO}_2$ recovery from [$1\text{-}^{13}\text{C}$]palmitate oxidation peaked at 4.25 h after dose in the 4 hr-eEx group and at 6 h after dose in the 2 hr-eEx and 4 hr-lEx groups. Cumulative [$1\text{-}^{13}\text{C}$]palmitate recovery at 3, 6, and 9 h after dose was $0.6 \pm 1\%$, $2.9 \pm 1\%$, and $5.6 \pm 2\%$ (Fig. 2), respectively, without acetate correction. When [$1\text{-}^{13}\text{C}$]palmitate recovery was corrected for sequestration using average group acetate recovery correction factor, the mean 3, 6, and 9 h postdose recoveries were $1.1 \pm 1\%$, $5.7 \pm 3\%$, and $10.7 \pm 4\%$, respectively. Corrected [$1\text{-}^{13}\text{C}$]palmitate using each individual subject's acetate recovery was $1.3 \pm 1\%$, $5.7 \pm 3\%$, and $10 \pm 3\%$ at 3, 6, and 9 h after dose (Table 3).

$^2\text{H}_2\text{O}$ recovery from d_{31} -palmitate oxidation peaked at 3.4 h after dose in all exercise groups alike. The d_{31} -palmitate recovery 3, 6, and 9 h after dose was $1.5 \pm 1\%$, $7.3 \pm 3\%$, and $10.6 \pm 3\%$, respectively. To correct for loss of tracer in the TCA cycle, d_{31} -palmitate recovery at each time point was divided by exercise group mean acetate recovery at the same time point. The 3, 6, and 9 h postdose d_{31} -palmitate recovery was $1.7 \pm 2\%$, $8.3 \pm 4\%$, and $12.0 \pm$

4%, respectively (Table 3). These values are not significantly different from uncorrected d_{31} -palmitate recoveries (mean difference = 1.76% ; $P = 0.55$), indicating little need for acetate corrections when ^2H -labeled fatty acids are used.

Validation

Correlation analysis showed that cumulative d_{31} -palmitate recovery uncorrected for acetate fixation was correlated with [$1\text{-}^{13}\text{C}$]palmitate recovery group corrected for [$1\text{-}^{13}\text{C}$]acetate fixation ($r^2 = 0.51$; $y = 0.55x + 4.6$; $\text{SEM} = 0.19$; $P < 0.02$) (Fig. 3). Because the y-intercept was not significant ($P = 0.58$), it was removed from the regression model ($r^2 = 0.2$; $y = 0.96x$; $\text{SEM} = 0.07$; $P < 0.0001$). When d_{31} -palmitate recovery was corrected for acetate sequestration using group average d_3 -acetate recovery, the correlation r^2 was 0.56 ($y = 0.74x + 4.6$), and when an individual subject's d_3 -acetate recovery was used to correct his/her own d_{31} -palmitate recoveries, the correlation r^2 was 0.54 ($y = 0.69x + 4.9$). The intercept in both of these instances was significantly different from zero ($P = 0.02$). The mean difference between the two tracers was lowest when uncorrected d_{31} -palmitate recoveries were used (mean difference = $0.4 \pm 3\%$; $P = 0.64$).

Votruba, Zeddun, and Schoeller (6) validated the use of d_{31} -palmitate for the measurement of dietary fatty acid oxidation against [$1\text{-}^{13}\text{C}$]palmitate under rest conditions. Mean cumulative recovery of [$1\text{-}^{13}\text{C}$]acetate at 10 h after dose was $53.7 \pm 10.4\%$. Mean cumulative [$1\text{-}^{13}\text{C}$]palmitate recovery corrected for acetate sequestration at 10 h after dose was $12.9 \pm 8.5\%$ and that of d_{31} -palmitate was $13.2 \pm 7.7\%$. Acetate corrected ^{13}C data correlated well with d_{31} -palmitate recovery in these subjects at rest ($r^2 = 0.88$). These data, when combined with our data, showed a strong correlation ($r^2 = 0.79$) between [$1\text{-}^{13}\text{C}$]palmitate recovery corrected for acetate recovery and d_{31} -palmitate recovery, suggesting that ^2H label can be used independent of acetate correction under conditions of rest and exercise alike (Fig. 4). Also, the exercise subjects showed no significant difference in their label recoveries compared with the recoveries of subjects at rest reported by Votruba, Zeddun, and Schoeller (6) ($P = 0.11$).

DISCUSSION

The present study demonstrates the validity of deuterium-labeled fatty acids to measure the oxidation of dietary fatty acids during exercise, expanding on the previ-

TABLE 3. Percentage recovery of palmitate at 9 h after dose corrected for label sequestration (mean \pm SD)

| Exercise | [^{13}C]palmitate, individual corrected | [^{13}C]palmitate, group corrected | [^2H]palmitate, individual corrected | [^2H]palmitate, group corrected | ^{13}C group corrected/ ^2H group uncorrected |
|-----------|--|---|---|--|---|
| 2 h | 11.2 ± 3 | 11.8 ± 4 | 13.5 ± 4 | 13.4 ± 4 | 1.0 ± 0.1 |
| Early 4 h | 13.6 ± 1 | 14.1 ± 4 | 21.1 ± 1 | 15.4 ± 1 | 1.1 ± 0.4 |
| Late 4 h | 7.9 ± 2 | 7.7 ± 1 | 9.8 ± 3 | 9.8 ± 3 | 1.0 ± 0.3 |

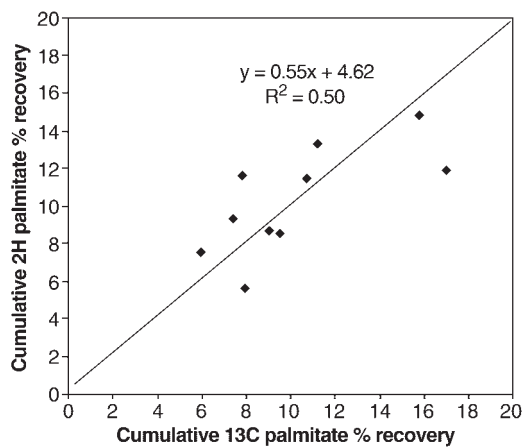


Fig. 3. Group acetate corrected ^2H - and $[1-^{13}\text{C}]$ palmitate percent recovery at 9 h after dose during exercise. Acetate recovery averaged for the exercise group was used to correct for label sequestration. When ^2H palmitate was correlated with $[1-^{13}\text{C}]$ palmitate recovery group corrected for acetate, the correlation r^2 was 0.51 ($y = 0.55x + 4.6$; SEM = 0.19; $P < 0.02$).

ously validated rest condition. The ^2H label not only offers an effective alternative to ^{13}C labeling, it also has the advantage of eliminating the need for an acetate correction, because acetate hydrogen sequestration in the TCA is minimal. The needs for frequent sampling and V_{CO_2} measurements are also eliminated by the use of ^2H label; hence, it can be used under free-living conditions without hindering the subject's normal daily activities.

Although this validation was performed using stable isotope-labeled fatty acids, radioisotope-labeled fatty acids have also been used to measure dietary fatty acid oxidation under rest and exercise conditions. Romanski, Nelson, and Jensen (13) dosed their subjects with ^3H triolein and ^{14}C triolein along with their meal to study the trafficking of dietary fat toward oxidation or storage for 24 h after dose. Although acetate corrections for both radioisotopes were not measured, the cumulative ^3H and ^{14}C

recoveries were correlated; thus, our results should apply equally well to these radioisotopes.

Carbon labeling of fatty acids appears as a natural choice for the measurement of oxidative processes, but it is subject to sequestration in the TCA, leading to reduced yield (14). Since Sidossis et al. (4) proposed the use of a correction factor for acetate sequestration in the TCA cycle intermediates, ^{13}C -labeled fatty acids have been used for the measurement of substrate metabolism, including fatty acid oxidation rates, but their accuracy has been debated. Acetate exits the path of the labeled substrate metabolism before entry into the TCA cycle and hence can account for label sequestration occurring in the TCA cycle. The accuracy of correction for sequestration depends on the position of the label in the fatty acid and the physiological conditions under which its metabolism is measured (15).

Unlike labeled carbons, most of the ^2H label from fatty acids is rapidly released as reducing power (NADH-H^+ and FADH_2) during β -oxidation and then again in the TCA cycle (4). Thereafter, the ^2H is oxidized to water and released into the total body water pool, which can be sampled in the urine. Because most of the NADH is formed during β -oxidation, only $\sim 25\%$ of the label enters the TCA cycle and only $\sim 10\%$ of the label is expected to be sequestered (6). This estimate is supported by our data, with $\sim 88\%$ (SEM = 1.4%) of ^2H label recovered when subjects were dosed with d_3 -acetate. Hence, ^2H -labeled fatty acids have the further advantage of not requiring an individual acetate recovery measurement and can be used independently of an acetate correction factor. An added advantage is that the time taken by the ^2H label to metabolize through the intermediate pools of the body is reduced. This is shown by the more rapid appearance of ^2H in urine than ^{13}C in breath CO_2 after acetate administration. This is probably attributable to the time required by ^{13}C to pass through the TCA cycle and internal bicarbonate pools, which is eliminated when ^2H label is used, because it mixes very rapidly with the body water pool (16).

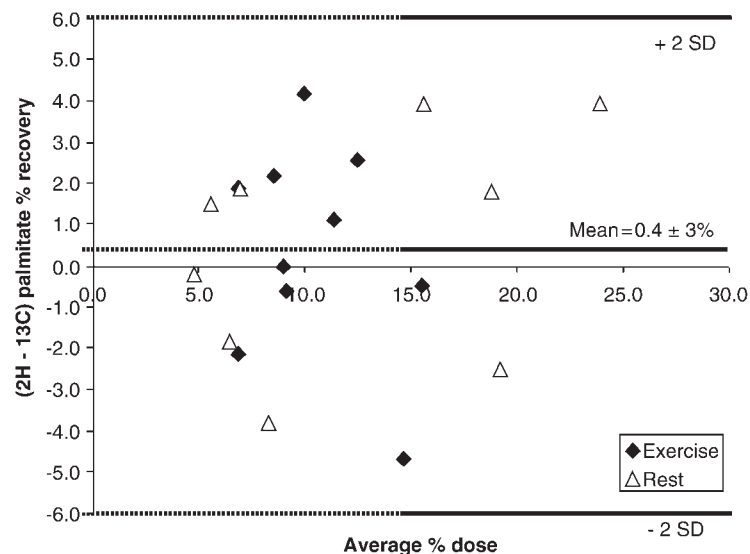



Fig. 4. Comparison of the average and difference between ^2H palmitate percent recovery and group acetate corrected $[1-^{13}\text{C}]$ palmitate percent recovery at 9 h after dose during exercise and rest. The mean difference between the two tracers was $0.03 \pm 2.6\%$ in the exercise group and $-0.22 \pm 2.6\%$ when the rest data were included. The residuals for the combined data showed a normal distribution.

Deuterium-labeled fatty acids when oxidized release the label in the body water pool, and sampling of urine measures the abundance of the isotope in the body water pool, whereas measurement of the abundance of $^{13}\text{C}_2$ in breath is an instantaneous measure of oxidation. Hence, administration of ^2H label does not require the frequent measurement of V_{CO_2} that is needed when using ^{13}C label for the calculation of recovery. The use of hydrogen-labeled fatty acids virtually obviates the need for a controlled environment during the collection of a subject's samples. Moreover, because the tracer accumulates in body water, it reduces the potential error of missed peak oxidation with carbon labeling should the peak excretion occur between breath samples, a particularly troublesome issue with rapidly oxidized substrates such as acetate. The use of hydrogen labeling, however, requires the measurement of body water for recovery calculation, correction for water turnover for long recovery times, and is subject to dilution in the large body water pool.

Correction of ^2H fatty acid oxidation measures for acetate sequestration did not yield any significant difference against uncorrected data compared with group corrected ^{13}C fatty acid oxidation. Because the coefficient of variation for d_3 -acetate recovery was only 5% between individuals of all exercise groups, correction using group acetate recovery versus individual recovery did not make a discernible difference in our data. This further emphasizes that deuterium-labeled fatty acids can be used to measure dietary fat oxidation without the need for measurement of acetate correction factor.

In conclusion, we demonstrated that ^2H -labeled fatty acids can be used to accurately measure the oxidation of dietary fat during exercise. Minimal sequestration of ^2H label in the TCA eliminates the need for an acetate oxidation measure and hence an additional stay in the hospital. Also, compared with ^{13}C label, ^2H label can be used without the need for frequent sampling, V_{CO_2} measurement, and restricted environment for measurements. Furthermore, high correlation between ^{13}C -labeled fatty acids (corrected for acetate sequestration) and ^2H -labeled fatty acids (uncorrected) suggests the accuracy of the measurement of fat oxidation using either method. 

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