Measuring plasma fatty acid oxidation with intravenous bolus injection of $^3$H- and $^{14}$C-fatty acid

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Abstract Accurate measures of plasma FA oxidation can improve our understanding of diseases characterized by impaired FA oxidation. We describe and compare the 24 h time-courses of FA oxidation using bolus injections of [1-$^{14}$C]palmitate versus [9,10-$^3$H]palmitate under postabsorptive, postprandial, and walking conditions. Fifty-one men and 95 premenopausal women participated in one condition (postabsorptive, postprandial, or walking), one tracer ([$^{13}$C- or $^3$H-labeled), and an acetate or palmitate study. Groups were matched for sex, age, and body mass index (BMI). At 24 h, cumulative [$^3$H]acetate recovery as $^3$H$_2$O was 80 ± 6%, 78 ± 2%, and 81 ± 6% in the postabsorptive, postprandial, and walking conditions, respectively (not significant). Model-predicted maximum [1-$^{14}$C]acetate recovery as expired $^{14}$CO$_2$ was 59 ± 12%, 52 ± 8%, and 65 ± 10% in the postabsorptive, postprandial, and walking condition, respectively (one way ANOVA, $P = 0.12$). When corrected with the corresponding acetate recovery factors, 24 h time-courses of FFA oxidation were similar between [1-$^{14}$C]palmitate and [9,10-$^3$H]palmitate in all three conditions. In contrast to previous meal ingestion studies, an acetate-hydrogen recovery factor was needed to achieve comparable oxidation rates using an intravenous bolus of [$^3$H]palmitate. In conclusion, intravenous boluses of [9,10-$^3$H]palmitate gave similar estimates of 24 h cumulative FA oxidation in age-, sex- and BMI-matched individuals.—Koutsari, C., A. H. Ali, M. S. Mundi, and M. D. Jensen. Measuring plasma fatty acid oxidation with intravenous bolus injection of 3H- and 14C-fatty acid. J. Lipid Res. 2013. 54: 254–264.

Supplementary key words isotopic tracers • acetate recovery factor • carbon-labeled • hydrogen-labeled • postabsorptive • postprandial • walking

FA tracers labeled on the carbon or hydrogen atoms are used for the study of plasma FFA oxidation in humans in vivo. Impaired plasma FFA oxidation occurs in disease states such as type 2 diabetes (T2DM) (1, 2). Therefore, accurate measures of FFA oxidation are important to help understand the pathophysiology of T2DM and related diseases.

Numerous investigators have used carbon and hydrogen isotope-labeled FA tracers to measure FA oxidation by means of continuous intravenous infusions (3–10) and dietary ingestion of the tracers (11–14). Rapid intravenous injection of an FFA tracer is useful when aspects of nonoxidative FA metabolism are investigated. Examples include the incorporation of plasma FAs into VLDL-triglyceride (TG) (15, 16) or the storage/uptake of plasma FAs into tissues (17–20). In addition, intravenous injections can be more convenient than a continuous infusion, because bolus injections obviate the need to keep the volunteers connected to an infusion pump, thus offering greater mobility and freedom during experimental days. To the best of our knowledge, there is no information on the use of carbon- and hydrogen isotope-labeled FAs in studies where the FFA tracers are administered as intravenous injections. Being able to incorporate whole-body FFA oxidation data into bolus injection experiments may increase the value of such studies.

When using carbon-labeled tracers ([$^{13}$C or $^{14}$C-labeled FAs), the appearance of the labeled carbon in expired CO$_2$ is used to calculate FA oxidation. For carbon-labeled FAs, the acetate recovery factor is used to correct for carbon-label retention in the bicarbonate pool and for label sequestration in isotopic exchange reactions in the tricarboxylic acid (TCA) cycle (4, 6–9, 21). Whereas there is no sequestration of the carbon label during $\beta$-oxidation, in the first turn of the TCA cycle, position-1 carbon label can be temporarily sequestered in the glutamate/glutamine, glucose, lactate, aspartate, and perhaps other pools (Fig. 1). In the second turn of the TCA cycle, 50% of the remaining position-1 carbon label is released as expired CO$_2$ before the possibility for sequestration, whereas the other 50% is

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Abbreviations: BMI, body mass index; CV, coefficient of variance; SA, specific activity; TCA, tricarboxylic acid; T2DM, type 2 diabetes.

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recovered averaged 51% during both rest and physical activity (11).

When using hydrogen-labeled tracers ($^2$H- or $^3$H-labeled FAs), the hydrogen atoms from the oxidation of the tracer appear as $^2$H$_2$O or $^3$H$_2$O in body water. During β-oxidation, most of the hydrogen labels (~75%) are transferred to NAD$^+$ and FAD (Fig. 2) and, eventually, they appear in body water. The remaining label (~25%) reaches the TCA cycle as part of the methyl group of the acetyl CoA molecule. Because the hydrogens of the methyl group are stereochemically identical, any of three hydrogens can have

released as CO$_2$ after the possibility for sequestration in the glutamate/glutamine pool.

With continuous intravenous infusion studies, the recovery of position-1 carbon-labeled acetate averages 56% in the postabsorptive state, 50% during hyperinsulinemia-hyperglycemia, and 80% during exercise (7). The percent recovery of labeled CO$_2$ from the oxidation of carbon-labeled acetate is dependent on the duration of the acetate tracer infusion (3, 4, 9) as well as the position of the carbon label on the acetate molecule (21). When position-1 carbon-labeled acetate was ingested with a meal, the carbon

![Diagram of beta-oxidation](image)

Fig. 1. The fate of the $^{14}$C label of [1-$^{14}$C]palmitoylCoA through β-oxidation and two turns of the TCA cycle.
Fig. 2. The fate of the four $^3$H labels of [3,4-$^3$H]palmitoyl-CoA through β-oxidation and two turns of the TCA cycle. The process of $^3$H label removal is similar to that for [9,10-$^3$H]palmitoyl-CoA. Any of the three hydrogens of the methyl group on the acetyl-CoA molecule can be the last $^3$H label. The fate of the three possible positions of the $^3$H label on the acetyl-CoA molecule is presented through two turns of the Krebs cycle.
METHODS

Study participants

After approval from the Mayo Institutional Review Board, a total of 51 men and 95 premenopausal women gave informed written consent to participate in the study. The following criteria were used to select the volunteers: body mass index (BMI) 18–36 kg/m²; no regular, vigorous physical activity; normal urinalysis; complete blood count; electrolytes; liver and kidney function tests; no medications known to influence lipid metabolism (including oral contraceptives); and no tobacco. All subjects were weight-stable for at least 2 months before the study.

Each volunteer participated in one condition (postabsorptive, postprandial, or walking), one tracer ([1-14C] or [3H]-labeled) and either the acetate or the palmitate study. The participants of the [3H]acetate studies were matched for sex, age, and BMI with the participants of the [9,10-3H]palmitate studies. Similarly, the participants of the [1-14C]acetate studies were matched for sex, age, and BMI with the participants of the [9,10-3H]palmitate studies. Also, volunteers within each protocol (postabsorptive, postprandial, and walking) were matched for sex, age, and BMI between the [1-14C]palmitate and [9,10-3H]palmitate tracers. The ratio of women to men in each study was set to 2:1.

Experimental design

Study protocols. All participants received their meals from the Mayo Clinic Clinical Research Unit (CRU) metabolic kitchen for 5 days prior to the study to ensure stable energy intake and consistent macronutrient composition (50% carbohydrate, 35% fat, and 15% protein). Volunteers were then admitted to the CRU at 1700 h and given a meal at 1800. Next morning (Day 1), at 0800, the participants in the acetate studies received an intravenous bolus injection of either 200 μCi [9,10-14H]palmitate or 60 μCi [1-14C]palmitate. All radioactive tracers were purchased from NEN Life Science Products, PerkinElmer, Boston, MA.

In the postabsorptive protocol, volunteers remained fasted until 1330 h on Day 1. They received a fat-free lunch at 1330 (70% of energy requirements) and a mixed supper (30% of energy requirements) at 1800. The volunteers rested throughout this protocol until the end of the study at 0800 next day (Day 2) and drank water ad libitum.

In the postprandial protocol, at 0620 h on Day 1, the participants began consuming small portions of a fat-free smoothie [fat-free frozen yogurt, skim milk, Beneprotein (Nestlé Nutrition), Polyose (Abbott Nutrition), and frozen unsweetened strawberries] at 20 min intervals until 1720 h. Overall, the smoothie portions provided 70% of each individual’s daily resting energy requirements. The smoothie provided 30% of energy as protein and 70% as carbohydrate. The participants received a mixed supper at 1800, which provided the remaining 30% of daily resting energy requirements. The volunteers rested throughout this protocol until the end of the study at 0800 on Day 2 and drank water ad libitum.

In the walking protocol, volunteers began walking on the treadmill at ~2 mph at 0700 h on Day 1. They continued walking for 5.5 h, i.e., until 1230 h. As in the postabsorptive protocol, volunteers remained fasted until 1330, and they received a fat-free lunch at 1330 and mixed supper at 1800. Volunteers rested until the end of the study at 0800 on Day 2 and drank water ad libitum.

When the 14C tracers (acetate or palmitate) were administered, breath samples were obtained at frequent intervals until the next morning at 0800 h for measurement of 14CO2 specific activity (SA). Breath CO2 production rates were measured by indirect calorimetry (DeltaTrac Metabolic Cart; Yorba Linda, CA) hourly until 1700 on Day 1 and at 0800 on Day 2. The metabolic cart was calibrated each morning of the study. Additional quality control for the metabolic carts included monthly pressure calibrations and gas calibrations together with every 6 month calibrations using an alcohol burn test.

When the 3H tracers (acetate or palmitate) were administered, blood samples were obtained at frequent intervals on Day 1 until the next morning at 0800 h for measurement of 3H2O SA (dpm/ml). In addition, urine was collected for 24 h after the tracer bolus to assess 3H2O losses in urine. The amount of 3H2O in total body water was corrected for 3H2O losses in urine and was used to determine cumulative [9,10-3H]palmitate or [3H]acetate oxidation. Total body water was measured with oral administration of 2H2O (Isotech; Miamisburg, OH) on a separate visit 1 to 2 weeks prior to the main study visit (22).

Assessment of residual radioactivity in plasma lipids. In small subsets of the study participants (postabsorptive protocol, n = 7; postprandial protocol, n = 6; walking protocol, n = 6), we assessed the residual radioactivity (14C and 3H) in plasma lipids for 8 days after the palmitate tracer bolus. One milliliter of plasma was extracted with chloroform-methanol 2:1, the solvent was evaporated, and the lipids were counted with scintillation counting. The results (dpm/ml plasma) were multiplied by plasma volume (PV = 55 ml × kg FFM) to calculate the total residual radioactivity in plasma. The results are expressed as percent of tracer dose administered.

Body composition

Fat free mass and body fat mass were measured using dual energy X-ray absorptiometry (DXA, DPX-IQ; Lunar Radiation; Madison, WI).
Analytical techniques

Quadruplicate aliquots of the [1-14C]palmitate, [1-14C]acetate, [9,10-3H]palmitate, and [3H]acetate infusates were counted using liquid scintillation counting to assess the exact amount of radiotracers administered.

Breath 14CO2 SA (dpm/mmol) was measured by air expired through a solution of benzenthionium hydroxide with phenolphthalein, trapping 0.5 mmol CO2. Plasma and urine 3H O SA (dpm/ml) were assessed with scintillation counting.

For the measurement of total body water with 3H2O, 500 µl of the urine sample was added to 40 mg activated charcoal in a 12 × 75 mm tube and mixed. After centrifugation, the supernatant was passed through a spin-x filter (0.22 µm) and 200 µl transferred to a 300 µl autosampler vial. Deuterium in the ‘decolorized’ urine was measured using a Thermo Delta V Advantage Isotope Ratio Mass Spectrometer (IR/MS) equipped with a high-temperature carbon-reduction elemental analyzer (TC/EA) inlet. One microliter of urine was reduced to hydrogen-deuterium by injection into the on-line glassy carbon TC/EA reactor held at 1400°C. Gases produced in the reactor were separated on a 5 Å molecular sieve gas solid chromatography column prior to introduction into the IR/MS. Quadruplicate aliquots of each sample were measured against a calibration curve normalized to the standard mean ocean water scale analyzed in the same sequence. Total body water was calculated using the formula by Schoeller et al. (22).

Calculations

The rate of 14CO2 production in breath (dpm/min) was determined by multiplying expired air 14CO2 SA (dpm/mmol) by CO2 production rate (mmol/min) at each time point. A nonlinear model was used to predict nocturnal CO2 production rates (23), because nocturnal CO2 production rates were not measured in this study. To calculate the fraction of [1,14C]palmitate or [1-14C]acetate that was oxidized, the area under the 14CO2 production rate versus time curve (dpm) was divided by the amount (dpm) of [1-14C]palmitate or [1-14C]acetate that each volunteer had received. The time course of the percent recovery of 14CO2 in breath from the oxidation of [1,14C]acetate was also modeled using the Statistics Toolbox 6 of MatLab software (The MathWorks; Natick, MA).

3H2O SA (dpm/ml) was multiplied by total body water (ml) to calculate the total amount of 3H-labeled tracer (dpm) that was recovered as 3H2O in body water. The loss of 3H2O in the body throughout the 24 h time course was also taken into account. To calculate the fraction of [9,10-3H]palmitate or [3H]acetate that was oxidized, the amount of 3H-labeled tracer (dpm) that was recovered as 3H2O in body water (including 3H2O loss in urine) was divided by the amount (dpm) of [9,10-3H]palmitate or [3H]acetate that each volunteer had received.

The recovery of 14CO2 in the breath from the oxidation of [1-14C]palmitate in each protocol is presented in two ways: a) corrected with the point-by-point group average acetate-carbon sequestration as assessed in the corresponding [1-14C]acetate oxidation protocol, and b) without correction for acetate-carbon sequestration. Similarly, the recovery of 3H2O in body water from the oxidation of [9,10-3H]palmitate is presented in two ways: a) corrected with the point-by-point group average acetate-hydrogen sequestration as assessed in the corresponding [3H]acetate oxidation protocol, and b) without correction for acetate-hydrogen sequestration.

Statistical analysis

Values are expressed as means ± SD, unless otherwise indicated. One-way ANOVA was used to compare the demographic characteristics of the volunteers among the three conditions. One-way ANOVA was also used to compare the model parameters, which were k (rate constant) and maximum percent recovery of [1-14C]acetate among the three conditions. A repeated-measures ANOVA was used to compare [1-14C]palmitate versus [9,10-3H]palmitate oxidation time courses within each condition. Tukey post hoc test was used to locate significant differences. Two-tailed nonpaired Student’s t test was used to compare cumulative [1-14C]palmitate versus [9,10-3H]palmitate oxidation at 6 h and 24 h after the bolus. P values of <0.05 were considered statistically significant. Inter-individual coefficient of variance (CV) was calculated as (SD/mean) × 100. Statistical analyses were performed with JMP 9.0.1 (SAS Institute, Inc.; Cary, NC) and Statistica 7.0 (StatSoft, Inc.; Tulsa, OK).

RESULTS

Participant characteristics

Table 1 presents the characteristics of the participants in the acetate and palmitate oxidation studies. Participants within each tracer study ([3H]acetate, [14C]acetate, [9,10-3H]palmitate, and [1-14C]palmitate) had similar characteristics among the postabsorptive, postprandial, and walking conditions. For all studies together, participants’ average age was between 30 and 40 years, average BMI fell into the overweight category, and percent body fat was ~35%.

Acetate recovery

The 24 h time course of the cumulative percent recovery of [3H]acetate as 3H2O in body water is depicted in Fig. 3 (left panel). Within 15 min after the bolus, 68 ± 6% (postabsorptive), 76 ± 10% (postprandial), and 75 ± 8% (walking) of the [3H]acetate tracer was recovered as 3H2O in body water. [3H]acetate recovery values at 2 h, 6 h, and 24 h after the tracer bolus are presented in Table 2. [3H]acetate recovery plateaued at around 3.5 h after the bolus. At 24 h, 3H2O recovery from [3H]acetate oxidation was 80 ± 6%, 78 ± 2%, and 81 ± 6% in the postabsorptive, postprandial, and walking conditions. There was no significant condition effect, i.e., the time courses were similar among the postabsorptive, postprandial, and walking conditions (repeated measures ANOVA condition effect: P = 0.33). The inter-individual CV of cumulative [3H]acetate recovery at 24 h ranged from 2% to 8% (Table 2).

The 24 h time courses of the cumulative percent recovery of [14C]acetate in breath CO2 is depicted in Fig. 3 (right panel). The recovery of 14CO2 from [14C]acetate oxidation was not as rapid as the recovery of 3H2O from the oxidation of [3H]acetate. The recovery of [14C]acetate as 14CO2 at 2 h, 6 h, and 24 h after the tracer bolus is presented in Table 2. There was a significant condition effect (ANOVA P = 0.017). Specifically, the cumulative recovery of [14C]acetate was overall greater during the walking than during the postprandial condition (Tukey P = 0.018).

The time course of the percent recovery of [1-14C]acetate in each condition was fit into the following exponential model: % [1-14C]acetate recovery = A × (1-e-kt×Time), where time is in hours, A is the maximum percent recovery,
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Figure 5 depicts the 24 h time courses of the cumulative percent recovery of [9,10-3H]palmitate and [1-14C]palmitate oxidation in the postabsorptive, postprandial, and walking protocols. These data were not corrected for carbon or hydrogen loss. Table 3 presents the percent [9,10-3H]palmitate and [1-14C]palmitate that was oxidized by 6 h and 24 h after the tracer boluses. In all three conditions, [1-14C]palmitate oxidation was significantly less than [9,10-3H]palmitate oxidation (repeated measures ANOVA tracer effect: all \( P < 0.0001 \)).

Previous studies have found that a hydrogen-labeled acetate recovery factor is not required when measuring dietary fat oxidation using hydrogen-labeled FAs (13, 14). To assess whether this was also true when tracers were administered intravenously, we compared the 24 h

\[ k \text{ (in h}^{-1}\text{)} \] is the rate constant of the increase in percent recovery. Examples of the fit in a randomly selected volunteer from each condition are presented in Fig. 4.

The predicted maximum percent recovery of [1-14C]acetate (model parameter A) was 59 ± 12% (postabsorptive), 52 ± 8% (postprandial), and 65 ± 10% (walking) (one way ANOVA \( P = 0.12 \)). The inter-individual CV of the predicted maximum percent [1-14C]acetate recovery ranged from 16.9% to 20.3% (Table 2).

The rate constant of the increase in percent [1-14C]acetate recovery (model parameter B) was 0.308 h\(^{-1}\) ± 0.028 h\(^{-1}\) (postabsorptive), 0.372 h\(^{-1}\) ± 0.027 h\(^{-1}\) (postprandial), and 0.561 h\(^{-1}\) ± 0.033 h\(^{-1}\) (walking) (one way ANOVA \( P < 0.0001 \); Tukey \( P < 0.0001 \) for postabsorptive vs. postprandial, postprandial vs. walking, and postabsorptive vs. walking).

**Table 1.** Clinical characteristics and body composition of the study participants in the plasma acetate recovery and palmitate oxidation studies

|                      | Postabsorptive | Postprandial | Walking | ANOVA | Postabsorptive | Postprandial | Walking | ANOVA | P     |
|----------------------|----------------|--------------|---------|-------|----------------|--------------|---------|-------|-------|
| Age (years)          | 36 ± 9         | 32 ± 9       | 31 ± 5  | 0.51  | 38 ± 7         | 31 ± 9       | 32 ± 7  | 0.20  |       |
| BMI (kg/m\(^2\))     | 27.7 ± 4.0     | 29.0 ± 7.4   | 27.7 ± 4.0 | 0.92 | 27.4 ± 5.5     | 29.1 ± 5.9   | 29.2 ± 2.5 | 0.75  |       |
| Weight (kg)          | 81 ± 8         | 84 ± 8       | 85 ± 8  | 0.92  | 79 ± 16        | 80 ± 15      | 90 ± 22 | 0.48  |       |
| Body fat (%)         | 34 ± 8         | 37 ± 11      | 33 ± 11 | 0.82  | 35 ± 7         | 36 ± 11      | 38 ± 9  | 0.87  |       |
| Total fat mass (kg)  | 27.2 ± 8.8     | 31.8 ± 15.1  | 28.0 ± 12.6 | 0.81 | 28.2 ± 8.1     | 28.9 ± 12.1  | 33.6 ± 9.5 | 0.58  |       |
| Fat free mass (kg)   | 52.2 ± 12.7    | 50.7 ± 6.8   | 55.6 ± 13.0 | 0.76 | 49.8 ± 12.1    | 49.4 ± 8.9   | 53.9 ± 15.1 | 0.80  |       |

**Table 3.** Percent [9,10-3H]palmitate and [1-14C]palmitate that was oxidized by 6 h and 24 h after the tracer boluses.

Values are means ± SD; BMI, body mass index.

**Figure 5.** The 24 h time course of the cumulative percent recovery of [1-14C]acetate in breath CO\(_2\). Error bars indicate SD.

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**Palmitate oxidation**

**Figure 5** depicts the 24 h time courses of the cumulative [9,10-3H]palmitate and [1-14C]palmitate oxidation in the postabsorptive, postprandial, and walking protocols. These data were not corrected for carbon or hydrogen loss. **Table 3** presents the percent [9,10-3H]palmitate and [1-14C]palmitate that was oxidized by 6 h and 24 h after the tracer boluses. In all three conditions, [1-14C]palmitate oxidation was significantly less than [9,10-3H]palmitate oxidation (repeated measures ANOVA tracer effect: all \( P < 0.0001 \)).

Previous studies have found that a hydrogen-labeled acetate recovery factor is not required when measuring dietary fat oxidation using hydrogen-labeled FAs (13, 14). To assess whether this was also true when tracers were administered intravenously, we compared the 24 h
time-courses of the [1-14C]palmitate oxidation corrected with the point-by-point group average percent [1-14C] acetate recovery with the time courses of the [9,10-3H] palmitate oxidation not corrected for hydrogen label loss (Fig. 6). In the postabsorptive and walking conditions, [1,14C]palmitate oxidation was significantly greater than [9,10-3H]palmitate oxidation (repeated measures ANOVA tracer effect: P < 0.0001 in both conditions). In contrast, in the postprandial state, there was no significant difference between the corrected [1-14C]palmitate oxidation

|                  | [3H]acetate recovery |                  | [1-14C]acetate recovery |                  |
|------------------|----------------------|------------------|-------------------------|------------------|
|                  | 2 h      | CV     | 6 h      | CV     | 24 h     | CV     | 2 h      | CV     | 6 h      | CV     | 24 h     | CV     | Model-predicted max | CV     |
| Postabsorptive   | 77 ± 6   | 7.8    | 78 ± 7   | 9.0    | 80 ± 6   | 7.6    | 32 ± 7   | 21.4   | 50 ± 10  | 20.9   | 59 ± 12   | 20.5   | 59 ± 12   | 20.3   |
| Postprandial     | 76 ± 5   | 6.1    | 76 ± 2   | 2.5    | 78 ± 2   | 2.0    | 32 ± 4   | 14.0   | 46 ± 7   | 14.5   | 54 ± 8    | 14.5   | 52 ± 8    | 15.4   |
| Walking          | 74 ± 5   | 6.9    | 80 ± 4   | 5.3    | 81 ± 6   | 8.0    | 50 ± 9   | 18.4   | 61 ± 10  | 16.3   | 68 ± 11   | 16.0   | 65 ± 11   | 16.9   |

Values are means ± SD; CV, coefficient of variance. For sample sizes in each protocol see Table 1.

Fig. 4. Examples of the exponential model fitting the time courses of the percent recovery of [1-14C]acetate in breath CO2 in a randomly selected volunteer in each condition.
and the uncorrected \([9,10-^3\text{H}]\)palmitate oxidation (repeated measures ANOVA tracer effect: \(P=0.23\)).

**Figure 7** depicts the 24 h time courses of the cumulative \([9,10-^3\text{H}]\)palmitate and \([1-^{14}\text{C}]\)palmitate oxidation when both tracers were corrected with the corresponding acetate recovery factors. No significant differences in cumulative oxidation between the \([9,10-^{14}\text{C}]\)palmitate and \([1-^{14}\text{C}]\)palmitate tracers were observed (repeated measures ANOVA tracer effect: \(P=0.26\) postabsorptive; \(P=0.31\) postprandial; \(P=0.89\) walking). Table 3 presents the percent \([9,10-^{14}\text{C}]\)palmitate and \([1-^{14}\text{C}]\)palmitate oxidized by 6 h and 24 h, when \([1-^{14}\text{C}]\)palmitate was corrected for label loss and when both \([9,10-^3\text{H}]\)palmitate and \([1-^{14}\text{C}]\)palmitate were corrected with the corresponding acetate recovery factors. In all three conditions, the oxidation time courses were virtually identical between the two tracers, when the recoveries of both acetate labels were applied to correct for label loss.

**Post-study residual radioactivity in plasma lipids**

There was very low residual radioactivity in plasma lipids at 48 h after the tracer bolus (postabsorptive 1.0 ± 0.1%; postprandial 1.4 ± 0.4%; walking 1.0 ± 0.1%). On day 4, values were postabsorptive 0.3 ± 0.1%; postprandial 0.5 ± 0.2%; walking 0.3 ± 0.2%. On day 8, values were 0.1 ± 0.1% in all three conditions.

**DISCUSSION**

The goal of the present study was to comprehensively assess the use of intravenous bolus injections of \([1-^{14}\text{C}]\)palmitate and \([9,10-^3\text{H}]\)palmitate to measure cumulative plasma FFA oxidation under postabsorptive, postprandial, and walking conditions. The novel findings were: 1) when corrected with the corresponding acetate recovery factor, 24 h time courses of cumulative FA oxidation were similar between \([1-^{14}\text{C}]\)palmitate and \([9,10-^3\text{H}]\)palmitate under postabsorptive, postprandial, and walking conditions; 2) maximum cumulative \([^{3}\text{H}]\)acetate recovery was ~80% in each condition; and 3) model-predicted maximum cumulative \([1-^{14}\text{C}]\)acetate recovery did not statistically differ among the postabsorptive (average 59%), postprandial (average 52%), and walking condition (average 65%).

Very few studies have investigated the use of hydrogen-labeled FAs for the measurement of FA oxidation in humans.

**TABLE 3.** Percent cumulative \([9,10-^{14}\text{C}]\)palmitate and \([1-^{14}\text{C}]\)palmitate oxidation at 6 h and 24 h after the tracer bolus, when acetate recovery was not taken into consideration (left) and when \([9,10-^{14}\text{C}]\)palmitate and \([1-^{14}\text{C}]\)palmitate oxidation were corrected with \([^{3}\text{H}]\)acetate and \([1-^{14}\text{C}]\)acetate recoveries, respectively (right)

| Time | Without Correction | With Correction | \(P\) | \(P\) |
|------|--------------------|----------------|------|------|
| 6 h  | \([9,10-^{14}\text{C}]\)palmitate | \([1-^{14}\text{C}]\)palmitate | \(P\) | \([9,10-^{14}\text{C}]\)palmitate | \([1-^{14}\text{C}]\)palmitate | \(P\) |
| Postabsorptive | 37 ± 6 | 22 ± 4 | <0.0001 | 47 ± 2 | 44 ± 8 | 0.14 |
| Postprandial | 26 ± 5 | 16 ± 4 | <0.0001 | 34 ± 7 | 34 ± 10 | 0.88 |
| Walking | 56 ± 5 | 45 ± 5 | <0.0001 | 71 ± 7 | 75 ± 8 | 0.20 |
| 24 h | \([9,10-^{14}\text{C}]\)palmitate | \([1-^{14}\text{C}]\)palmitate | \(P\) | \([9,10-^{14}\text{C}]\)palmitate | \([1-^{14}\text{C}]\)palmitate | \(P\) |
| Postabsorptive | 41 ± 5 | 30 ± 5 | <0.0001 | 51 ± 7 | 51 ± 9 | 0.99 |
| Postprandial | 32 ± 7 | 21 ± 6 | 0.001 | 41 ± 9 | 39 ± 10 | 0.64 |
| Walking | 60 ± 6 | 50 ± 5 | <0.0001 | 74 ± 7 | 73 ± 7 | 0.86 |

Values are means ± SD. \(P\), unpaired, two-tailed Student’s \(t\)-test.
Furthermore, they examined dietary, rather than endogenous, FA oxidation (13, 14). Votruba, Zeddun, and Schoeller (14) reported that when $d_{31}$-palmitate and $[1^{13}C]$-palmitate were ingested in a liquid meal, cumulative acetate-corrected $^{13}C$-palmitate oxidation was similar to cumulative acetate-uncorrected $d_{31}$-palmitate oxidation. Raman et al. (13) further reported that hydrogen-acetate recovery correction was unnecessary when $d_{31}$-palmitate was used to assess dietary fat oxidation during physical activity. Similarly, in the present study in the postprandial condition, we found that the time course of the cumulative acetate-uncorrected $[9,10^{3}H]$-palmitate oxidation did not significantly differ from that of acetate-corrected $[1^{14}C]$-palmitate oxidation. However, acetate-corrected $[1^{14}C]$-palmitate oxidation was $\sim20\%$ greater than acetate-uncorrected $[9,10^{3}H]$-palmitate oxidation (Fig. 6, middle panel). It is possible that because FA oxidation is suppressed postprandially, the inclusion of the relatively small acetate-hydrogen recovery factor does not have a major quantitative impact on the reduced FA oxidation values. However, applying the $[^{3}H]$-acetate and $[1^{14}C]$-acetate recovery factors to $[9,10^{3}H]$-palmitate and $[1^{14}C]$-palmitate oxidation, respectively, provided the best agreement between the two FA tracers in the postprandial protocol in our study (Fig. 7, middle panel).

Applying the $[^{3}H]$-acetate and $[1^{14}C]$-acetate recovery factors to account for label sequestration resulted in the same $^{3}H$- and $^{14}C$-labeled palmitate oxidation rates in the postabsorptive resting and walking conditions. It is well-established that carbon-labeled FA tracers need to be corrected for carbon retention and fixation in the TCA cycle. Therefore, it was not surprising that without acetate-carbon correction, the time course of cumulative $[1^{14}C]$ palmitate oxidation was $\sim40\%$ lower than that of $[9,10^{3}H]$ palmitate oxidation (Fig. 5). However, the reverse was observed when $[1^{14}C]$-acetate recovery was used but the $[^{3}H]$-acetate recovery was not taken into consideration. In this case, the 24 h time course of $[1^{14}C]$-palmitate oxidation

![Postabsorptive](image1.png) ![Postprandial](image2.png) ![Walking](image3.png)

**Fig. 6.** Twenty-four hour time courses of cumulative $[9,10^{3}H]$-palmitate and $[1^{14}C]$-palmitate oxidation. $[1^{14}C]$-palmitate oxidation was corrected with the point-by-point group average percent $[1^{14}C]$-acetate recovery obtained from the corresponding $[1^{14}C]$-acetate protocol. $[9,10^{3}H]$-palmitate oxidation data were not corrected for hydrogen label sequestration. Error bars indicate SD.

![Postabsorptive](image4.png) ![Postprandial](image5.png) ![Walking](image6.png)

**Fig. 7.** Twenty-four hour time courses of cumulative $[9,10^{3}H]$-palmitate and $[1^{14}C]$-palmitate oxidation when both tracers were corrected with the corresponding acetate recovery factor. Error bars indicate SD.
was \( \sim 20\% \) greater than that of \([9,10-^3\text{H}]\)palmitate oxidation (Fig. 6). Thus, although hydrogen label sequestration is not as extensive as carbon label sequestration, in order to optimally measure FFA oxidation using a \([9,10-^2\text{H}]\) palmitate bolus, we did need to correct \(^3\text{H}\) recovery in body water with the appropriate hydrogen-acetate recovery factor in the postabsorptive, postprandial, and walking conditions.

As shown in Figs. 1 and 2, the opportunity for hydrogen label sequestration during FA oxidation is less than that for carbon label. If all of the hydrogen label that is transferred to NAD\(^+\) and FAD during \(\beta\)-oxidation eventually appears in body water, then only \( \sim 25\% \) of the hydrogen label enters the TCA cycle where it may be sequestered in isotopic exchange reactions. Furthermore, if we assume that the percent acetate-hydrogen label sequestration in the TCA cycle is equal to the acetate-carbon label sequestration, we can estimate that the average acetate-hydrogen recoveries should be \( \sim 90\% \) in the postabsorptive condition \([100-(25\% \times 41\%)]\), \( \sim 87\% \) in the postprandial condition \([100-(25\% \times 48\%)]\), and \( \sim 91\% \) in the walking condition \([100-(25\% \times 35\%)]\). In the present study, cumulative \(^2\text{H}\)acetate recovery was \( \sim 80\% \) in each condition, which is somewhat lower than the theoretical values. A previous study reported a cumulative \(^3\text{H}\)acetate recovery of \( \sim 88\% \), when acetate was orally administered and volunteers were subjected to low-intensity exercise (13). It is unknown whether the lower acetate-hydrogen recovery in the present study is related to the different route of tracer administration (intravenous vs. oral), the different population, and/or the different methodologies employed (\(^2\text{H}\)- vs. \(^3\text{H}\)-labeled tracers).

Investigators using a continuous intravenous tracer infusion found that acetate-carbon recovery was greater during exercise than during rest (7–9). A nonlinear relationship has also been described between acetate-carbon recovery and oxygen consumption (7). During exercise, TCA cycle activity is accelerated in relation to the exchange reactions, perhaps reducing the sequestration of acetate-carbon label in exchange reactions. In our experiments, the maximum \([1-{^{13}\text{C}}]\)acetate recovery in \(^{14}\text{CO}_2\) was not statistically different among the post-absorptive, postprandial, and walking conditions. However, at the slow walking speed of \( \sim 2 \text{ mph} \) (oxygen consumption of \( \sim 8.5 \text{ ml/kg/min} \) vs. \( \sim 3.5 \text{ ml/kg/min} \) in the resting condition), it is likely that we did not achieve the high level of TCA cycle activity needed to detect greater acetate-carbon recovery than the resting condition. An alternative explanation for the similar carbon-labeled acetate recovery between the resting and the walking conditions is the route of administration (bolus injection vs. continuous infusion) and the long observation period in our study. In line with our findings, dietary carbon-labeled acetate recovery has been shown to be similar \( (\sim 51\% ) \) between resting and physical activity conditions (11). Collectively these results emphasize the importance of using identical experimental protocols between acetate and FA tracers in oxidation studies.

We found that the appearance of \(^{14}\text{CO}_2\) from \([1-{^{14}\text{C}}]\)acetate increased at an \( \sim 50\% \) faster rate in the walking protocol than in the other two conditions. Faster (rather than greater) carbon-label recovery during walking is probably due to more-rapid equilibration of the labeled carbon with the bicarbonate/\(\text{CO}_2\) pool(s). Notably, \([^{13}\text{C}]\)acetate recovery was always much slower than \(^3\text{H}\)acetate recovery (Fig. 3), probably because the hydrogen label mixes very rapidly with the body water pool, whereas longer time is required for the carbon label to transit the bicarbonate pools.

The inter-individual variability of acetate-carbon recovery (16.9–20.4\%) was greater than previous reports in continuous infusion studies (12.0–16.1\%) (5) or dietary fat oxidation studies (10.6–12.2\%) (11). The somewhat greater inter-individual variance in our studies partly reflects the fact that we included women and men with a wide range of adiposity, but it might also relate to the fact that the tracer was administered as an intravenous bolus injection. Interestingly, the CVs of acetate-hydrogen (2.0–8.0\%) were substantially lower than those of acetate-carbon recovery. This suggests that using hydrogen-labeled tracers to study cumulative FA metabolism may be more favorable than using carbon-labeled tracers, because the metabolism of the hydrogen label is characterized by a lower inherent variability.

Because of the high inter-individual CV that they observed in their continuous-infusion acetate-carbon recovery study, Schrauwen et al. (4) advised investigators to measure the acetate recovery factor for every individual. The present studies were part of a complex protocol assessing the nonoxidative disposal of plasma FFA into different tissues. Because of the complexity of the studies, it was not feasible to assess an acetate recovery factor for each individual and in a larger sample. However, we took precautions to control for factors that affect acetate-carbon recovery, such as sex, age, and adiposity (5). Another limitation was that the use of \(^{13}\text{C}\)- and \(^3\text{H}\)-labeled FAs was not performed in the same group of individuals, so a paired analysis of agreement of the two tracers could not be performed (24). Finally, it was not feasible to assess intra-subject variability. Despite of the above limitations, the present study provides physiologically relevant and novel information on the use of two different FA labels administered intravenously under three different nutritional conditions.

In conclusion, \([9,10-^3\text{H}]\)palmitate and \([1-{^{14}\text{C}}]\)palmitate tracers administered as an intravenous bolus injection gave similar estimates of 24 h cumulative plasma FA oxidation in age-, sex-, and BMI-matched individuals under postabsorptive, postprandial, and walking conditions. Although the sequestration of \(^3\text{H}\) label was substantially lower than that of \(^{14}\text{C}\), the need to use an acetate-hydrogen recovery factor was not eliminated. Therefore, each FA tracer had to be corrected with its corresponding acetate recovery factor. Future studies should validate hydrogen-labeled and carbon-labeled FA tracers administered as bolus injections in the same group of individuals.
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REFERENCES

1. Blaak, E. E., D. P. van Aggel-Leijssen, A. J. Wagenmakers, W. H. Saris, and M. A. van Baak. 2000. Impaired oxidation of plasma-derived fatty acids in type 2 diabetic subjects during moderate-intensity exercise. Diabetes. 49:2102–2107.

2. Blaak, E. E., B. H. Wolfenbuttel, W. H. Saris, M. M. Pelsers, and A. J. Wagenmakers. 2001. Weight reduction and the impaired plasma-derived free fatty acid oxidation in type 2 diabetic subjects. J. Clin. Endocrinol. Metab. 86:1638–1644.

3. Mittendorfer, B., L. S. Sidossis, E. Walser, D. L. Chinkes, and R. R. Wolfe. 1998. Regional acetate kinetics and oxidation in human volunteers. Am. J. Physiol. 274:E978–E983.

4. Schrauwen, P., D. P. Aggel-Leijssen, M. W. Lichtenbelt, M. Baak, and A. P. Gijzen. 1998. Validation of the [1,2-13C] acetate recovery factor for correction of [13C2] palmitate oxidation rates in humans. J. Physiol. 515:215–223.

5. Schrauwen, P., E. E. Blaak, D. P. Van Aggel-Leijssen, L. B. Borghouts, and A. J. Wagenmakers. 2000. Determinants of the acetate recovery factor: implications for estimation of [13C]substrate oxidation. Clin. Sci. 98:587–592.

6. Sidossis, L. S., A. R. Coggan, A. Gastaldelli, and R. R. Wolfe. 1995. Pathway of free fatty acid oxidation in human subjects. J. Clin. Invest. 95:278–284.

7. Sidossis, L. S., A. R. Coggan, A. Gastaldelli, and R. R. Wolfe. 1995. A new correlation factor for use in tracer estimations of plasma fatty acid oxidation. Am. J. Physiol. 269:E649–E656.

8. Trimmer, J. K., G. A. Casazza, M. A. Horning, and G. A. Brooks. 2001. Recovery of (13)CO2 during rest and exercise after [1-(13)C]acetate, [2-(13)C]acetate, and NaH(13)CO3 infusions. Am. J. Physiol. Endocrinol. Metab. 281:E683–E692.

9. van Hall, G., M. Sacchetti, and G. Radegran. 2002. Whole body and leg acetate kinetics at rest, during exercise and recovery in humans. J. Physiol. 542:263–272.

10. Pouteau, E., P. Maugere, D. Darmaun, J. S. Marchini, H. Pilquet, H. Dumon, P. Nguyen, and M. Krempf. 1998. Role of glucose and glutamine synthesis in the differential recovery of 13CO2 from infused [2-13C] versus [1-13C] acetate. Metabolism. 47:549–554.

11. Bergouignan, A., D. A. Schoeller, S. Votrubca, C. Simon, and S. Blanc. 2008. The acetate recovery factor to correct tracer-derived dietary fat oxidation in humans. Am. J. Physiol. Endocrinol. Metab. 294:E645–E653.

12. Bergouignan, A., G. Trudel, C. Simon, A. Chopard, D. A. Schoeller, I. Momken, S. B. Votrubca, M. Desage, G. C. Burdge, G. Gauquelin-Koch, et al. 2009. Physical inactivity differentially alters dietary oleate and palmitate trafficking. Diabetes. 58:367–376.

13. Raman, A., S. Blanc, A. Adams, and D. A. Schoeller. 2004. Validation of deuterium-labeled fatty acids for the measurement of dietary fat oxidation during physical activity. J. Lipid Res. 45:2339–2344.

14. Votrubca, S. B., S. M. Zeddun, and D. A. Schoeller. 2001. Validation of deuterium labeled fatty acids for the measurement of dietary fat oxidation: a method for measuring fat-oxidation in free-living subjects. Int. J. Obes. Relat. Metab. Disord. 25:1240–1245.

15. Eaton, R. P., M. Berman, and D. Steinberg. 1969. Kinetic studies of plasma free fatty acid and triglyceride metabolism in man. J. Clin. Invest. 48:1560–1579.

16. Friedberg, S., J., R. F. Klein, D. L. Trout, M. D. Bogdonoff, and E. H. Estes, Jr. 1961. The incorporation of plasma free fatty acids into plasma triglycerides in man. J. Clin. Invest. 40:1846–1855.

17. Koutsari, C., A. H. Ali, M. S. Mundi, and M. D. Jensen. 2011. Storage of circulating FFA in adipose tissue of postabsorptive humans: quantitative measures and implications for body fat distribution. Diabetes. 60:2032–2040.

18. Koutsari, C., M. S. Mundi, A. H. Ali, and M. D. Jensen. 2012. Storage rates of circulating free fatty acid into adipose tissue during eating or walking in humans. Diabetes. 61:329–338.

19. Peterson, L. R., P. Herrero, J. McGill, K. B. Schechtman, Z. Kisrieva-Ware, D. Lesniak, and R. J. Gropler. 2008. Fatty acids and insulin modulate myocardial substrate metabolism in humans with type 1 diabetes. Diabetes. 57:32–40.

20. Rigazio, S., H. R. Lehto, H. Tuunanen, K. Nagren, M. Kankaanpaa, C. Simi, R. Borra, A. G. Naum, R. Parkkola, J. Knuuti, et al. 2008. The lowering of hepatic fatty acid uptake improves liver function and insulin sensitivity without affecting hepatic fat content in humans. Am. J. Physiol. Endocrinol. Metab. 295:E413–E419.

21. Wolfe, R. R., and F. Jahoor. 1990. Recovery of labeled CO2 during the infusion of C-1 vs. C-2 labeled acetate: implications for tracer studies of substrate oxidation. Am. J. Clin. Nutr. 51:248–252.

22. Schoeller, D. A., E. vanSanten, D. W. Peterson, W. Dietz, J. Jaspen, and P. D. Klein. 1980. Total body water measurement in humans with 18O and 2H labeled water. Am. J. Clin. Nutr. 33:2686–2695.

23. Romanski, S. A., R. Nelson, and M. D. Jensen. 2000. Meal fatty acid uptake in adipose tissue: gender effects in non-obese humans. Am. J. Physiol. 279:E455–E462.

24. Bland, J. M., and D. G. Altman. 1986. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet. 1:307–310.