Role of Glucose and Glutamine Synthesis in the Differential Recovery of $^{13}CO_2$ From Infused [2-^{13}C] Versus [1-^{13}C] Acetate

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Carbon exchange in the Krebs cycle may result in underestimation of substrate oxidation measured with ^{13}C-labeled substrates, since carbon labeled in position 2 of acetyl-coenzyme A (CoA) could be incorporated into glucose (via gluconeogenesis) and glutamine. Five healthy volunteers were therefore infused with [1-^{13}C] and [2-^{13}C] acetate at a rate of 0.5 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ for 165 minutes on two different occasions in randomized order. Whole body acetate turnover did not differ between the two tracers: 7.9 ± 0.3 and 7.5 ± 0.6 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ (nonsignificant [NS]) for [1-^{13}C] and [2-^{13}C] acetate, respectively. Isotopic ^{13}C enrichment was higher in expired CO_2 (0.177 ± 0.021 *v* 0.089 ± 0.009 atom percent excess [APE], $P < .01$) and lower in glucose (0.074 ± 0.017 *v* 0.291 ± 0.061 mole percent excess [MPE], $P < .01$) for [1-^{13}C] acetate compared with [2-^{13}C] acetate, respectively, at the end of the infusions. Glutamine isotopic enrichment was slightly but not significantly higher when infusing [1-^{13}C] acetate versus [2-^{13}C] acetate (0.348 ± 0.038 *v* 0.495 ± 0.069 MPE, NS, respectively). At the end of the experiment, the recovery of $^{13}CO_2$ from [1-^{13}C] acetate was 44.8% ± 2.7%, and from [2-^{13}C] acetate, 22.6% ± 1.3%. A significant correlation was observed between the differences in ^{13}C enrichment of CO_2 for the two tracers and glucose ($\Delta CO_2 = 0.424 \cdot \Delta glucose + 0.001$, $R^2 = .9856$, $P = .0007$) or glutamine ($\Delta CO_2 = 0.621 \cdot \Delta glutamine + 0.004$, $R^2 = .9573$, $P = .0038$) during the infusion. These results suggest that (1) although gluconeogenesis appears to be more responsible than glutamine for the differential recovery of [2-^{13}C] versus [1-^{13}C] acetate, other secondary pathways are probably also implicated; and (2) different recovery correction factors should be applied when measuring substrate oxidation with a stable isotope tracer depending on the expected position of ^{13}C in acetyl-CoA.

CARBON-LABELED TRACERS have been used extensively for measurement of substrate oxidation.[1] In vivo in humans, the method relies on the assumption that the rate of labeled carbon dioxide excretion directly reflects the rate of substrate oxidation. This assumption was recently challenged by Wolfe and Jahoor.[2] As pointed out by Strisower et al,[3] although a net production of two carbon dioxide molecules should be expected from the entry of the two carbons of acetyl coenzyme A (acetyl-CoA) into the tricarboxylic acid (TCA) cycle, there is a chance that when both of these carbons are labeled, both labels will not end up as labeled carbon dioxide, particularly if the label is in position 2 of acetyl-CoA. Recently, Wolfe and Jahoor[2] clearly demonstrated that when [1-^{13}C] or [2-^{13}C] acetate were infused into human subjects in the postabsorptive state, a dramatic reduction in ^{13}C recovery as $^{13}CO_2$ was observed with [2-^{13}C] versus [1-^{13}C] tracer (53% *v* 81%). From these findings, it was suggested that this difference in recovery related to the labeled position in acetyl-CoA should be taken into account in the calculation of substrate oxidation measured with carbon-labeled tracers.[2] This difference could be explained by a variable exchange rate of acetyl-CoA carbons within the TCA cycle depending on the label position. Carbons in position 2 of acetyl-CoA are more likely directed toward gluconeogenesis, while carbons in position 1 are mainly converted to carbon dioxide.[2] To further explore the relationship between the difference in oxidation with [1-^{13}C] or [2-^{13}C] acetate and carbon exchange within the oxaloacetate pool that is a precursor of gluconeogenesis, Wolfe and Jahoor[2] infused one subject with both acetate tracers during exogenous glucose infusion. However, the same difference in $^{13}CO_2$ recovery was again observed between the two tracers. This result could be explained if one assumes that a major fraction of hepatic glycogen synthesis occurs via the "indirect" gluconeogenic pathway[4,5]; alternatively, this could be accounted for by carbon exchange of acetyl-CoA with other compounds such as glutamine.[6]

The aim of this study was to assess recovery coefficients proposed for the correction of data in calculation of substrate oxidation. Moreover, we tested the hypothesis that an uptake in glutamine synthesis and in the gluconeogenesis pathway of the carbon derived from acetyl-CoA labeled in position 2 indeed occurred in vivo, as determined from measurement of ^{13}C incorporation into plasma glutamine and glucose using the gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS) technique, and could explain most of the difference in $^{13}CO_2$ recovery of the two acetate tracers.

SUBJECTS AND METHODS

Subjects

Five healthy subjects (three women and two men) were included in the study. They were not taking any medication and were disease-free on the basis of physical examination. They had no personal or family history of diabetes mellitus. They were aged 22 to 28 years and of normal weight (58.2 ± 3.1 kg; body mass index, 19.3 ± 0.6 $kg \cdot m^{-2}$). The potential risks were fully explained to each subject before written

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consent was obtained. The experimental protocol was approved by the Ethics Committee of Nantes University Hospital.

Experimental Design

To avoid any disturbance of endogenous acetate metabolism by "exogenous" acetate production from the bacterial colonic fermentation of carbohydrate, the subjects were asked to consume a low-fiber diet (<5 g per day) for 3 days before entry into the study. The study was conducted after a 12-hour overnight fast. A hydrogen breath test was performed before starting the studies, and no subject showed any significant colonic fermentation ($H_2 < 10$ ppm).

The subjects came to the clinical research center on two separate days. [1-^{13}C] acetate or [2-^{13}C] acetate (both 99% enrichment; Tracer Technologies, Somerville, MA) were infused intravenously into the subjects in random order within 2 weeks. On the morning of the experiments, a short polyethylene catheter (20 G; Vygon, Paris, France) was inserted into an antecubital vein for infusion of the tracer. A second catheter was placed on the back of the opposite hand for blood sampling. The hand was warmed to 55°C with an electric heating pad to obtain arterialized venous blood.[7] A continuous infusion of [1-^{13}C] acetate or [2-^{13}C] acetate was started at a rate of 0.5 μmol · kg^{-1} · min^{-1}. No priming dose was used. Arterialized blood samples were obtained at baseline and after 30, 60, 90, 120, 135, 150, and 165 minutes of infusion. Expired air was collected at the same time points, and respiratory gas exchange was measured using an indirect calorimeter (Delta Trac, Helsinki, Finland) during the last hour of infusion.

Analytical Procedures

Acetate analysis. Analysis of plasma acetate enrichment was performed using our previously published method.[8] Briefly, plasma samples (500 μL) were deproteinized with 10 mg sulfosalicylic acid (Sigma, St Quentin Fallavier, France) and centrifuged at 2,200× *g* for 10 minutes. The supernatant was transferred to a vial containing 30 μL HCl (10N; Panreac, Barcelona, Spain), and 3 mL diethyl ether (Merck, Darmstadt, Germany) was added. The vial was vortexed for 15 minutes and centrifuged for 10 minutes at 1,200× *g* for extraction. Eight microliters of *tert*-butyl-dimethyl-silyl-imidazole (Fluka Chemika, Buchs, Switzerland) was added to the separated organic phase. Then, the sealed vial was heated at 60°C for 30 minutes. The sample was cooled and evaporated to 500 μL.

A volume of 2 μL was injected into a gas chromatograph (model 5890A; Hewlett Packard, Palo Alto, CA) connected to a quadrupole mass spectrometer (5971A; Hewlett Packard). Chromatographic separation was performed with a 30-m × 0.25-mm capillary column (DB5; J/W Scientific, Folsom, CA). The temperature program was as follows: initial temperature at 80°C for 1 minute, and then 10°C · min^{-1} up to 180°C. Methane chemical ionization and selected ion monitoring were used on ions at *m/z* 175 and 176. Calibration curves were obtained from known isotopic enrichment solutions in the range of 0.5% to 15% for [1-^{13}C] acetate and [2-^{13}C] acetate. The within-assay (n = 10) and between-assay (n = 4) coefficients of variation were 5% and 7%, respectively.

Glucose analysis. To assess isotopic enrichment in glucose, a penta-acetate derivative of glucose was prepared from plasma samples (200 μL) as previously described[1] with acetic anhydride (Prolabo, Paris, France) and pyridine (Merck, Stockholm, Denmark).

One microliter was injected into a gas chromatograph (5890A) connected to a 900°C combustion furnace online with an isotopic ratio mass spectrometer (Delta S; Finnigan MAT, Bremen, Germany) for GC/C/IRMS. Chromatographic separation was attained using a 30-m × 0.252-mm capillary column (DB5; J/W Scientific) heated at 220°C for 10 minutes. The apparatus was calibrated against Pee Dee Belemnite (PDB) carbonate. The accuracy of glucose derivative enrichment was checked against a standard of glucose penta-acetate (Aldrich, Gillingham, UK) with known ^{13}C enrichment obtained from a direct combustion and IRMS analysis. Because of the dilution of ^{13}C enrichment due to carbons from the derivatization agent, true glucose enrichment was determined from a standard curve obtained from beet (1.0839 atom percent [AP]) and corn starch (1.1009 AP) glucose. The values for enrichment of the standards were obtained by direct combustion without derivatization in an elemental analyzer coupled to an IRMS (Finnigan MAT).

Glutamine analysis. Plasma glutamine was separated from glutamate as previously described.[9,10] Briefly, a 500-μL aliquot of plasma was deproteinized with 300 μL 10% 5-sulfosalicylic acid (Sigma, St Louis, MO). After centrifugation, the supernatant was mixed with 120 μL 1-mol/L potassium hydroxide solution (Aldrich, Milwaukee, WI) and 3 mL pH 9 buffer. The solution was poured onto an anion-exchange resin column (formate form, AG1X8, 200-400 mesh; Bio-Rad, Richmond, CA). The column was rinsed with pH 9 buffer (3 mL). All eluate was collected and underwent a "clean-up" procedure: The glutamine fraction was acidified with 1 mL 1-mol/L hydrochloric acid solution (Sigma). It was then poured onto a cation-exchange resin column (Hydrogen form, Dowex-50W, 100-200 mesh; Sigma) and rinsed with distilled water (10 mL). The eluate was discarded. Then, 2 mL 3-mol/L ammonium hydroxide solution (Sigma) was poured onto the column to elute the amino acids, and the eluate was dried under nitrogen. The *n*-acetyl-*n*-propyl (NAP) derivatives of glutamate were prepared as previously reported[9] and dissolved in ethyl acetate (50 μL; Rathburn, Walkeburn, Scotland); 1 μL was then injected into the capillary column (ultra 1; Hewlett Packard, Les Ulis, France) of the GC/C/IRMS. Chromatographic conditions were as follows: initial temperature at 80°C, with a ramp at 25°C · min^{-1} up to 250°C, and a plateau at 250°C for 1 minute. After chromatographic separation and total combustion by the furnace interface, isotopic enrichment was measured against PDB by the isotopic ratio mass spectrometer. Isotopic enrichment was calculated using a calibration curve prepared with standards of known ^{13}C glutamine enrichment and analyzed on the same day as the plasma samples. Typical values for the ^{13}C glutamine calibration curve were 0.0007 and 0.98 for the intercept and slope, respectively, when the measured enrichment (mole percent excess [MPE]) was plotted as a function of the expected enrichment (MPE).

CO_2 analysis. Total expired carbon dioxide production was determined by indirect calorimetry. To measure the isotopic enrichment of expired CO_2, an 80-μL breath sample was injected into the GC/C/IRMS using a PoraPLOT column (Chrompack, The Netherlands) at 30°C. A laboratory CO_2 standard calibrated against the international PDB carbonate was used.

Calculation Methods

Rate of appearance of acetate. The total rate of appearance (Ra) of acetate was calculated according to the equation for steady state, Ra = i(Et/Ep − 1), where i is the infusion rate in micromoles per kilogram per minute, and Et and Ep are the isotopic enrichment of the tracer solution ([1-^{13}C] acetate or [2-^{13}C] acetate) and of plasma, respectively, expressed in MPE.

$^{13}CO_2$ enrichment. The measured data for $^{13}CO_2$ enrichment were expressed in δ^{13}C (in ‰), and the values obtained in breath CO_2 from the GC/C/IRMS were converted to AP,[11] $AP = 100R_{pdb}(0.001\delta^{13}C + 1)/(1 + R_{pdb}[0.001\delta^{13}C + 1])$, where R_{pdb} is the $^{13}C/^{12}C$ ratio of the international PDB standard ($R_{pdb} = 0.0112372$) and δ^{13}C is ^{13}C enrichment of the CO_2 samples. Isotopic enrichment of expired CO_2 was expressed in terms of AP excess (APE), $APE = AP_s - AP_0$, where AP_s is the AP of the sample at different sampling times, and AP_0 is the AP at time zero.

$^{13}CO_2$ excretion rate. $^{13}CO_2$ excretion at time 165 minutes (in micromoles per kilogram per minute) was estimated[1] as $^{13}CO_2$ excretion = $(ECO_2 \cdot \dot{V}CO_2)/M$, where ECO_2 is the isotopic enrichment of expired

CO_2 in APE, $\dot{V}CO_2$ is the total rate of CO_2 production (micromoles per minute), and M is the weight of the subject (kilograms).

Fractional recovery. At time 165 minutes, the fractional recovery in breath CO_2 of label derived from infusion of ^{13}C acetate was calculated: fractional recovery = $(ECO_2 \cdot \dot{V}CO_2)/i$.

Glucose enrichment. ^{13}C glucose enrichment was first expressed in δ^{13}C and then converted to AP by the above-mentioned formula. Because each combusted molecule of glucose yields six CO_2 molecules, the data were expressed in MP by multiplying the AP values by six. Glucose enrichment was expressed in MPE.

Glutamine enrichment. ^{13}C glutamine enrichment was expressed in δ^{13}C and converted to AP. The APE was calculated at each time as already explained. APE values were expressed in MPE with a calibration curve made using standard solutions of known ^{13}C glutamine enrichment.

Correlation between glucose or glutamine and CO_2 isotopic enrichment. The penta-acetate glucose enrichment difference (Δglucose) at a given sampling time was defined as the derivative glucose isotopic enrichment (in δ^{13}C ‰) during [2-^{13}C] acetate infusion minus its enrichment during [1-^{13}C] acetate infusion. Similarly, the NAP derivative glutamine enrichment difference (Δglutamine) at a given sampling time was defined as the derivative glutamine isotopic enrichment (in δ^{13}C ‰) during [2-^{13}C] acetate infusion minus its enrichment during [1-^{13}C] acetate infusion. Furthermore, the CO_2 enrichment difference (ΔCO_2) at a given sampling time was defined as $^{13}CO_2$ enrichment (in δ^{13}C ‰) during [1-^{13}C] acetate infusion minus its enrichment when infusing [2-^{13}C] acetate. Linear regression analysis between the derivative glucose or glutamine enrichment difference and CO_2 enrichment difference was performed by the least-squares method.

Statistical Analysis

Results are reported as the mean ± SEM. One- and two-way ANOVA for multiple comparison was performed using SuperANOVA software (Abacus Concepts, Berkeley, CA), and a two-tailed paired *t* test was used with the Instat Statistical Software package (GraphPad, San Diego, CA) to determine significant differences between groups. Linear regression and correlation analysis were performed using Instat software.

RESULTS

Individual plasma isotopic enrichment and acetate turnover data are shown in Table 1. Steady state was obtained in all cases within the first 30 minutes of the unprimed tracer infusion. The acetate flux rate was 7.9 ± 0.3 μmol · kg^{-1} · min^{-1} for [1-^{13}C] acetate and 7.5 ± 0.6 μmol · kg^{-1} · min^{-1} for [2-^{13}C] acetate.

Table 1. Plasma Isotopic Enrichment (MPE) and Turnover Rate (μmol · kg^{-1} · min^{-1}) of Acetate Measured at Plateau With [1-^{13}C] Acetate and [2-^{13}C] Acetate Intravenous Infusions (0.5 μmol · kg^{-1} · min^{-1}) in Five Healthy Subjects

Subject No./Sex	[1-^{13}C] Acetate		[2-^{13}C] Acetate	
	Enrichment	Turnover	Enrichment	Turnover
1/F	6.4 ± 0.5	7.8 ± 0.5	4.7 ± 0.3	9.4 ± 0.8
2/F	6.3 ± 0.3	8.2 ± 0.5	5.6 ± 1.2	8.1 ± 2.0
3/M	5.3 ± 0.4	8.3 ± 0.7	6.9 ± 0.8	6.6 ± 0.8
4/M	6.4 ± 0.3	6.9 ± 0.4	6.3 ± 0.5	7.2 ± 0.6
5/F	7.0 ± 0.7	8.2 ± 0.9	8.9 ± 0.4	6.1 ± 0.3
Mean ± SEM	6.3 ± 0.3*	7.9 ± 0.3*	6.5 ± 0.7	7.5 ± 0.6

Abbreviations: F, female; M, male.
*NS, [1-^{13}C] acetate *v* [2-^{13}C] acetate.

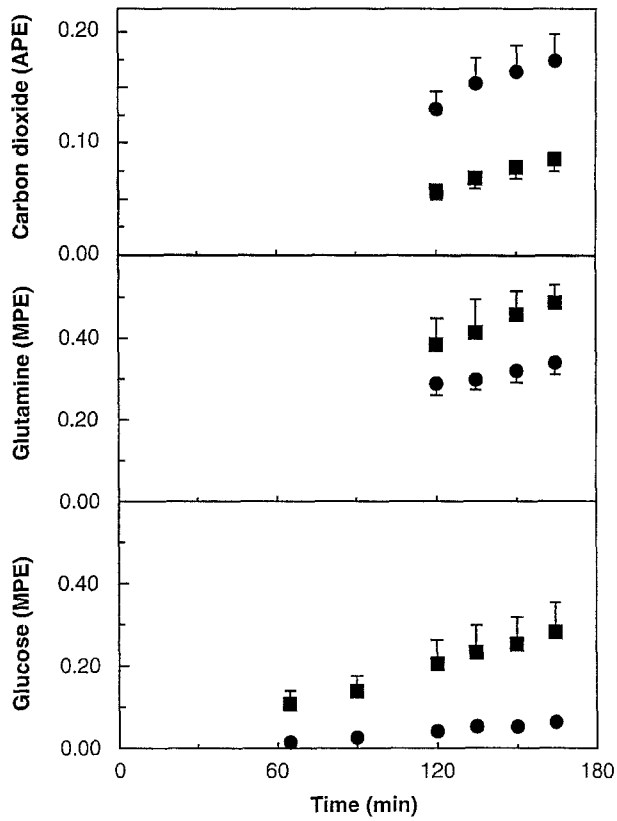

Fig 1. Isotopic enrichment of expired CO_2 (in APE) and plasma glucose and glutamine (in MPE) during a [1-^{13}C] acetate (●) and [2-^{13}C] acetate infusion.

No significant difference was observed for either enrichment at plateau or flux between the two tracers.

In contrast, enrichment in expired $^{13}CO_2$ was different depending on the tracer used ($P < .01$, two-way ANOVA, Fig 1). At the end of the experiments, [2-^{13}C] acetate infusion produced an isotopic enrichment of expired CO_2 (0.089 ± 0.009 APE) only half as high as that produced by [1-^{13}C] acetate (0.177 ± 0.021 APE, $P < .01$; Tables 2 and 3). During both experiments, enrichment increased from time zero to 165 minutes. The rate of $^{13}CO_2$ excretion was higher from [1-^{13}C] acetate infusion versus [2-^{13}C] acetate infusion (0.24 ± 0.02 *v* 0.11 ± 0.01 μmol · kg^{-1} · min^{-1}, $P < .01$). The fractional ^{13}C recovery was 44.8% ± 2.7% and 22.6% ± 1.3% for [1-^{13}C] and [2-^{13}C] acetate infusion, respectively ($P < .01$; Table 4).

The isotopic enrichment of plasma glucose increased with time for both tracers over the course of the infusion and was more than four times higher ($P < .01$, two-way ANOVA) with [2-^{13}C] acetate (0.291 ± 0.061 MPE) compared with [1-^{13}C] acetate (0.074 ± 0.017 MPE) at time 165 minutes, and nearly reached a plateau at the end of the study (Fig 1 and Table 2). ^{13}C enrichment in plasma glutamine increased during both tracer infusions (Fig 1). At the end of the infusion, glutamine enrichment was lower, although not significantly different, throughout the study (two-way ANOVA) with [1-^{13}C] acetate versus [2-^{13}C] acetate (0.495 ± 0.069 *v* 0.348 ± 0.038 MPE, respectively; Table 2). The ratio of isotopic enrichment in glutamine when infusing [1-^{13}C] acetate versus [2-^{13}C] acetate

Table 2. Isotopic Enrichment of Expired CO_2 (APE) and Glucose (MPE) and Glutamine (MPE) After 165 Minutes of [1-^{13}C] and [2-^{13}C] Acetate Intravenous Infusion

	[1-^{13}C] Acetate			[2-^{13}C] Acetate		
Subject No.	CO_2	Glucose	Glutamine	CO_2	Glucose	Glutamine
1	0.189	0.056	0.387	0.083	0.216	0.512
2	0.232	0.098	0.420	0.108	0.316	0.432
3	0.132	0.035	0.260	0.071	0.212	0.405
4	0.135	0.060	0.271	0.074	0.215	0.399
5	0.195	0.120	0.403	0.108	0.496	0.727
Mean ± SEM	0.177 ± 0.021	0.074 ± 0.017	0.348 ± 0.038	0.089 ± 0.009	0.291 ± 0.061	0.495 ± 0.069

was lower than the corresponding ratio for glucose ($P < .005$; Table 3).

Differences for enrichment (MPE) in plasma ^{13}C glucose and expired $^{13}CO_2$ between infusion of [1-^{13}C] and [2-^{13}C] acetate at five consecutive times were shown to fit a linear regression with the following equation: $\Delta CO_2 = 0.424 \cdot \Delta\text{glucose} + 0.001$ ($R^2 = .9856$, $P = .0007$). Similarly, a linear regression was observed between plasma ^{13}C glutamine and expired $^{13}CO_2$ enrichment (MPE) differences: $\Delta CO_2 = 0.621 \cdot \Delta\text{glutamine} + 0.004$ ($R^2 = .9573$, $P = .0038$).

DISCUSSION

In the current study, similar values for an acetate turnover rate of about 8 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ were observed when healthy human subjects were infused in the postabsorptive state with [1-^{13}C] or [2-^{13}C] acetate. In contrast, $^{13}CO_2$ excretion was found to be higher with [2-^{13}C] acetate versus [1-^{13}C] acetate infusion. When infusing [2-^{13}C] acetate, a greater fraction of the ^{13}C tracer was incorporated into glucose and glutamine molecules. This finding would have implications for measurement of the oxidation of acetate or many other substrates, since acetyl-CoA is the ultimate end point of the oxidation of all nutrients.

From the outset, it must be emphasized that because steady state was not attained in expired $^{13}CO_2$ nor in plasma ^{13}C glucose and ^{13}C glutamine at the end of tracer infusion, the results of the present study should be interpreted with caution.

Whatever the tracer used, the present study found the same whole-body turnover rate of acetate (≈8 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) as our earlier studies using [1-^{13}C] acetate.[8,12] Infusing [1-^{14}C] acetate intravenously in humans and dogs, Skutches et al[13] and Bleiberg et al[14] found similar flux rates.

In a previous study using a $^{13}CO_2$ recovery of 0.81,[2] we showed that 70% of acetate was oxidized in the postabsorptive state, implying that about 30% of acetate turnover underwent nonoxidative disposal.[12] If this nonoxidative disposal is correct, the use of acetate recovery recently proposed by Sidossis et al[6,15] to calculate fatty acid oxidation may not be valid, as it will overestimate fat oxidation. Indeed, in their study,[15] it was assumed that 100% of infused acetate molecules enter the TCA cycle even at the end of 86 hours of fasting. This assumption is probably incorrect, since a significant fraction of acetate flux can be incorporated into nonoxidative pathways such as (1) production of ketone bodies, as 15% of hepatic ketone body production was shown to arise from acetate in the dog[14]; and (2) fatty acid and cholesterol synthesis.[16,17] When infusion [1-^{13}C] acetate, Sidossis et al[6,15] observed 56% ^{13}C recovery in expired CO_2. They proposed that the "loss" of labeled carbon was due to exchange with bicarbonate pools with a slow rate of turnover, but also to carbon incorporation into glutamine, glucose, pyruvate, and aspartate molecules via label exchange in the TCA cycle.[6,15] We measured glutamine and glucose label incorporation upon infusion of ^{13}C acetate. Assuming a flux rate of 6 and 11 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ for glutamine and glucose, respectively,[1,18] and knowing the infusion rate of [1-^{13}C] acetate, we can estimate that only 4.2% and 1.6% of infused ^{13}C was incorporated into glutamine and glucose, respectively, after 165 minutes of tracer infusion. From the calculated CO_2 recovery of 44.8%, we could thus explain the fate of 50.6% of the labeled carbon at 165 minutes. Even if we assume that exchanges with aspartate and pyruvate account for the same loss of label (≈6%) and about 20% into bicarbonate pools,[2,6] the result would be only about 77% of label recovery of [1-^{13}C] acetate in those metabolites at 165 minutes. One could speculate that the "missing" label represents nonoxidative disposal of acetate, including incorporation into ketone bodies or lipids. Thus, the recovery coefficient of ^{13}C acetate suggested by

Table 3. Enrichment Ratios After 165 Minutes of [2-^{13}C] Acetate Versus [1-^{13}C] Acetate for CO_2, Glucose, and Glutamine

	Enrichment Ratio		
Subject No.	CO_2	Glucose	Glutamine
1	0.44	3.88	1.32
2	0.46	3.21	1.03
3	0.54	6.08	1.56
4	0.55	3.56	1.47
5	0.55	4.12	1.80
Mean ± SEM	0.51 ± 0.03	4.2 ± 0.6*	1.4 ± 0.1

*$P < .005$, glucose *v* glutamine.

Table 4. $^{13}CO_2$ Excretion Rate ($\mu mol \cdot kg^{-1}1$)) in Breath and Fractional Recovery (%) of Carbon 13 in CO_2 When Infusing [1-^{13}C] or [2-^{13}C] Acetate at 0.5 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ for 165 Minutes

	[1-^{13}C] Acetate		[2-^{13}C] Acetate	
Subject No.	$^{13}CO_2$ Excretion	Recovery	$^{13}CO_2$ Excretion	Recovery
1	0.270	51.0	0.105	23.4
2	0.251	45.6	0.104	23.2
3	0.186	40.4	0.092	19.6
4	0.179	38.3	0.099	20.7
5	0.293	48.8	0.158	26.4
Mean ± SEM	0.24 ± 0.02*	44.8 ± 2.7*	0.11 ± 0.01	22.6 ± 1.3

*$P < .01$, [1-^{13}C] acetate *v* [2-^{13}C] acetate.

Sidossis et al for measuring fatty acid oxidation should be higher than the proposed 56%, if label "escaping" the TCA cycle is taken into account.

In the current study, the recovery of $^{13}CO_2$ from [2-^{13}C] acetate infusion (≈23%) was half that observed with [1-^{13}C] acetate infusion (≈45%) at 165 minutes, as previously reported by Wolfe and Jahoor.[2] This difference of 22% is related in part to exchange of labeled carbon between metabolites and the TCA cycle.[2] Carbon 2 of acetyl-CoA is not eliminated as CO_2 before three rotations of the TCA cycle, while carbon 1 entering the TCA cycle is mostly oxidized to CO_2 within the second rotation. Label at position 2 of acetyl-CoA is more likely involved in labeling TCA metabolites. Accordingly, glucose enrichment was significantly higher (about fourfold) with [2-^{13}C] acetate versus [1-^{13}C] acetate after infusing the tracer for 165 minutes. Strisower et al found a lower (threefold) difference, yet this was measured in vitro and in rats. The difference in label position within the TCA cycle accounted for the slight but nonsignificant ($P > .05$) difference in glutamine enrichment observed with [1-^{13}C] versus [2-^{13}C] acetate infusion. In addition, the plots for the correlation between glucose or glutamine and CO_2 isotopic enrichment differences suggest that gluconeogenesis and glutamine synthesis both contribute to the difference in CO_2 recovery, as previously suggested by others.[1,6] Assuming rates of glutamine and glucose production similar to those measured in earlier studies (already discussed) and knowing the ^{13}C enrichment and infusion rate of [2-^{13}C] acetate, we estimate that at time 165 minutes 5.9% and 6.4% of infused ^{13}C went into glutamine and glucose, respectively. The cumulative recovery of label in CO_2, glutamine, and glucose was thus only 34.9% at time 165 minutes, which pointed to a greater label escape versus [1-^{13}C] acetate infusion. If we assume that about 12% of label entered aspartate and pyruvate and about 20% entered the bicarbonate pool, we would achieve 67% recovery when infusing [2-^{13}C] acetate for 165 minutes versus 77% with [1-^{13}C] acetate. Based on our findings, incorporation of carbon 13 into glucose and glutamine cannot be the sole process explaining the difference in CO_2 recovery between the two tracers. Although the gluconeogenic route contributes more than glutamine synthesis to the loss of tracer, as seen from the ratios for isotopic enrichment, other biochemical pathways must clearly be involved in the approximately 10% difference in label loss between the two acetate tracers.

In summary, differential labeling of acetyl-CoA can lead to artifactually different rates of substrate oxidation due to distinct biochemical fates of labeled carbon. The use of appropriate recovery factors could diminish the discrepancy observed in measuring $^{13}CO_2$ excretion from fatty acid oxidation with the same fatty acid labeled in two different positions, for example, [1-^{13}C] and [16-^{13}C] palmitate as reported by Clandinin et al[19] or [1-^{13}C] and [8-^{13}C] triolein as reported by Metges and Wolfram.[20] Different recovery coefficients should be used to estimate substrate oxidation depending on the label position in the acetyl-CoA molecule and also on the rate of label exchange within the TCA cycle.

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