Recovery of $^{13}$CO$_2$ during rest and exercise after 
[1-$^{13}$C]acetate, [2-$^{13}$C]acetate, and NaH$^{13}$CO$_3$ infusions

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Received 3 August 2000; accepted in final form 3 May 2001

Trimmer, Jeff K., Gretchen A. Casazza, Michael A. Horning, and George A. Brooks. Recovery of $^{13}$CO$_2$ during rest and exercise after [1-$^{13}$C]acetate, [2-$^{13}$C]acetate, and NaH$^{13}$CO$_3$ infusions. Am J Physiol Endocrinol Metab 281: E683–E692, 2001.—For estimating the oxidation rates (Rox) of glucose and other substrates by use of $^{13}$C-labeled tracers, we obtained correction factors to account for label dilution in endogenous bicarbonate pools and TCA cycle exchange reactions. Fractional recoveries of $^{12}$C label in respiratory gases were determined during 225 min of rest and 90 min of leg cycle ergometry at 45 and 65% peak oxygen uptake ($V_{\text{O}_2 \text{peak}}$) after continuous infusions of [1-$^{13}$C]acetate, [2-$^{13}$C]acetate, or NaH$^{13}$CO$_3$. In parallel trials, [6,6-$^{2}$H]glucose and [1-$^{13}$C]glucose were given. Experiments were conducted after an overnight fast with exercise commencing 12 h after the last meal. During the transition from rest to exercise, CO$_2$ production increased ($P < 0.05$) in an intensity-dependent manner. Significant differences were observed in the fractional recoveries of $^{13}$C label as $^{13}$CO$_2$ at rest (NaH$^{13}$CO$_3$, 77.5 ± 2.8%; [1-$^{13}$C]acetate, 49.8 ± 2.4%; [2-$^{13}$C]acetate, 26.1 ± 1.4%). During exercise, fractional recoveries of $^{13}$C label from [1-$^{13}$C]acetate, [2-$^{13}$C]acetate, and NaH$^{13}$CO$_3$ were increased compared with rest. Magnitudes of label recoveries during both exercise intensities were tracer specific (NaH$^{13}$CO$_3$, 93%; [1-$^{13}$C]acetate, 80%; [2-$^{13}$C]acetate, 65%). Use of an acetate-derived correction factor for estimating glucose oxidation resulted in Rox values in excess of $P < 0.05$) of glucose rate of disappearance during hard exercise. We conclude that, after an overnight fast: 1) recovery of $^{13}$C label as $^{13}$CO$_2$ from [1-$^{13}$C]acetate is decreased compared with bicarbonate; 2) the position of $^{13}$C acetate label affects carbon dilution estimations; 3) recovery of $^{13}$C label increases in the transition from rest to exercise in an isotope-dependent manner; and 4) application of an acetate correction factor in glucose oxidation measurements results in oxidation rates in excess of glucose disappearance during exercise at 65% of $V_{\text{O}_2 \text{peak}}$. Therefore, bicarbonate, not acetate, correction factors are advocated for estimating glucose oxidation from carbon tracers in exercising men.

stable isotopes; free fatty acid oxidation; glucose oxidation; carbon dioxide; exertion

SUBSTRATE OXIDATION RATES have been extensively estimated in vivo during rest and exercise by measuring the appearance of labeled CO$_2$ (*CO$_2$) in the breath after the infusion of $^{13}$C- or $^{14}$C-labeled substrates (e.g., [1-$^{13}$C]glucose and [14C]palmitate). However, the appearance of *CO$_2$ is not merely a function of substrate oxidation; rather, recovery of infused tracer carbon is dependent on 1) the oxidation rate of the specified substrate (15), 2) the exchange and temporary fixation of labeled carbon in pathways of intermediary metabolism (19, 30, 32), 3) the equilibration of label with endogenous bicarbonate pools (2, 15, 21), and 4) the duration and quantity of carbon tracer infusion (2, 21, 22, 25). Therefore, valid estimations of plasma metabolite oxidation rates require that the equilibration of *CO$_2$ in the bicarbonate pool and fixation or retention of carbon label in intermediary metabolic pathways be considered (19, 30, 32).

To account for carbon label dilution, correction factors have been calculated from the recovery of *CO$_2$ after intravenous infusion of tracer-NaHCO$_3$ (2, 13, 15, 27) or -acetate (12, 25, 27, 28). The fraction of tracer bicarbonate or acetate carbon not recovered in *CO$_2$ was thought to represent carbon retention in bicarbonate or pools of slowly turning over metabolites formed via CO$_2$ fixation (12, 17, 27); accordingly, specific correction factors have been derived to correct oxidation rates for CO$_2$ retention. However, the different metabolic fates of bicarbonate and acetate carbon have produced different correction factors (27). After observing carbon label fixation in excess of that explained by bicarbonate kinetics, Heiling et al. (11) questioned the validity of bicarbonate correction factors. Recently, Sidossis et al. (27, 28) and others (6, 11, 33) have suggested that NaH$^{13}$CO$_3$-derived correction factors underestimated free fatty acid (FFA) oxidation rates, because exchange of label within the TCA cycle is not accounted for when bicarbonate is used. Subsequently, an acetate-derived correction factor to account for label fixation between entry of acetyl-CoA into the TCA cycle and dilution in the bicarbonate pool was proposed. Previously, Wolfe and Jahoor (33) and Pouteau et al. (22) reported different recoveries of labeled CO$_2$ when [1-$^{13}$C]- and [2-$^{13}$C]acetate were infused and observed...
that carbon in the second position was directed toward gluconeogenesis, whereas carbon in the first position was preferentially liberated into the CO2 pools. Therefore, the specific correction factor used in the calculation of oxidation rates should represent the position of the carbon label on the molecule of interest, or the specific metabolic intermediary, as the labeled substrate enters the TCA cycle. Finally, Sidossis et al. (27, 28) and Wolfe and Jahoor (33) have further suggested that an acetate correction factor could be used for the calculation of plasma glucose oxidation rates. Indeed, there is no need to consider the acetate correction to apply exclusively to glucose oxidation, because any metabolite giving rise to acetate (e.g., fatty acids) will course the TCA cycle and give rise to $^13$CO$_2$ that will equilibrate with body CO$_2$ and bicarbonate pools.

The purpose of the present investigation was to compare differences in the fractional recoveries of $^{13}$C label in respiratory CO$_2$ after the infusion of labeled NaHCO$_3$ or positionally labeled acetate during rest and exercise and apply the derived “correction factors” to the calculation of glucose oxidation during rest and exercise.

METHODS

Subjects

Thirteen endurance-trained male subjects were recruited from the University of California, Berkeley campus, by posted notice and electronic mailing. Subjects were divided into two matched groups for the calculation of acetate ($n = 5$) or bicarbonate ($n = 8$)-derived correction factors and the measurement of glucose turnover and oxidation. Trained subjects were used to extend the period of time that constant metabolic flux rates and, therefore, recoveries of $^{13}$CO$_2$ could be determined during exercise. Subjects were considered endurance trained if they had been competing in United States Cycling Federation or collegiate mountain or road endurance racing if they had been competing in the United States. The subjects were nonsmokers, diet and weight stable with a body fat percentage $<10\%$, had a 1-s forced expiratory volume $>70\%$ of vital capacity, and were injury and disease free as determined by medical questionnaire and physical examination. The protocol was approved by the University of California, Berkeley Committee for the Protection of Human Subjects (Protocols CPHS 98–4–83 and 99–5-67), and subjects gave informed written consent.

Screening Tests

$^\text{V}O_2$ peak was determined on two occasions by means of a progressive leg cycle ergometer protocol (Monark Ergomedic 829E) beginning at 100 W and increasing 25 or 50 W every 3 min until voluntary cessation. Two $^\text{V}O_2$ peak tests were performed before the isotope trials to assure a true maximum effort. Respiratory gases were continuously collected and analyzed via an open-circuit indirect calorimetry system (AMETEK S-3A1 O$_2$ and AMETEK CD-3A CO$_2$ analyzers), and respiratory parameters were determined every minute by a real-time, on-line, PC-based system (10). Three-day dietary records were collected before and after completion of the testing period to assess dietary habits and monitor individual caloric intake and macronutrient composition. Analysis of dietary records was performed using the Nutritionist III program (N-Squared Computing, Salem, OR). Body composition was determined by skinfold measurements, as previously reported (10).

Experimental Design

After screening, stable isotope infusion trials were performed on each subject (see Tracer Protocol). During the 24 h preceding each isotope trial, subjects refrained from exercise and consumed a standardized diet [3,240 kcal; 66% carbohydrate (CHO), 19% fat, and 14% protein] prepared by laboratory staff. The dietary protocol included a final snack (566 kcal; 52% CHO, 33% fat, and 15% protein) consumed 12 h before the onset of exercise. Subjects reported to the laboratory at 7:00 AM on the morning of the isotope trial, 7.5 h after their last meal. After collection of background blood and breath samples, tracer infusion began, and subjects rested for 3.75 h, followed by 90 min of leg ergometer cycling at either 45% (moderate) or 65% (hard) of $^\text{V}O_2$ peak. Subjects in each infusion protocol group participated in a total of four isotope trials. Trials were performed in a randomized order with no fewer than 5 days between experiments. Subjects were instructed to maintain their initial dietary and training regimens throughout the testing period.

Tracer Protocol

On the morning of the isotope trials, a catheter was placed into a dorsal hand vein and subsequently heated for collection of arterialized blood. A second catheter was inserted into a contralateral forearm vein for the continuous infusion (Baxter Travenol 6200 infusion pump) of the isotope solutions. After collection of background breath samples, individuals in the acetate protocol received a continuous infusion of [1-$^{13}$C]- or [2-$^{13}$C]acetate; subjects in the bicarbonate group received a continuous infusion of NaH$^{13}$CO$_3$ or [6,6-$^2$H]-$^1$[1-$^{13}$C]glucose. Acetate and bicarbonate infusion protocols were intended to maintain isotopic enrichment of $^{13}$CO$_2$ during exercise. Consequently, the [1-$^{13}$C]acetate and [2-$^{13}$C]acetate were infused at 2.0 mg/min during rest and were increased 9 and 12 times during exercise at 45 and 65% $^\text{V}O_2$ peak, respectively. The NaH$^{13}$CO$_3$ was infused at 1.2 mg/min during rest and was increased 6 and 8 times during exercise at 45 and 65% $^\text{V}O_2$ peak, respectively. During rest, tracer glucose was infused at 4 mg/min and was increased 2 times during both exercise intensities. The isotopes (Cambridge Isotope Laboratories, Woburn, MA) were diluted in 0.9% sterile saline, pyrogenicity and sterility tested (University of California, San Francisco, School of Pharmacy), and, on the day of the experiment, were passed through a 0.2-$\mu$m Millipore filter (Nalgene, Rochester, NY) before infusion.

Blood Sampling and Analysis

Blood was sampled at minutes 0, 180, 195, 210, and 225 of the 3.75-h rest period and at minutes 30, 45, 60, 75, and 90 of exercise. Samples were immediately chilled on ice and centrifuged at 3,000 g for 20 min, and the supernatant was collected and frozen until analysis (31). Hematocrit was measured at each sampling point, and subjects were instructed to drink tap water to ensure that metabolite and hormone concentrations were not affected by changes in plasma volume. Blood glucose and lactate concentrations were determined in duplicate (31).

Isotopic Enrichment Analyses

Glucose enrichments were measured using gas chromatography-mass spectrometry (GC model 5890 series II and MS
Calculations

Plasma glucose rates of appearance and disappearance. Glucose rates of appearance (Ra) and disappearance (Rd) were calculated using equations defined by Steele and modified for use with stable isotopes, as previously described (10). Glucose kinetics were calculated as follows:

\[ Ra (mg \cdot kg^{-1} \cdot min^{-1}) = \frac{F - V(C_1 + C_2)/2[(IE_2 - IE_1)/t_2 - t_1]}{[(IE_2 + IE_1)/2]} \] (1)

\[ Rd (mg \cdot kg^{-1} \cdot min^{-1}) = R_a - [V(C_2 - C_1)/(t_2 - t_1)] \] (2)

where \( F \) represents the specific isotope infusion rate; \( V \) is volume distribution of glucose (180 ml/kg); \( C_1 \) and \( C_2 \) are concentrations at sampling times \( t_1 \) and \( t_2 \); and \( IE_1 \) and \( IE_2 \) are the excess isotopic enrichments of 2m glucose at times \( t_1 \) and \( t_2 \). Measured isotopic enrichments were corrected for background by comparison to blood samples taken before isotope infusion.

Respiratory CO\(_2\) enrichment. Respiratory \( V\dot{CO}_2 \) during rest and exercise was determined by indirect calorimetry. Fractional recovery of \(^{13}\text{C}\) label in respiratory \( CO_2 \) from infused \([1-^{13}\text{C}]\)acetate, \([2-^{13}\text{C}]\)acetate, and \( \text{NaH}^{13}\text{CO}_3 \) was calculated from the following equation:

fractional recovery of label = \( \frac{E^{13}\text{CO}_2 \times V\dot{CO}_2}{F} \) (3)

where \( E^{13}\text{CO}_2 \) is the enrichment of respiratory \(^{13}\text{C}\)CO\(_2\) above background, \( V\dot{CO}_2 \) is the volume of expired CO\(_2\), and \( F \) is the infusion rate of \([1-^{13}\text{C}]\)acetate, \([2-^{13}\text{C}]\)acetate, and \( \text{NaH}^{13}\text{CO}_3 \).

Glucose oxidation. The rate of glucose oxidation (Glcox) was calculated using the IRMS-measured enrichment of expired CO\(_2\) and the equations

fractional glucose oxidation = \( [(E^{13}\text{CO}_2)/(V\dot{CO}_2)(100)]/(F \times k) \) (4)

\[ \text{Glcox} (mg \cdot kg^{-1} \cdot min^{-1}) = (\text{glucose} \ R_a) \text{(fractional glucose oxidation)} \] (5)

where \( E^{13}\text{CO}_2 \) is the \(^{13}\text{C}\) isotopic enrichment of expired CO\(_2\); \( V\dot{CO}_2 \) is the volume of CO\(_2\) expired per minute; \( F \) is the isotopic infusion rate; \( k \) is the correction factor for the retention of CO\(_2\) in body pools derived from the recovery of \(^{13}\text{C}\) during bicarbonate or acetate infusion (Eq. 3); and Glcox is in milligrams per kilogram per minute, in which \( R_d \) was estimated from \(^2\text{H}\) and fractional oxidation for \(^{13}\text{C}\) tracers, respectively.

Statistical Analyses

Data are presented as means ± SE. Significance of mean differences between exercise intensities and isotope infusates was determined with two-factorial ANOVA measures. Significance changes over time were determined using repeated-measures factorial ANOVA and post hoc analysis. Post hoc comparisons were made with Fisher’s protected least significant difference test. Representative values of substrate kinetics were obtained by averaging values from the final 30 min of rest and exercise. All statistical analyses were performed on the computer program SuperANOVA (Abacus Concepts, Berkeley, CA). Statistical significance was set at \( P = 0.05 \).

RESULTS

Subject Characteristics, Dietary Records, and Physiological Response to Exercise

Anthropometric data on subjects are reported in Table 1. No differences were observed between isotope infusate groups. Macronutrient and energy contents of individual diets were consistent during the experimental period, as determined from 3-day dietary records collected during the first and final weeks of testing (first week: 60.1 ± 2.4% CHO, 25.7 ± 2.6% fat, 14.2 ± 0.7% protein, 3,256.2 ± 176.5 kcal vs. final week: 58.2 ± 2.9% CHO, 26.1 ± 2.7% fat, 15.7 ± 1.4% protein, 3,079.0 ± 201.5 kcal). During exercise, heart rate and respiratory exchange ratio increased independently of isotope infusate (Table 2). The cycle ergometer workloads required for the specific exercise intensities were not affected by isotope infusate and are reported in Table 2.

Isotopic Enrichments

Stable plasma glucose enrichments were obtained during the final 30 min of rest and exercise at 45% \( V\dot{O}_2\) peak during \([6,6-^{2}\text{H}]-\text{and} \ [1-^{13}\text{C}]\text{glucose infusions (Fig. 1, A and B). Compared with 45%} V\dot{O}_2\text{peak, tracer glucose enrichments were reduced during exercise at 65%} V\dot{O}_2\text{peak (Fig. 1, A and B). During bicarbonate infusion, isotopic enrichment of respiratory} ^{13}\text{CO}_2 \text{was stable during the final 30 min of rest and exercise (Fig. 2A). However, during the infusion of tracer acetate, respiratory} ^{13}\text{CO}_2 \text{enrichment continued to increase throughout rest and both exercise intensities (Fig. 2B). Despite equivalent rates of} ^{13}\text{C} \text{infusion, resting} ^{13}\text{CO}_2 \text{enrichment was 79% greater during} \ [1-^{13}\text{C}]\text{acetate infusion compared with} \ [2-^{13}\text{C}]\text{acetate infusion (Fig. 2B). During the transition from rest to both exercise intensities,} ^{13}\text{CO}_2 \text{enrichment increased in an intensity-independent manner (Fig. 2B). A difference in} ^{13}\text{CO}_2 \text{enrichment between} \ [1-^{13}\text{C}]-\text{and} \ [2-^{13}\text{C}]\text{acetate...}}

Table 1. Subject characteristics during the 6-wk experimental period

<table>
<thead>
<tr>
<th>Variable</th>
<th>Bicarbonate/Glucose</th>
<th>([1-^{13}\text{C}]/[2-^{13}\text{C}]\text{Acetate})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>25.6 ± 1.6</td>
<td>27.2 ± 1.0</td>
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<tr>
<td>Height, cm</td>
<td>175.8 ± 2.3</td>
<td>181.9 ± 2.8</td>
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<tr>
<td>Weight, kg</td>
<td>72.8 ± 2.9</td>
<td>75.5 ± 1.9</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>9.1 ± 1.5</td>
<td>9.0 ± 1.2</td>
</tr>
<tr>
<td>(V\dot{O}_2\text{peak})/min</td>
<td>4.54 ± 0.14</td>
<td>4.59 ± 0.12</td>
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<tr>
<td>(V\dot{O}_2\text{peak})/ml kg⁻¹min⁻¹</td>
<td>62.4 ± 1.8</td>
<td>60.8 ± 1.4</td>
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Values are means ± SE; \( n = 5 \) (acetate); \( n = 8 \) (bicarbonate). \( V\dot{O}_2\text{peak}, \text{peak} O_2 \text{consumption.}

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infusion was also observed during both exercise intensities. During the final 30 min of exercise at both 45 and 65% of \( V_\text{O2peak} \), \( ^{13} \text{CO}_2 \) enrichment was \(-0.16\%\) in the \([1-^{13}C]\)-acetate trials compared with 0.09% during the \([2-^{13}C]\)-acetate trials (Fig. 2B). In the bicarbonate trials, \( ^{13} \text{CO}_2 \) enrichment was unchanged during the transition from rest to exercise at 45 and 65% \( V_\text{O2peak} \) (Fig. 2A).

### Fractional Recovery of Carbon Label

Representative fractional recoveries were calculated by averaging values obtained during the final 30 min of rest and exercise. During rest, the fraction of infused \( ^{13} \text{C} \) label recovered from bicarbonate was increased compared with \([1-^{13}C]\)- and \([2-^{13}C]\)-acetate; similarly, the fraction of \( ^{13} \text{C} \) label recovered from the \([1-^{13}C]\)-acetate infusion was increased compared with \([2-^{13}C]\)-acetate (Table 3). During exercise, the fractional recovery of \( ^{13} \text{C} \) label was independent of exercise intensity, and the data were pooled. The correction factors were calculated from the \([1-^{13}C]\)- and \([2-^{13}C]\)-acetate and bicarbonate recoveries reported in Table 3. Similar to rest, the fraction of \( ^{13} \text{C} \) label recovered during exercise was isotope dependent (Table 3). In \([1-^{13}C]\)- and \([2-^{13}C]\)-acetate trials, label recoveries were 60 and 148% greater during exercise compared with rest, whereas fractional \( ^{13} \text{C} \) recovery increased only 21% during exercise in the bicarbonate trials. In addition, the exercise effect on the fractional recovery of tracer was significantly different (\( P < 0.05 \)) among the three tracer protocols. Because blood lactate concentrations were stable during rest and exercise (31), acidosis was unlikely to have affected the excretion of label as \( \text{CO}_2 \).

Apparent in Fig. 2B, but not apparent in Fig. 2A because only the final 30 min of exercise are shown, is the fact that, after initiation of tracer infusion, excretion of tracer in breath increased exponentially and then plateaued (3). In the present report, we chose to utilize a prolonged (3.75 h) tracer equilibration period to make measurements in a "steady state." However, in previous, short-term protocols, metabolite oxidation could be estimated from parallel experiments in which kinetics of \( ^{13} \text{CO}_2 \) excretion could be predicted.

### Glucose Oxidation

Excretion of label in breath \( \text{CO}_2 \), i.e., Glc ox, increased in an intensity-dependent manner during the transition from rest to exercise (Fig. 3). At rest, the use of the \([1-^{13}C]\)- and \([2-^{13}C]\)-acetate correction factors increased calculated Glc ox compared with no correction (N/C) and the bicarbonate correction (Fig. 3). In addition, resting Glc ox was increased by use of the \([2-^{13}C]\)- compared with the \([1-^{13}C]\)-acetate correction factor. Glc ox corrected for \([2-^{13}C]\)-acetate recovery during exercise at 45% \( V_\text{O2peak} \) was also increased compared with Glc ox calculated from N/C and the bicarbonate correction factor. During exercise at 65% \( V_\text{O2peak} \), the use of either acetate correction factor increased calculated Glc ox compared with N/C and the bicarbonate correction. In addition, Glc ox, corrected for \([2-^{13}C]\)-acetate recovery, was increased compared with \([1-^{13}C]\)-acetate.

### Comparison of Corrected Glc ox and Glucose Disposal Rates

Compared with values at rest, glucose disposal rates increased approximately two and three times during exercise at 45 and 65% \( V_\text{O2peak} \), respectively (Fig. 3). In addition, glucose \( R_d \) demonstrated a significant intensity effect during exercise (Fig. 3). Resting glucose \( R_d \) was about two-, three-, and fourfold greater than Glc ox calculated using either \([1-^{13}C]\)-acetate, bicarbonate, or N/C, respectively (Fig. 3). Conversely, there was no statistical difference between glucose \( R_d \) and Glc ox calculated using the \([2-^{13}C]\)-acetate correction factor at rest. During exercise at 45% \( V_\text{O2peak} \), Glc ox increased, and no difference was observed between glucose \( R_d \) and Glc ox, whether Glc ox was corrected or uncorrected (Fig. 3). However, during exercise at 65% \( V_\text{O2peak} \), Glc ox was estimated to account for 130 and 163% of glucose \( R_d \) with either the \([1-^{13}C]\)- or \([2-^{13}C]\)-acetate correction factors, respectively (Fig. 3).

### DISCUSSION

The calculation of plasma FFA and Glc ox rates requires the use of correction factors to account for dilution of label in endogenous bicarbonate pools and path-
ways of intermediary metabolism. Moreover, the increased metabolic flux rates and changes in pool size during exercise require the application of different correction factors from those used under resting conditions. In the present study, we compared correction factors derived from the fractional recoveries of $^{13}$C tracer bicarbonate and acetate. Furthermore, we evaluated the appropriateness of the $^{13}$C tracer correction factors for estimation of Glc$_{ox}$ rates during rest and submaximal exercise. For rest and exercise, labeled acetate did not produce stable $^4$CO$_2$ excretion rates over the 90-min observation periods studied. Furthermore, for hard exercise, use of the acetate correction factor resulted in Glc$_{ox}$ rates significantly larger than

Fig. 1. Isotopic enrichment (IE) for [6,6-$^2$H]glucose (D$_2$ glucose; A), and [13C]glucose (B) over time during rest and exercise. Values are means ± SE; n = 8. ○, 45% Peak O$_2$ consumption (VO$_{2peak}$); □, 65% VO$_{2peak}$. *Significantly different from 45%; †significantly different over time of exercise, (P < 0.05).
glucose $R_d$, and we conclude that bicarbonate-derived, not acetate-derived, correction factors should be used.

Recovery of $^{13}$C Label During Rest and Exercise

Fractional recovery of $^{13}$C label is dependent on label source and position as well as the metabolic rate of the subjects. In terms of empirical evaluations of substrate oxidation rates, appreciation of differences in label distribution and tissue physiology during rest and exercise is necessary. During resting postprandial conditions, the splanchnic bed is relatively well perfused with substrate for both gluconeogenesis and $\beta$-oxidation (1). In contrast, muscle is poorly perfused and is not gluconeogenic but relies on $\beta$-oxidation. When ex-

Fig. 2. Respiratory $^{13}$CO$_2$ isotopic enrichment during bicarbonate (A) and acetate (B) infusion over time during rest and exercise. APE, atom percent excess. Values are means ± SE; n = 8 (bicarbonate), n = 5 (acetate). *Significantly different from rest; †significantly different from 45% $V_2$peak; ‡significantly different from $[1-^{13}$C$] acetate ($P < 0.05$).
Table 3. Percent recovery of $^{13}$C tracer during the final 30 min of rest and exercise

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<tbody>
<tr>
<td>Rest</td>
<td>49.9 ± 2.4†</td>
<td>26.1 ± 1.2</td>
<td>77.2 ± 1.7††</td>
</tr>
<tr>
<td>45% VO$_2$peak</td>
<td>79.2 ± 1.1††</td>
<td>64.0 ± 0.7*‡</td>
<td>94.0 ± 0.8*†‡</td>
</tr>
<tr>
<td>65% VO$_2$peak</td>
<td>81.8 ± 0.7††</td>
<td>65.2 ± 2.1*‡</td>
<td>92.3 ± 2.2*†‡</td>
</tr>
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</table>

Values are means ± SE recorded during the final 30 min of rest and exercise; $n = 5$ (acetate); $n = 8$ (bicarbonate). *Significantly different from rest; †significantly different from [2-13C]acetate; ‡significantly different from [1-13C]acetate; $P < 0.05$.

Exercise starts, muscle perfusion increases as do gluconeogenesis and glycolysis, whereas β-oxidation is down-regulated (4). In liver, the rate of gluconeogenesis increases to support glucose homeostasis (14). Thus, although the fractional recovery of $^{13}$CO$_2$ is similar across the splanchnic bed and skeletal muscle (21), the tissue sources and pathways responsible for recovery of tracer CO$_2$ differ.

In the current investigation, recoveries of carbon label from bicarbonate were increased compared with acetate during rest and both exercise intensities. Previously, several groups also reported that the recovery of carbon label from bicarbonate exceeded recovery from acetate (7, 12, 27). After infusion of bicarbonate and equilibration with the endogenous CO$_2$ pool, carbon label is recovered primarily in respiratory CO$_2$ (2, 13, 15, 16, 20). In contrast, the metabolic fate of exogenous acetate is entry into the mitochondria and oxidation and dilution in the TCA cycle (17, 21, 22, 25). As the consequence of equilibration in TCA cycle pools in various tissues, label can be incorporated into glucose, glycogen, lipid, and amino acids. Those pools will have different turnover and oxidation rates that will likely differ from the metabolite of interest. Thus recovery of acetate carbon label is reduced compared with bicarbonate carbon label.

With regard to the large relative recovery of bicarbonate label (≥80%), we recognize that, due to the “first pass” phenomenon of $^{13}$CO$_2$ excretion after venous infusion, $^{13}$CO$_2$ enrichment could be increased in venous compared with arterial blood. Disagreement therefore exists regarding the sites of appropriate bicarbonate tracer administration and the physiological interpretation of label recovery when a venous infusion site is used (9, 18). However, Steele et al. (29) reported that the enrichment of infused bicarbonate was consistent throughout the venous and arterial circulation within 10 min of infusion, regardless of the vascular entry site, and concluded that the intravascular site of tracer administration was of minimal consequence. Similarly, Coxon and Robinson (7, 8) demonstrated that, within 3 min of a NaH$^{13}$CO$_3$ bolus, the specific activity of mixed venous blood was identical to that of expired $^{13}$CO$_2$ and arterial $^{13}$CO$_2$. Thus, given the 3-h equilibration period before the first sample in the present study, differences between venous and arterial $^{13}$CO$_2$ enrichment due to first pass loss in the pulmonary bed seem unlikely.

The fractional recoveries of bicarbonate and acetate labels increased during exercise (Fig. 2, A and B; Table 3). However, although there were differences in fractional recovery rates among labels (Table 3), exercise intensity did not significantly affect fractional recovery of carbon label. Thus our results are inconsistent with those of previous studies that reported positive relationships between energy expenditure and label recovery (20, 27). However, the range of exercise intensities employed in the present study may have been insufficient to elicit physiological differences, or recovery rates may have been maximal at 45% VO$_2$peak. For bicarbonate protocols, the fractional recovery of label increased to ~100% during exercise at 45% VO$_2$peak. Consequently, it is not surprising that recovery of infused label as CO$_2$ did not increase further at 65% VO$_2$peak.

The increased recovery of acetate label during exercise compared with rest suggests that the rate of TCA cycle exchange reactions during exercise did not increase in proportion to the increase in TCA cycle turnover. Such a conclusion is supported by the reduced incorporation of $^{13}$C acetate label into glucose during exercise compared with rest (28). The increased recovery of label as $^{14}$CO$_2$ observed during exercise after acetate infusion could also be attributed to excretion of label that was converted to glucose in the liver and kidneys during the preexercise rest period. Prelabeling of glucose and other metabolite pools before exercise may help explain the Glc$_{exo}$ measures in excess of glucose Rd during exercise. A possible solution to the prelabeling effect would be to start the acetate or bicarbonate tracer infusion at the onset of exercise. However, the time required for adequate equilibration among bicarbonate and other pools would make oxidation measures during hard exercise impossible in human subjects.
During the bicarbonate protocols, $^{13}$C enrichment of respiratory CO$_2$ appeared to reach stable isotopic equilibrium within 3 h after the start of infusion. Similarly, within 45 min of exercise, $^{13}$CO$_2$ recovery reached isotopic equilibrium. Thus we believe it is reasonable to assume that the correction factors calculated from the recovery of bicarbonate label provided an accurate index of CO$_2$ dilution in bicarbonate pools.

Conversely, respiratory $^{13}$CO$_2$ enrichment during acetate infusion did not appear to adequately label the bicarbonate pools or equilibrate with TCA cycle exchange reactions during the time course of the present experiment. The incomplete equilibrium was apparent from the continual increase in the fractional recovery of carbon label throughout the 3.45 h of rest and 90 min of exercise. Mittendorfer et al. (21) reported that acetate carbon recovery in respiratory $^{13}$CO$_2$ continued to increase throughout an extended infusion protocol, reaching equilibrium only after 15 h. The slow equilibration of $^{13}$CO$_2$ suggests that recovery of acetate during the time frame of a typical isotope trial does not sufficiently account for label dilution. Therefore, correction factors derived from acetate carbon recovery will overestimate plasma oxidation rates unless considerable time is allowed for acetate to reach equilibrium. In an attempt to correct for the effect of CO$_2$ retention in bicarbonate pools when tracer acetate was given, several groups (23, 33) have infused $^{[13]$C]acetate after a $^{[13]$C]bicarbonate bolus. The fractional recovery of tracer in breath in these studies was similar to the mean of the bicarbonate and acetate recoveries (54%) (24) or values obtained after bicarbonate infusion alone (23, 33) in the present study. The question then arises, would a mixed-tracer approach be optimal for exercise studies? However, the infusion of two $^{13}$C tracers makes it impossible to distinguish between effects of either tracer in a mixed-study design. Moreover, due to changes in CO$_2$ and bicarbonate pool sizes and turnover times during exercise, there can be no assurance that a bicarbonate prime given at rest will saturate the respective pools in exercising subjects. Furthermore, the apparent steady state achieved using a bicarbonate bolus followed by a constant $^{13}$C tracer infusion (acetate or bicarbonate) may represent equilibration of the descending $^{13}$CO$_2$ enrichment from the bolus and the ascending $^{13}$CO$_2$ enrichment from the constant infusion and not the “real” enrichment of the bicarbonate pool for a given tracer.

**Positional Discrimination of Acetate Label Recovery**

Before oxidation, plasma FFA and glucose are converted to acetyl-CoA and enter the TCA cycle in the mitochondria of respiring cells. The recovery of labeled carbon from the TCA cycle is influenced by label position in mitochondrial acetyl-CoA pools (19, 30). Furthermore, all labeled carbon entering the TCA cycle through acetyl-CoA, regardless of tracer substrate (e.g., glucose or FFA), will be incorporated into oxaloacetate (OAA). $^{[1-13]$C]acetyl-CoA carbon will be retained on the C-1 or C-4 position of OAA and be predominantly excreted as CO$_2$ in the second spin of the TCA cycle. In contrast, $^{[2-13]$C]acetyl-CoA carbon may be retained in TCA cycle intermediates until the third spin of the cycle. As a result of positional discrimination of carbon in the TCA cycle, there is an increased probability that $^{[2-13]$C]acetate compared with $^{[1-13]$C]acetate label will enter the glucose pool or become fixed in an exchange reaction between α-ketoglutarate and glutamate before recovery in respiratory CO$_2$ (22, 26, 27).

In the present study, recovery of $^{[1-13]$C]acetate label was increased 91 and 24% compared with $^{[2-13]$C]acetate during rest and exercise, respectively. Similarly, Wolfe and Jahoor (33) and Clandinin et al. (5) reported recovery of respiratory $^{13}$CO$_2$ from $^{[1-13]$C]- and $^{[2-13]$C]pyruvate, and $^{[16-13]$C]palmitate was increased when label was transferred to the first carbon of acetyl-CoA compared with the second carbon via pyruvate dehydrogenase and β-oxidation, respectively. Therefore, the specific correction factor used in the calculation of oxidation rates should be derived for the position of the carbon label on the molecule, or the specific metabolic intermediary, as the substrate in question enters the TCA cycle.

**Calculation of Substrate Oxidation Rates**

The use of the acetate and bicarbonate correction factors in the current investigation during exercise at 45% V$_{O_2$ peak} resulted in glucose oxidation rates equal to glucose R$_d$. However, when the $^{[1-13]$C}- or $^{[2-13]$C]acetate correction factors were applied during hard exercise (65% V$_{O_2$ peak}), estimated Glc$_{ox}$ rates were 130–165% greater than [6,6-$^2$H]glucose-measured glucose R$_d$ (Fig. 3). The calculation of oxidation rates in excess of glucose R$_d$ suggests that the fractional recovery of acetate carbon during hard exercise was underestimated. The incomplete equilibration of acetate, as was evident from the continual increase in the fractional recovery of acetate carbon throughout the rest and exercise periods, would consequently overestimate the acetate correction factor. Therefore, it appears reasonable to assume that the Glc$_{ox}$ rates calculated using acetate correction factors were overestimated due to incomplete equilibration of CO$_2$, dilution of acetate carbon in TCA cycle exchange reactions, and oxidation of labeled metabolites with slow turnover rates. In agreement, Pouteau et al. (22) and Mittendorfer et al. (21) observed incomplete equilibration of respiratory $^{13}$CO$_2$ after a 3-h infusion protocol and suggested that acetate-derived correction factors were not appropriate for use in substrate oxidation calculations during the time course of a typical tracer experiment. Similarly, our data suggest that the calculation of an acetate correction factor requires a prolonged infusion of label to assure isotopic equilibrium and minimize the overestimation of substrate oxidation rates. However, physiological conditions of the subject would change during a prolonged period of equilibration (31).
The various correction factors applied to CO2 excretion after [13C]glucose infusion raise the apparent Glc ox rates (Fig. 3). Despite this correction effect, because glycogen and lactate are the main fuels for exercise, Glc ox rates are only a fraction of the total CHO oxidation rates during exercise (33 and 25% of CHO energy expenditure during exercise at 45 and 65% VO2 peak, respectively). Furthermore, Glc ox is also less than total CHO oxidation in resting subjects. This result (Fig. 3) indicates that glucose is not the only CHO source oxidized at rest.

In summary, results of the current investigation indicate that the fractional recovery of carbon label is significantly greater after tracer-bicarbonate compared with tracer-acetate infusion. Moreover, we observed that an acetate correction factor overestimates Glc ox rates during hard exercise despite the similar metabolic fate of [2-13C]acetate carbon and [1-13C]glucose carbon in the TCA cycle. In addition, the apparent error is exaggerated when the [2-13C]- rather than the [1-13C]acetate correction factor is used to calculate Glc ox rates despite the similar metabolic fate of [2-13C]acetate carbon and [1-13C]glucose carbon in the TCA cycle. Therefore, we conclude that a bicarbonate-derived correction factor is appropriate for calculating glucose oxidation rates in exercising men.

The investigators thank the subjects for their participation in and compliance with the dietary and exercise protocols. We are also exercising men.

The transport of radioactive labeled carbon dioxide from the body.


REFERENCES
