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Retention of intravenously infused [^{13}C]bicarbonate is transiently increased during recovery from hard exercise

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Henderson GC, Fattor JA, Horning MA, Faghihnia N, Luke-Zeitoun M, Brooks GA. Retention of intravenously infused [^{13}C]bicarbonate is transiently increased during recovery from hard exercise. *J Appl Physiol* 103: 1604–1612, 2007. First published August 16, 2007; doi:10.1152/japplphysiol.00309.2007.—The effects of exercise on energy substrate metabolism persist into the postexercise recovery period. We sought to derive bicarbonate retention factors (*k*) to correct for carbon tracer oxidized, but retained from pulmonary excretion before, during, and after exercise. Ten men and nine women received a primed-continuous infusion of [^{13}C]bicarbonate (sodium salt) under three different conditions: *1*) before, during, and 3 h after 90 min of exercise at 45% peak oxygen consumption ($\dot{V}O_{2peak}$); *2*) before, during, and 3 h after 60 min of exercise at 65% $\dot{V}O_{2peak}$; and *3*) during a time-matched resting control trial, with breath samples collected for determination of $^{13}CO_2$ excretion rates. Throughout the resting control trial, *k* was stable and averaged 0.83 in men and women. During exercise, average *k* in men was 0.93 at 45% $\dot{V}O_{2peak}$ and 0.94 at 65% $\dot{V}O_{2peak}$, and in women *k* was 0.91 at 45% $\dot{V}O_{2peak}$ and 0.92 at 65% $\dot{V}O_{2peak}$, with no significant differences between intensities or sexes. After exercise at 45% $\dot{V}O_{2peak}$, *k* returned rapidly to control values in men and women, but following exercise at 65% $\dot{V}O_{2peak}$, *k* was significantly less than control at 30 and 60 min postexercise in men (0.74 and 0.72, respectively, $P < 0.05$) and women (0.75 and 0.76, respectively, $P < 0.05$) with no significant postexercise differences between men and women. We conclude that bicarbonate/CO_2 retention is transiently increased in men and women for the first hour of postexercise recovery following endurance exercise bouts of hard but not moderate intensity.

prior exercise; postexercise; exertion; physical activity; $NaHCO_3$; correction factor; glucose oxidation; fatty acid oxidation; leucine oxidation

IT IS OF INTEREST TO MEASURE the kinetics of metabolic processes that contribute to energy flux under various conditions to advance our understanding of the control of metabolism. Of particular importance is the oxidation of energy substrates such as fatty acids, glucose, lactate, and amino acids. Continuous tracer infusion of ^{13}C-labeled metabolites, with concomitant blood and breath sampling, is commonly utilized to study metabolism in humans. Because bicarbonate (HCO_3^-) and carbon dioxide (CO_2) are intimately related in vivo, calculation of the percent of $^{13}CO_2$ that would have been produced metabolically but would not have appeared in breath during the course of the experiment can be derived from [^{13}C]bicarbonate or [^{14}C]bicarbonate infusion under the same experimental conditions as the ^{13}C-metabolite infusion trial. The percentage of infused bicarbonate tracer carbon label that is recovered in breath CO_2, expressed as a decimal, is defined as the bicarbonate retention factor (*k*). This factor may also be referred to as the bicarbonate recovery factor or bicarbonate correction factor. Commonly, the apparent oxidation rate of energy substrate is then divided by *k* to calculate the corrected oxidation rate, reflecting the fact that metabolic $^{13}CO_2$ production does not necessarily equal pulmonary $^{13}CO_2$ excretion.

In studies utilizing ^{13}C-labeled tracers in which whole body rate of substrate oxidation is calculated utilizing measurements of $^{13}CO_2$ excretion, investigators choose between utilizing a bicarbonate correction factor or an acetate correction factor. Here we report the derivation of bicarbonate correction factors. Sidossis et al. (36) described the acetate correction factor as being derived analogously to the manner in which the bicarbonate correction factor is derived, that is, by the percent recovery of infused [^{13}C]acetate as pulmonary $^{13}CO_2$ excretion. Although subject to effects of labeling position (41) and duration of tracer infusion (28, 44), acetate correction factors apply a larger adjustment to oxidation rates than is done by a bicarbonate retention factor (41). In our study we chose to use a bicarbonate correction factor because a rationale for its use has been previously established and because it yields a more conservative estimate of tracer-derived substrate oxidation.

In 1951 Kornberg et al. (20) reported that ~80% of an intravenously infused [^{14}C]bicarbonate bolus could be recovered as $^{14}CO_2$ in the breath of unanesthetized cats, and later similar results were obtained with human study participants (15) by the same method. In their study Kornberg et al. accounted for about half of [^{14}C]bicarbonate retention in a combination of urea and bone, and they proposed that additional label may have been retained in carboxylic acids. Bicarbonate/CO_2 pool sizes, exchange rates between pools, turnover rates of pools that may act as a sink for labeled CO_2, and irreversible excretion through nonpulmonary routes may each potentially change under different physiological states and could have substantial effects on *k*.

In 1959 Shipley et al. (35) noted an effect of nutritional state reporting that rats retained a larger fraction of [^{14}C]bicarbonate bolus when fasted compared with fed. Soon after, in 1963 Morris and Simpson-Morgan (30) noted in discussion of their data an observation that $^{14}CO_2$ excretion during continuous [^{14}C]bicarbonate infusion in rats seemed to increase when the rats spontaneously moved within the restraint apparatus, perhaps the first hint that considerations of bicarbonate retention would be important to exercise physiologists. Later, other groups published results in support of and that elaborated on

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the original findings of effects of nutritional status (2, 11, 13, 29, 35, 39) and effects of physical activity (1, 5, 7, 24, 27, 38, 41, 48) on carbon-labeled bicarbonate retention. Numerous reports of [^{13}C]tracer-derived measurements of substrate oxidation in humans during exercise have been published (10, 18, 27, 40) reflecting interest in understanding the acute effects of exercise bouts on energy metabolism. With recognition that the effects of exercise bouts on metabolism can persist into the postexercise recovery period, studies of postexercise metabolism utilizing tracer-derived measurements of substrate oxidation have been published (4, 6, 8, 22, 23, 38, 42, 43, 46, 47) and more are expected as findings importantly indicate that prior exercise alters resting energy substrate metabolism.

Quantifying *k* by measuring cumulative recovery of a [^{14}C]bicarbonate intraperitoneal bolus injection, Brooks and Gaesser (6) showed increased retention of [^{14}C]bicarbonate (lower *k*) following exhaustive endurance exercise in female rats, and Leese et al. (24) showed increased retention of orally administered [^{13}C]bicarbonate bolus following an hour of hard endurance exercise [73% peak oxygen consumption ($\dot{V}O_{2peak}$)] in a combined group of men and women. As well, utilizing a primed-continuous [^{13}C]bicarbonate infusion in men, Tarnopolsky et al. (38) showed increased bicarbonate retention following resistance exercise bouts, but *k* was depressed in recovery for only a brief period. Alternatively, Devlin et al. (8) judged their postexercise *k* values to be in the range those expected in a resting control trial, while others have assumed that postexercise *k* values were similar during preexercise rest and recovery (22, 23). On the basis of the existing literature regarding postexercise bicarbonate retention (6, 8, 24, 38), it seemed unclear whether the postexercise recovery period following sustained endurance exercise bouts should be assigned a different *k* value than postabsorptive rest and, if it should, how long the state of altered bicarbonate retention would persist. For future studies of postexercise recovery in which substrate oxidation will be assessed by continuous tracer infusion, correction factors for bicarbonate retention will be needed along a time course as can be derived from continuous tracer infusion, and, additionally, any differences in bicarbonate retention between men and women and between exercise intensities may also become important. Therefore, we sought to derive bicarbonate retention factors and to test the hypothesis that bicarbonate retention in men and women would be increased for 3 h following endurance exercise bouts, in an intensity-dependent manner, with retention being greater following hard-intensity exercise (65% $\dot{V}O_{2peak}$) than following moderate-intensity exercise (45% $\dot{V}O_{2peak}$).

METHODS

Study participants. Twenty healthy, moderately active, nonsmoking, weight-stable volunteers (10 men and 10 women) were recruited from the University of California, Berkeley campus and surrounding community by posted notice and e-mail. Potential study participants underwent subsequent screening tests if they were disease free as determined by physical examination and health history questionnaire, were not taking medications known to affect energy metabolism, had a body mass index (BMI) of less than 28, were neither sedentary individuals nor elite athletes, and had normal lung function as determined by 1-s forced expiratory volume of greater than or equal to 70% of vital capacity. Female study participants reported regular menstrual cycles (24–32 days) and were not taking oral contraceptives. Eight women were studied in the early follicular phase (*days 3–8*). For one woman, one trial was carried out on *day 11* of her menstrual cycle because she was unavailable between *days 3* and *8*. One woman withdrew from the study before completion of bicarbonate tracer infusion trials and therefore the number of female study participants included in the final analysis was reduced from 10 to 9. The procedures and risks were thoroughly explained to the study participants, and their written, informed consent was obtained. The University of California, Berkeley Committee for the Protection of Human Subjects approved the study protocol (CPHS no. 2004-6-103).

Screening tests. Study participants underwent two progressive exercise tests to assess $\dot{V}O_{2peak}$ before beginning the study, and an additional $\dot{V}O_{2peak}$ assessment was carried out upon completion of the study to confirm that study participants' fitness levels had not changed over the course of experimentation. Exercise was performed on a leg-cycle ergometer (Monark Ergometric 839E, Vansbro, Sweden). A continual progressive protocol was used to determine $\dot{V}O_{2peak}$ with an increase in power output at 3-min intervals until volitional exhaustion. Body composition was assessed by skinfold measurement (16, 17) at seven sites (abdominal, triceps, chest/pectoral, midaxillary, subscapular, suprailiac, and thigh). The body composition assessment was completed both before enrollment of study participants and again at completion of the study. Dietary energy and macronutrient intake were monitored at the beginning, middle, and end of the study by separate 3-day diet records; analysis was performed with Diet Analysis Plus, version 6.1 (ESHA Research, Salem, OR).

Experimental design. With at least 1 wk between trials for men and 1 mo between trials for women, study participants were studied under each of three conditions, each on separate occasions, assigned in a random order. Men and women were studied *1*) before, during, and 3 h after ~90 min of exercise at 45% $\dot{V}O_{2peak}$ (Fig. 1*A*); *2*) before, during, and 3 h after ~60 min at 65% $\dot{V}O_{2peak}$ (Fig. 1*B*); and *3*) during a time-matched resting control trial (Fig. 1*C*). After catheterization, study participants lay semisupine quietly reading or watching movies. After exercise, study participants dismounted the ergometer and sat into a chair where they remained for 30 min and were then transferred to an examination table where they remained semisupine for the remainder of postexercise recovery. Trips to the restroom were accomplished by transporting study participants in a wheelchair. Duration of the first randomly assigned exercise trial, either 45 or 65% $\dot{V}O_{2peak}$, was set at 90 or 60 min, respectively. The appropriate duration for the subsequent exercise trial at the remaining exercise condition was predicted with the goal of matching exercise energy expenditure (EEE) between exercise bouts using oxygen consumption ($\dot{V}O_2$) and respiratory exchange ratio (RER) data from the $\dot{V}O_{2peak}$ assessments. Study participants rested the day prior to the day in which exercise or time-matched rest was performed and received standardized diets (see *Experimental protocol* below) to cover energy needs. On the day of tracer infusion trials, study participants received breakfast 3 h before exercise.

Experimental protocol. Study participants were instructed to consume a standardized diet and water ad libitum each day prior to tracer infusion trials. The study participants were asked to abstain from structured physical exercise sessions but to continue typical activities of daily living and were fed for a physical activity level of 1.5 according to the current dietary reference intake guidelines of the Institute of Medicine for estimated energy requirement (14). Dietary energy intake was individualized for each study participant (men 2,788 ± 44 kcal/day; women 2,131 ± 41 kcal/day), and macronutrient composition was made similar between individuals for carbohydrate (men 49.8 ± 0.3%; women 50.0 ± 0.2%), lipid (men 32.0 ± 0.3%; women 32.4 ± 0.3%), and protein (men 18.2 ± 0.3%; and women 17.6 ± 0.2%). On the day of tracer infusion trials, study participants arrived at the laboratory at 7:00 AM overnight fasted and ate a standardized breakfast that was 16.3 ± 0.2% of the daily standardized energy intake for men and 16.3 ± 0.6% of the daily standardized

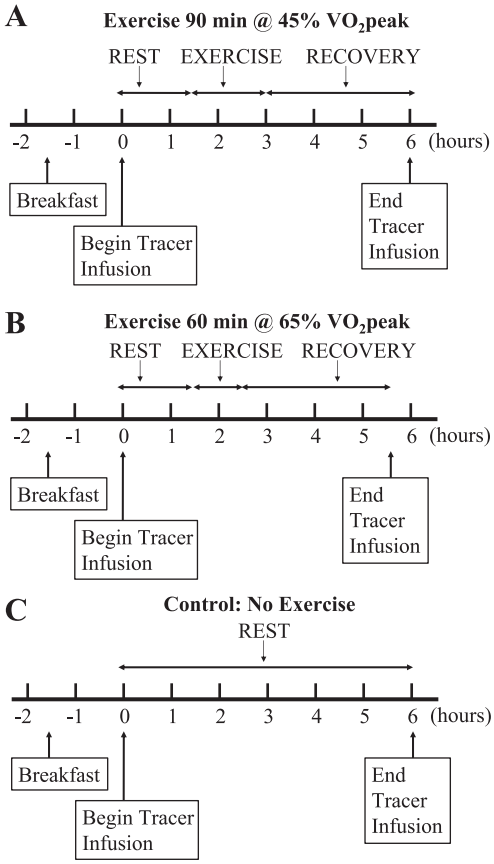

Fig. 1. Experimental design. Each study participant completed 3 different tracer infusion trials in randomized order, one involving moderate-intensity exercise, 45% $\dot{V}O_{2peak}$ (*A*), one involving hard-intensity exercise, 65% $\dot{V}O_{2peak}$ (*B*), and one involving a matched time of day, no exercise control (*C*). $\dot{V}O_{2peak}$, relative exercise intensity expressed as a percentage of peak O_2 consumption.

energy intake for women. We chose to feed our study participants standardized breakfasts 3 h before exercise to mimic typical nonlaboratory conditions.

The stable isotope tracer [$NaH^{13}CO_3$, 99% atom percent excess (APE)] was purchased from Cambridge Isotope Laboratories (Andover, MA) and subsequently prepared in 0.9% sterile saline in airtight glass bottles and tested for sterility and pyrogenicity at the University of California, San Francisco, School of Pharmacy. On the morning of tracer infusion trials, after collection of background breath samples, a catheter was placed in an arm vein for continuous infusion of stable isotope tracer. A [^{13}C]bicarbonate ($NaH^{13}CO_3$) priming dose of 96 mg for men and 72 mg for women was given immediately prior to continuous infusion of [^{13}C]bicarbonate at 1.2 mg/min in men and 0.9 mg/min in women. Bicarbonate tracer infusion rates were increased sixfold above rest during exercise at 45% $\dot{V}O_{2peak}$ and eightfold above rest at 65% $\dot{V}O_{2peak}$. Tracer infusion rates were immediately set back to initial resting rates at completion of exercise bouts for assessment of [^{13}C]bicarbonate retention during 3 h of postexercise recovery. Immediately prior to tracer infusion during each trial, bicarbonate concentration in the infusate was measured with an enzyme-linked reaction (Pointe Scientific, Lincoln Park, MI) and UV absorbance measurement to input more accurate infusion rates into the calculations of bicarbonate retention.

At each sampling time point respiratory gas exchange was determined in real time for determination of $\dot{V}O_2$ and carbon dioxide production ($\dot{V}CO_2$) using a Parvo Medics system. An aliquot of expired breath was collected in evacuated Exetainer tubes from a mixing chamber for subsequent determination of $^{13}CO_2$ isotopic enrichment by isotope ratio mass spectrometry that was performed by Metabolic Solutions (Nashua, NH). Breath samples were collected before exercise (75 and 90 min after the start of tracer infusion), during exercise (45 and 60 min at 65% $\dot{V}O_{2peak}$ and 75 and 90 min at 45% $\dot{V}O_{2peak}$), and after exercise (5, 15, 30, 60, 90, 120, 150, and 180 min) for a total of 12 sampling time points. During the nonexercise trials, breath samples were collected during 6 h of rest (75, 90, 135, 150, 165, 180, 210, 240, 270, 300, 330, 360 min after the start of tracer infusion) for a total of 12 sampling time points. Additionally, breath was collected before tracer infusion to obtain background isotopic enrichments (IE) of $^{13}CO_2$. At each sampling time point, heart rate was recorded from an electrocardiograph (Quinton Q750, Seattle, WA) and blood pressure was measured by auscultation. Bicarbonate retention data are not reported for 5 min postexercise because this time point immediately follows a change in tracer infusion rate.

Calculations. Fractional recovery of intravenously infused $NaH^{13}CO_3$ as expired breath $^{13}CO_2$ was calculated from the following equation

$$k = (E^{13}CO_2 \times \dot{V}CO_2)/F$$

where k represents the fractional recovery of label (bicarbonate retention factor), $E^{13}CO_2$ is the isotopic enrichment of expired breath $^{13}CO_2$ in APE divided by 100 to express as a decimal, $\dot{V}CO_2$ is the rate of pulmonary carbon dioxide production, and F is the [^{13}C]bicarbonate tracer infusion rate. Isotopic enrichments were corrected for background enrichments in breath samples collected before tracer infusion.

Statistical analyses. Data are presented as means ± SE. Descriptive statistics were compared between men and women by unpaired *t*-tests. Comparisons between trials and across time points were made by analysis of variance with repeated measures with post hoc comparisons using Fisher's protected least significant difference test. The 75- and 90-min time points, the initial rest samples, were collected under similar conditions in each of the three trials, and because the pulmonary gas exchange and bicarbonate retention results were not significantly different between trials for these time points the data for these time points are presented with the three trials pooled. Selected planned comparisons between men and women for bicarbonate retention factors and parameters of pulmonary gas exchange were made by unpaired *t*-tests. Statistical analyses were performed using SPSS Graduate Pack 11.0 software. Statistical significance was set at $\alpha = 0.05$.

RESULTS

Characteristics of study participants. Physical characteristics of study participants are reported in Table 1. There were no significant differences between men and women regarding age, BMI, exercise habits, or $\dot{V}O_{2peak}$ per unit fat-free mass (FFM).

Table 1. *Characteristics of study participants*

	Men	Women
Age, yr	24.5±1.1	26.2±1.7
Height, cm	178.5±1.6*	162.7±2.1
Weight, kg	73.1±2.4*	58.4±1.9
BMI, kg/m^2	22.9±1.6	22.1±0.7
Body fat, %	10.4±1.2*	22.1±0.9
$\dot{V}O_{2peak}$, l/min	4.1±0.2*	2.8±0.1
$\dot{V}O_{2peak}$, ml·kg^{-1}·min^{-1}	56.6±2.0*	49.0±2.1
$\dot{V}O_{2peak}$, ml·kg FFM^{-1}·min^{-1}	63.0±2.2	62.6±2.7
Exercise (h/wk)	6.9±0.9	6.9±1.0

Values are means ± SE. Men, $n = 10$; women, $n = 9$. $\dot{V}O_{2peak}$, peak O_2 consumption; BMI, body mass index. *Significantly different between men and women, $P < 0.05$.

In both men and women, $\dot{V}O_{2peak}$ and body composition did not vary significantly between pre- and poststudy measurements. The habitual exercise of ~7 h/wk was predominantly described as a moderate exertion level by both the men and women (more intense than walking and less intense than competitive sporting competition). Height, weight, and $\dot{V}O_{2peak}$ (l/min and $ml \cdot kg^{-1} \cdot min^{-1}$) were significantly larger in men than in women ($P < 0.05$). Body fat percent was significantly greater in women than in men ($P < 0.05$). Habitual dietary energy intake and macronutrient composition, as determined by multiple 3-day diet records, did not vary significantly between the three separate assessments and, therefore, averaged values are reported. Habitual dietary energy intake was significantly larger in men than women ($P < 0.05$, men 2,537 ± 118 kcal/day; women 1,937 ± 79 kcal/day). Habitual dietary macronutrient composition was not significantly different between sexes for carbohydrate (men 53.2 ± 1.9%; women 54.6 ± 1.4%), lipid (men 30.5 ± 1.6%; women 28.9 ± 1.1%), and protein (men 16.3 ± 0.9%; and women 16.5 ± 0.8%).

Characteristics of exercise bouts. Exercise sessions are described in Table 2. EEE values were significantly greater in men than women ($P < 0.05$). EEE was calculated by subtracting the resting energy expenditure, calculated from the control trial, from the energy expenditure of the exercise sessions. In men and in women the EEE was successfully matched between exercise trials in that there was no significant difference between the EEE for the 45% $\dot{V}O_{2peak}$ trials and 65% $\dot{V}O_{2peak}$ trials. There were no significant sex-related differences in heart rate response to either exercise intensity, although, as expected, heart rate was significantly higher ($P < 0.05$) at 65% $\dot{V}O_{2peak}$ than at 45% $\dot{V}O_{2peak}$. Because there was no difference between men and women for physical fitness level ($\dot{V}O_{2peak}$ per unit FFM), $\dot{V}O_2$ was not significantly different between sexes during exercise when expressed per unit FFM at 45% or at 65% $\dot{V}O_{2peak}$ (Table 2).

Pulmonary gas exchange. Although not different per unit of FFM, the absolute rates of pulmonary $\dot{V}O_2$ were larger in men (Fig. 2*A*) than in women (Fig. 2*B*), and the absolute rates of $\dot{V}CO_2$ were also larger in men (Fig. 2*C*) than in women (Fig. 2*D*) during periods of rest, exercise, and postexercise recovery. These sex-related differences are consistent with the male study participants having larger body weights and FFMs than the female study participants. In men (Fig. 2*A*) and women (Fig. 2*B*), individual time point values for $\dot{V}O_2$ remained elevated above control values at 5 and 15 min after exercise but were not significantly different than control values for the subsequent remainder of the 3-h recovery period. $\dot{V}CO_2$ remained elevated above control values at 5 and 15 min after exercise in men (Fig. 2*C*) and 5 min after exercise in women (Fig. 2*D*) but also was not significantly different than control values for the subsequent remainder of the 3-h recovery period. The average $\dot{V}CO_2$ over the duration from 30 to 180 min postexercise was not significantly different than the corresponding control $\dot{V}CO_2$ following exercise at either intensity in men (0.25 ± 0.01, 0.25 ± 0.01, and 0.26 ± 0.01 l/min in control, 45% $\dot{V}O_{2peak}$, and 65% $\dot{V}O_{2peak}$ trials, respectively) or women (0.19 ± 0.01, 0.19 ± 0.004, and 0.19 ± 0.005 l/min in control, 45% $\dot{V}O_{2peak}$, and 65% $\dot{V}O_{2peak}$ trials, respectively). Although statistical significance was not reached at the individual time points, the average $\dot{V}O_2$ over the duration from 30 min to 180 min postexercise was significantly elevated in men following both exercise bouts (0.30 ± 0.01, 0.32 ± 0.01, and 0.33 ± 0.01 l/min in control, 45% $\dot{V}O_{2peak}$, and 65% $\dot{V}O_{2peak}$ trials, respectively, $P < 0.05$), but was not significantly elevated above control in women (0.23 ± 0.01, 0.24 ± 0.01, and 0.25 ± 0.01 l/min in control, 45% $\dot{V}O_{2peak}$, and 65% $\dot{V}O_{2peak}$ trials, respectively). There were no significant differences between exercise intensities for the average postexercise $\dot{V}O_2$ or $\dot{V}CO_2$ in either men or women.

RER in both men (Fig. 3*A*) and women (Fig. 3*B*) decreased across the resting control trial from the samples corresponding to preexercise time points (75 and 90 min) to the end of the trial ($P < 0.05$). During exercise at either intensity, the average RER was lower in women than in men, but there were no significant differences between men and women for average pre- or postexercise RER values. In both men and women, RER did not significantly change over time from 30 min postexercise until the end of the trial (3 h postexercise), and RER values at all time points were within the theoretical limits of 0.707 and 1.00 (25, 49). Although there was no effect of exercise intensity between isoenergetic exercise bouts, in both men and women the average postexercise RER following both exercise intensities was lower than the corresponding control values.

Retention of [^{13}C]bicarbonate. Isotopic enrichments of $^{13}CO_2$ in men (Fig. 4*A*) and women (Fig. 4*B*) continued to increase throughout the resting control trial from the samples corresponding to preexercise time points to the end of the trial ($P < 0.05$). However, the drift in isotopic enrichment was counterbalanced by a slight downward trend for $\dot{V}CO_2$ throughout the

Table 2. *Characteristics of exercise bouts*

	45% $\dot{V}O_{2peak}$		65% $\dot{V}O_{2peak}$	
	Men	Women	Men	Women
% $\dot{V}O_{2peak}$	47.1±0.7†	47.0±1.1†	66.9±1.1	66.8±1.2
$\dot{V}O_2$, l/min	1.94±0.10*†	1.32±0.06†	2.73±0.16*	1.86±0.10
$\dot{V}O_2$, $ml \cdot kg^{-1} \cdot min^{-1}$	26.4±0.7*†	22.6±0.7†	37.1±1.2*	32.0±1.2
$\dot{V}O_2$, $ml \cdot kg\ FFM^{-1} \cdot min^{-1}$	29.5±0.9†	29.0±0.8†	41.5±1.4	41.0±1.4
RER	0.87±0.01*†	0.84±0.005†	0.92±0.005*	0.89±0.01
Duration, min	89.4±0.5†	90.3±0.8†	60.5±0.3	61.2±0.5
EEE, kcal	693.2±40.3*	454.7±23.1	703.6±41.5*	472.7±29.6
Heart rate, bpm	124.3±2.9†	123.3±5.1†	161.9±2.6	154.7±4.8

Values are means ± SE. Men, $n = 10$; women, $n = 9$. % $\dot{V}O_{2peak}$, relative exercise intensity expressed as a percentage of peak O_2 consumption; RER, respiratory exchange ratio; EEE, energy expenditure of exercise, adjusted by subtracting resting energy expenditure. *Significantly different between men and women, $P < 0.05$. †Significantly different from corresponding values in 65% $\dot{V}O_{2peak}$ trial, $P < 0.05$.

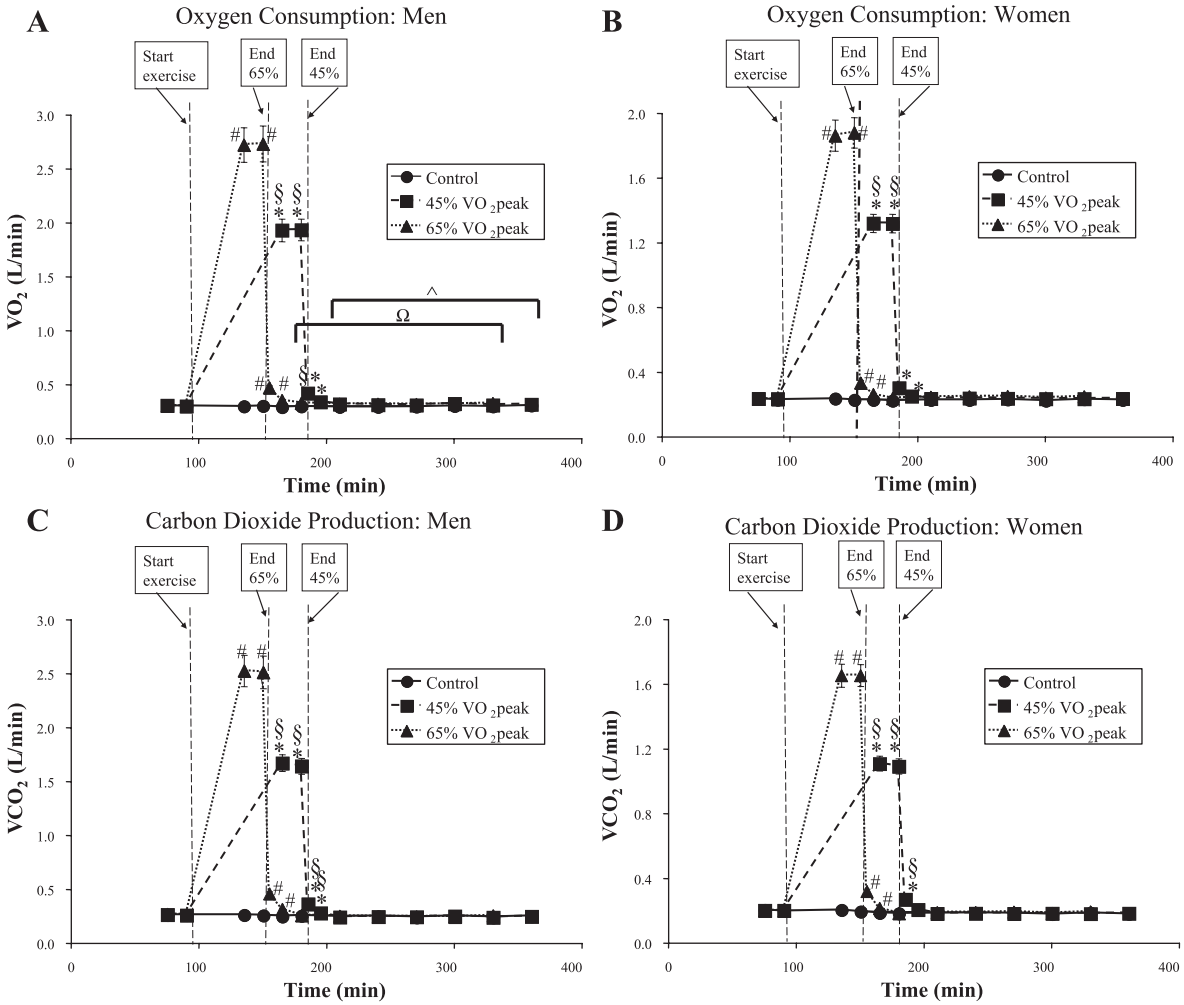

Fig. 2. Pulmonary gas exchange. Oxygen consumption ($\dot{V}O_2$) in men (*A*) and women (*B*) and carbon dioxide production ($\dot{V}CO_2$) in men (*C*) and women (*D*). Values are means ± SE. Men, $n = 10$; women $n = 9$. Time (min), duration elapsed since beginning tracer infusion. *45% $\dot{V}O_{2peak}$ trial significantly different from corresponding time points in control trial, $P < 0.05$. #65% $\dot{V}O_{2peak}$ trial significantly different from corresponding time points in control trial, $P < 0.05$. §45% $\dot{V}O_{2peak}$ trial significantly different from corresponding time points in 65% $\dot{V}O_{2peak}$ trial, $P < 0.05$. ^Average postexercise (30 min to 180 min postexercise) in 45% $\dot{V}O_{2peak}$ trial significantly different from corresponding average in control trial, $P < 0.05$. ΩAverage postexercise (30 to 180 min postexercise) in 65% $\dot{V}O_{2peak}$ trial significantly different from corresponding average in control trial, $P < 0.05$.

trial such that pulmonary $^{13}CO_2$ production did not change significantly from the beginning to the end of the resting control trial. The relative recovery of infused $NaH^{13}CO_3$ in breath $^{13}CO_2$ is reported in Fig. 5*A* (men) and Fig. 5*B* (women). This percentage, if expressed as a decimal (% divided by 100) represents the bicarbonate retention factor (Table 3) that acts as a correction factor (*k*) in calculations of substrate oxidation. The bicarbonate retention factor (Fig. 5) did not change significantly from the first sampling time point (75 min after beginning of infusion) to the end of the resting control trial (360 min). Preexercise *k* (average of 75- and 90-min time points) was not statistically different between men and women and was 0.81 ± 0.005 for men and 0.83 ± 0.02 for women. Average *k* across the entire resting control trial, calculated as the average of the 12 time points, was not different between men and women and was 0.83 ± 0.01 for men and 0.83 ± 0.02 for women. The bicarbonate retention factor rose during exercise and was not significantly different between exercise intensities or between men and women. At 45% $\dot{V}O_{2peak}$, *k* was 0.93 ± 0.02 in men and 0.91 ± 0.02 in women, and at 65% $\dot{V}O_{2peak}$ *k* was 0.94 ± 0.01 in men and 0.92 ± 0.01 in women. During postexercise recovery following exercise at 45% $\dot{V}O_{2peak}$, *k* rapidly returned to resting control values and was not significantly different from control from 30 min after cessation of exercise on out to the end of the trial (180 min of postexercise recovery). However, after exercise at 65% $\dot{V}O_{2peak}$, *k* did not immediately return to resting control values but rather declined to a smaller value at the 30- and 60-min time points in both men (Fig. 5*A*) and women (Fig. 5*B*), representing a greater level of bicarbonate/CO_2 retention in the first hour of postexercise recovery from exercise at 65% $\dot{V}O_{2peak}$. Whereas average resting control *k* values were ~0.83 as described above, at 30 min postexercise *k* had significantly declined ($P < 0.05$) to 0.74 ± 0.02 in men and 0.75 ± 0.01 in women. At 60 min *k* remained depressed ($P < 0.05$) at 0.72 ± 0.02 in men and 0.76 ± 0.02 in women. From 90 min postexercise onward, *k* was no longer significantly different from control values during postexercise recovery following exercise at 65% $\dot{V}O_{2peak}$, with the isolated exception of 150 min postexercise in men (note: 150 min postexercise in 65%

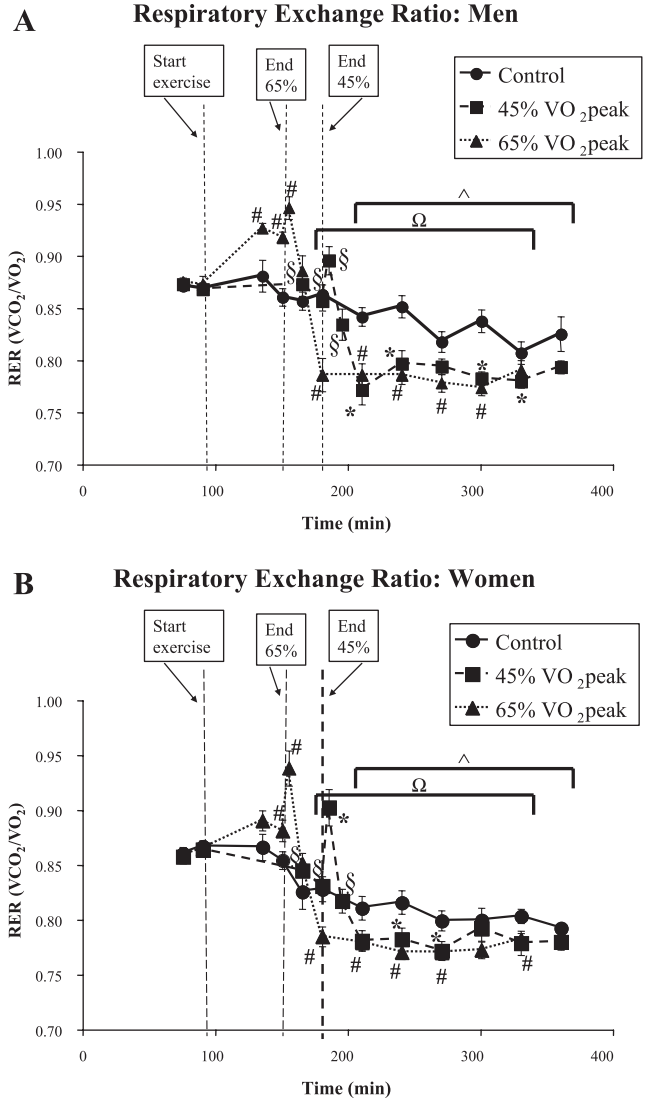

Fig. 3. Respiratory exchange ratio (RER) in men (*A*, $n = 10$) and women (*B*, $n = 9$). Values are means ± SE. Time (min), duration elapsed since beginning tracer infusion. *45% $\dot{V}O_{2peak}$ trial significantly different from corresponding time points in control trial, $P < 0.05$. #65% $\dot{V}O_{2peak}$ trial significantly different from corresponding time points in control trial, $P < 0.05$. §45% $\dot{V}O_{2peak}$ trial significantly different from corresponding time points in 65% $\dot{V}O_{2peak}$ trial, $P < 0.05$. ^Average postexercise (30 to 180 min postexercise) in 45% $\dot{V}O_{2peak}$ trial significantly different from corresponding average in control trial, $P < 0.05$. ΩAverage postexercise (30 min to 180 min postexercise) in 65% $\dot{V}O_{2peak}$ trial significantly different from corresponding average in control trial, $P < 0.05$.

$\dot{V}O_{2peak}$ trial is labeled as "300 min" after start of infusion in the figures). Average *k* values for rest and for the predominant time periods in which *k* varied significantly from resting control values (exercise and *hour 1* of recovery) are reported in Table 3. Comparisons between average *k* values from specific periods of the tracer infusion trials, as shown in Table 3, revealed no significant differences between men and women. Therefore, *k* values are reported for men and women pooled together, as well.

DISCUSSION

We provide data describing the degree to which intravenously infused $NaH^{13}CO_3$ is excreted as pulmonary $^{13}CO_2$ in men and women at rest, during exercise, and during the postexercise recovery period. From these data we draw conclusions regarding the physiological link between systemic CO_2 production and pulmonary CO_2 excretion, and, furthermore, we report correction factors that may be utilized in studies of energy substrate metabolism in which ^{13}C-labeled tracers are infused.

Exercise intensity. We designed this study to correspond to studies of postexercise recovery in which exercise durations have been adjusted to match EEE between bouts (3, 9, 21, 26, 33, 45, 46) in the expectation that future studies of postexercise recovery utilizing ^{13}C tracers might also utilize this aspect of study design in choosing exercise durations. Such practice makes it possible to draw conclusions regarding the effect of exercise intensity on postexercise resting metabolism without being confounded by different degrees of energy balance manipulation by exercise. In agreement with others who have shown no effect of changing the intensity of endurance exercise on *k* during exercise in men (1, 41), we report here no

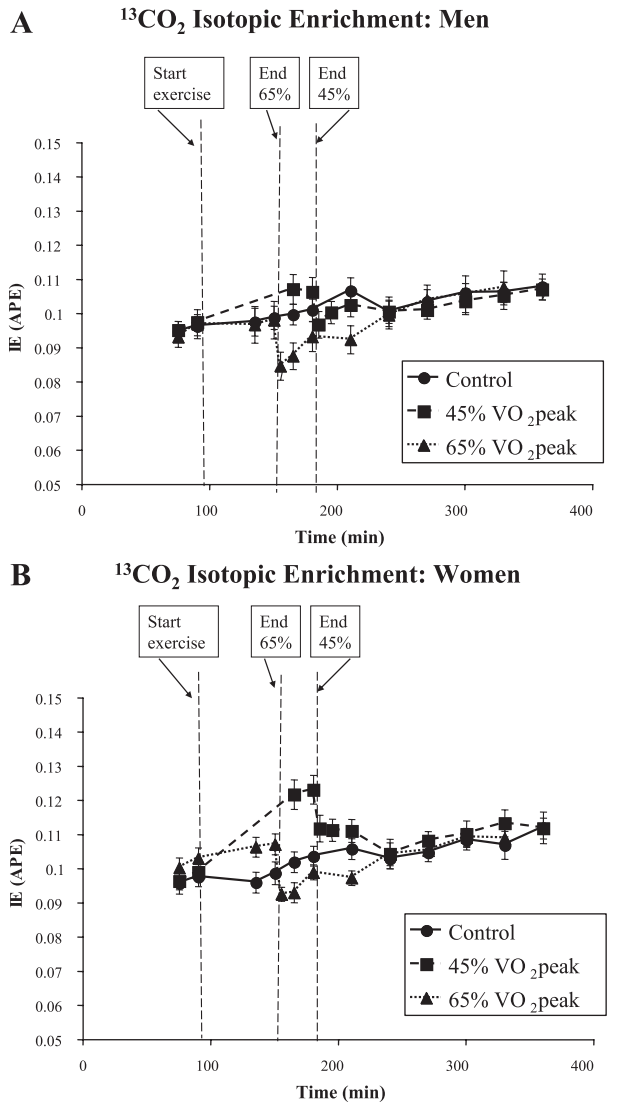

Fig. 4. Isotopic enrichment (IE) of breath $^{13}CO_2$ in men (*A*, $n = 10$) and women (*B*, $n = 9$). Values are means ± SE. Time (min), duration elapsed since beginning tracer infusion. APE, atom percent excess.

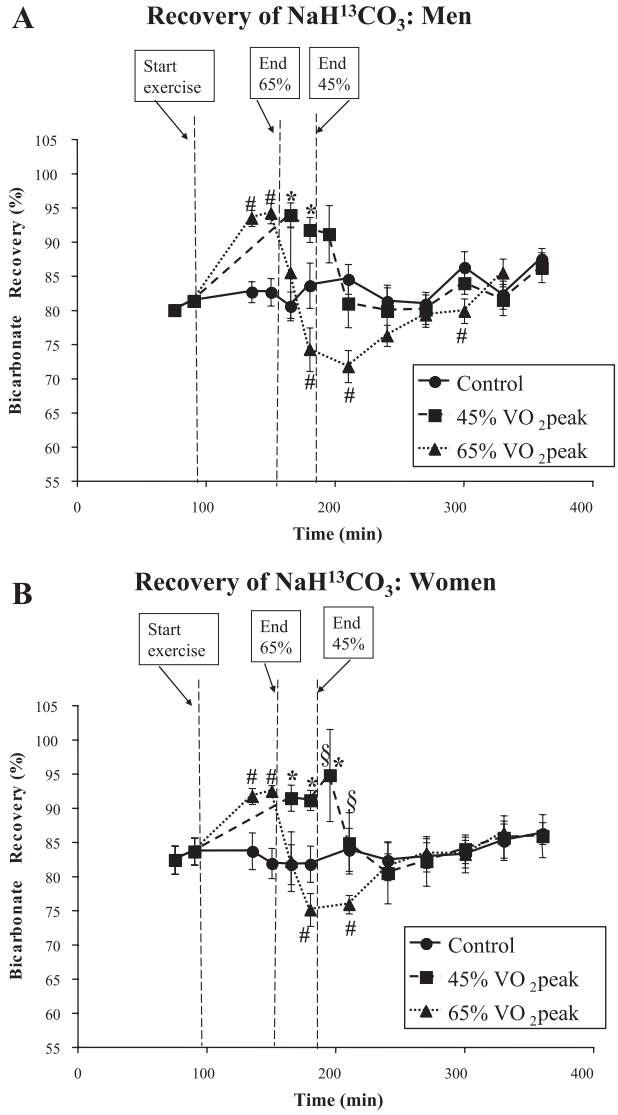

Fig. 5. Relative recovery of infused [^{13}C]bicarbonate in men (*A*, $n = 10$) and women (*B*, $n = 9$). Values are means ± SE. Time (min), duration elapsed since beginning tracer infusion. *45% $\dot{V}O_{2peak}$ trial significantly different from corresponding time points in control trial, $P < 0.05$. #65% $\dot{V}O_{2peak}$ trial significantly different from corresponding time points in control trial, $P < 0.05$. §45% $\dot{V}O_{2peak}$ trial significantly different from corresponding time points in 65% $\dot{V}O_{2peak}$ trial, $P < 0.05$.

significant difference between exercise intensities in either men or women, both being elevated to similar extents above rest values. However, a unique effect of hard exercise becomes apparent in the postexercise recovery period in which *k* transiently decreases to values significantly below the resting control. These findings show that exercise intensity actually does affect bicarbonate retention, but the effects are manifested subsequently following cessation of the activity. Thus we interpret such findings to imply that particular care is to be taken in studies of postexercise recovery and that correcting apparent oxidation rates upwardly compared with resting control data would be appropriate if the prior exercise bout was of a relatively hard intensity (e.g., as we show for 65% $\dot{V}O_{2peak}$). Although previous results from studies utilizing bolus administration of bicarbonate tracer following hard endurance exercise bouts did show increased retention (lower *k*) during postexercise recovery in female rats (6) and in a mixed group of men and women (24), our present results show that applying these correction factors to studies utilizing lower-intensity exercise bouts would cause overestimation of substrate oxidation in the postexercise recovery period.

Time course of tracer kinetics. In studies utilizing bolus tracer administration, *k* is calculated from cumulative $^{13}CO_2$ total excretion. In contrast, when continuous tracer infusion is employed, as we did in this study, nearly instantaneous $^{13}CO_2$ excretion rates are used to calculate *k*. Hence, utilizing primed-continuous $NaH^{13}CO_3$ infusion allows for description of bicarbonate retention along a changing time course as is not achieved in studies utilizing bolus injection. Our results show that the increased retention of infused bicarbonate tracer following hard exercise bouts (6, 24) is manifested transiently during postexercise recovery in men and women and that *k* then subsequently returns to resting control values. In future studies of postexercise recovery, description of the changing time course of metabolite kinetics will extend our understanding of the effects of physical activity on energy substrate metabolism in men and women. Measurements of the rate of pulmonary excretion of $^{13}CO_2$ during ^{13}C-tracer infusion will play an important role in progression of understanding the partitioning of energy substrates between storage and oxidation during and after exercise. Our present results show that the relationship between $^{13}CO_2$ excretion rates and metabolic $^{13}CO_2$ production after exercise would be affected by the exercise intensity and the duration of time passed since cessation of the exercise bout.

Possible sites of bicarbonate retention. Skeletal muscle (32) and arterial blood (37) bicarbonate pools decrease in size during exercise, in an intensity-dependent manner (37), and therefore these pools would be depleted at the start of the postexercise recovery period and would subsequently expand, providing a nonpulmonary destination for metabolically produced CO_2. Our results describing fractional recovery of bicarbonate tracer (Fig. 5) indicate that the rate of CO_2 production rises relative to the rate of CO_2 fixation pathways during exercise and that after moderate-intensity exercise (45% $\dot{V}O_{2peak}$) the relative rates of CO_2 production and fixation rapidly return to those of rest. However, following hard-intensity exercise bouts (65% $\dot{V}O_{2peak}$), in both men and women CO_2 fixation relative to CO_2 production is elevated during the first hour of the postexercise recovery period compared with rest (indicated by lower *k*). This intensity-dependent effect is consistent with the greater depletion of bicarbonate

Table 3. *Bicarbonate retention factors (k)*

	Men	Women	Men + Women
Rest	0.83±0.01	0.83±0.02	0.83±0.01
Exercise, 45% $\dot{V}O_{2peak}$	0.93±0.02*	0.91±0.02*	0.92±0.01*
Exercise, 65% $\dot{V}O_{2peak}$	0.94±0.01*	0.92±0.01*	0.93±0.01*
Hour-1 postexercise, 45% $\dot{V}O_{2peak}$	0.81±0.02†	0.83±0.04	0.82±0.02†
Hour-1 postexercise, 65% $\dot{V}O_{2peak}$	0.73±0.02*	0.76±0.01*	0.74±0.01*

Values are means± SE. Men, $n = 10$; women, $n = 9$, men + women, $n = 19$. Hour-1 postexercise, average of 30 min and 60 min postexercise *Significantly different from corresponding time points in control trial, $P < 0.05$. †Significantly different from corresponding time points in 65% $\dot{V}O_{2peak}$ trial, $P < 0.05$.

that would occur in association with the lower systemic pH during exercise of harder intensities (37) and therefore could possibly be due to an expanding bicarbonate pool size during recovery from hard exercise. However, pulmonary $\dot{V}_{CO_2}$ simply declines to its plateau value (same as resting control trial) rather than rebounding to lower rates of pulmonary CO_2 excretion during recovery from hard exercise. As well, RER also does not rebound to values significantly below its plateau values following exercise at 65% $\dot{V}_{O_{2peak}}$, and, furthermore, RER does not travel below the theoretical limit of 0.707 (25, 49) during postexercise recovery. So it might be presumptuous for us to assume that the actual volume of CO_2 produced metabolically is underestimated by the volume expired ($\dot{V}_{CO_2}$) during the time of depressed *k* following 65% $\dot{V}_{O_{2peak}}$ exercise, and the possibility remains that increased bicarbonate retention in the postexercise recovery period following hard exercise would not be attributable to pool size expansion, but rather to increased turnover of relatively unlabeled carbon pools that would simultaneously fix CO_2 enriched from ^{13}C-tracer infusion and liberate CO_2 with a lower IE back into circulation.

Application of correction factors in tracer studies. As discussed in the introduction, use of an acetate correction factor is an alternative to use of a bicarbonate correction factor. Validity of an acetate correction requires that there be zero nonoxidative acetate metabolism in all tissues during labeled energy substrate infusion. These assumptions may be difficult to test in vivo, and, to our knowledge, there have been no attempts to quantify pathways of acetate disposal during rest, exercise, and recovery from exercise. To the contrary, Pouteau et al. (31) have reasoned that nonoxidative acetate metabolism may be substantial in the postabsorptive state. Although the rationale would be that an acetate correction should be applied to any tracer that traverses the TCA cycle, including glucose (34), Trimmer et al. (41) showed that an acetate correction factor excessively corrects oxidation of [1-^{13}C]glucose, which would generate [2-^{13}C]acetyl CoA and, therefore, enter the TCA cycle. The theoretical basis for use of an acetate correction factor for calculations of substrate oxidation is that simultaneous anapleurosis and catapleurosis across exchange reactions in the TCA cycle deplete the cycle of ^{13}C label before $^{13}CO_2$ production occurs, and because acetate-derived acetyl-CoA would traverse the TCA cycle loss of label in the TCA cycle could be theoretically quantified. However, it is worth noting that [^{13}C]bicarbonate does also label the TCA cycle, such as via the pyruvate carboxylase reaction, in the same positions as would be labeled by [1-^{13}C]acetate, [1-^{13}C]palmitate, etc. (at carbons 1 and 4 of oxaloacetate). In fact, in kinetic studies of mitochondrial metabolism, both bicarbonate (12) and acetate (19) tracers have been infused to label TCA cycle intermediates and measure their flux. Although labeling of the TCA cycle by bicarbonate would be less than with [^{13}C]acetate in many tissues within the body, a bicarbonate tracer would to some extent correct for loss of carbon label from the TCA cycle across exchange reactions, but the bicarbonate correction is simply more conservative in this respect. Although employing an acetate correction factor to control for dilution of label within the TCA cycle may be theoretically reasonable, on the basis of previous comparisons between bicarbonate and acetate retention factors (41), we have chosen to continue with the definition of in vivo ^{13}C-metabolite oxidation as $^{13}CO_2$ excretion corrected for $^{13}CO_2$ fixation studies.

Summary and conclusions. We report findings regarding retention of intravenously infused $NaH^{13}CO_3$ in men and women before, during, and following isoenergetic exercise bouts of different intensities and during time-matched resting control trials. Results were not yet available in either humans or laboratory animals describing postexercise bicarbonate retention along a time course, and so we systematically studied postexercise *k* in both sexes with exercise bouts of two different intensities. Although relative retention of bicarbonate decreases during exercise, we have now shown that bicarbonate retention is transiently increased in men and women following 1 h of hard endurance exercise but is not increased following an isoenergetic bouts of exercise at a lower intensity. Because the effects of exercise bouts on energy substrate metabolism persist into the postexercise recovery period, calculations of substrate oxidation rates from administration of ^{13}C-labeled metabolites following exercise bouts will be relevant to the pursuit of a more complete understanding of the effects of physical activity on metabolism. In studies of postexercise metabolism utilizing ^{13}C tracers, care must be taken in correction of pulmonary $^{13}CO_2$ excretion rates because the relationship between metabolic $^{13}CO_2$ production and pulmonary $^{13}CO_2$ excretion may vary depending on the exercise intensity and the time passed since cessation of activity.

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