Changes in breath $^{13}CO_2/^{12}CO_2$ consequent to exercise and hypoxia

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BARSTOW, THOMAS J., DAN M. COOPER, SAMUEL EPSTEIN, AND KARLMAN WASSERMAN. *Changes in breath $^{13}CO_2/^{12}CO_2$ consequent to exercise and hypoxia.* J. Appl. Physiol. 66(2): 936–942, 1989.—Because the natural enrichment of carbohydrate with ^{13}C is greater than that of lipid, we hypothesized that the natural enrichment of exhaled CO_2 with ^{13}C (E_N) could be used to gauge endogenous substrate utilization in exercising human subjects. To test this, E_N and the respiratory exchange ratio (R) which equals the respiratory quotient (RQ) in the steady state, were measured simultaneously in seven subjects. Rest and exercise protocols, performed under conditions of room air (sea level) and hypoxic (inspired O_2 fraction = 0.15) breathing, were chosen to cause a variety of patterns of oxidative substrate utilization. Work rates were performed both below and above the subject's lactate threshold (LT). Work above the LT was expected to cause the greatest increase in E_N reflecting greater utilization of glucose. There was significant intersubject ($P < 0.05$) but not intrasubject variability in resting E_N. By 40 min of exercise, E_N increased significantly ($P < 0.05$) over resting values in all exercise protocols during both room air and hypoxia conditions. In the room air studies, we found no difference in E_N during the below-LT work, even though there were significant increases in O_2 uptake ($\dot{V}O_2$). In contrast, above-LT work resulted in significantly greater increases in E_N by 20 and 40 min of exercise ($P < 0.05$). Contrary to our expectations, we observed no separate effect by hypoxia on the E_N during exercise. Both E_N and R tended to increase from rest to exercise, but during exercise there was no overall correlation between R and the E_N. E_N reflects changes in endogenous substrate utilization over relatively long periods of time such as at rest, but delays in the appearance of $^{13}CO_2$ at the mouth due to dilution in body CO_2 pools, and possibly isotopic fractionation, preclude the usefulness of E_N as an indicator of endogenous fuel mix during short-term exercise.

natural enrichment; carbon isotopes; respiratory exchange ratio; fuel mix

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A STABLE ISOTOPE of carbon, ^{13}C, occurs naturally in both organic and inorganic substances at a variety of abundances relative to ^{12}C. The abundance of ^{13}C is higher in glucose than in lipid (5, 6). Enrichment of exhaled CO_2 with $^{13}CO_2$ (E_N) accurately reflects the pattern of enrichment of venous and arterial blood bicarbonate (8, 20). Thus, one might predict that changes in the relative contribution of carbohydrate to lipid oxidation could be detected by changes in the ratio of $^{13}C/^{12}C$ in exhaled CO_2. In human subjects during resting conditions the E_N has been shown to reflect the relative rates of oxidation of both endogenous (20) and exogenous (14, 18) substrate. Furthermore, Pirnay et al. (19) and Krzentowski et al. (13) used changes in E_N to calculate the oxidation of orally administered (exogenous) carbohydrate given during exercise. We therefore wondered if changes in E_N could be used in endogenous substrate oxidation during exercise. In fact, we hypothesized that E_N may more accurately reflect substrate oxidation than the respiratory exchange ratio [R, the ratio of CO_2 elimination ($\dot{V}CO_2$) to O_2 uptake ($\dot{V}O_2$)]. During unsteady states, such as for the first several minutes of exercise, changing CO_2 stores preclude R from equaling the tissue respiratory quotient [RQ, which reflects the proportion of carbohydrate to lipid oxidation (9, 12, 15)]. Under these conditions E_N, which may be insensitive to transient hyper- or hypoventilation, could potentially give a better indication of the oxidative fuel mixture. Because ^{13}C tracers are increasingly used in studies of metabolism during exercise, it is also important to document the magnitude and patterns of exercise-induced changes in E_N.

Preliminary reports suggest that the naturally occurring $^{13}C/^{12}C$ of breath CO_2 does change during exercise (16, 20, 26), but they are inconclusive as to the time course of change in E_N during exercise, and how these changes might reflect differences in the oxidative fuel mix. To test whether changes in E_N could be used to gauge endogenous substrate utilization during exercise, we examined $^{13}CO_2/^{12}CO_2$ in normal subjects under conditions known from previous studies to represent different mixes of substrate utilization, namely, rest and exercise of varying work rates under conditions of room air breathing and hypoxia (4, 23). Gas exchange was measured continually throughout the exercise, simultaneously with periodic determinations of E_N. Thus, any changes in the R would be readily apparent and could be compared to changes in E_N.

METHODS

Experimental design. The subjects were studied at rest and at three different levels of constant work rate exer-

cise under conditions of both room air (sea level) and hypoxia [inspired O_2 fraction (FI_{O_2}) = 0.15] on separate days (Fig. 1). At the low work rate [WR-1; 50% of the subject's lactate (or anaerobic) threshold (LT)], we expected to find little or no increase in E_N because there may be little change in whole body substrate utilization at low work rates. The second work rate (WR-2) was selected so as to be below the subject's LT during normoxic exercise, but above it during hypoxic breathing. We have previously demonstrated that under conditions identical with WR-2, breathing FI_{O_2} of 0.15 can itself increase blood glucose turnover (4). Therefore, we predicted for WR-2 an additional increase in $^{13}CO_2/^{12}CO_2$ under the hypoxic compared with the room air condition. Finally, a heavy work rate (WR-3) was chosen to be above the subject's room air LT. We predicted that E_N would be further elevated at this high work rate where glucose turnover is increased (4).

Subjects. The subject population consisted of seven male volunteers, ranging in age from 21 to 37 years, who were in good health and without any previous history of chronic respiratory or other disease. Each subject's age, weight, and room air and hypoxic gas breathing $\dot{V}O_{2\,max}$ and LT values are presented in Table 1. Informed consent was obtained from each subject before entry into the study.

Protocol. Each subject participated in six separate exercise sessions, each in a separate day. The first session consisted of two ramp cycle ergometer tests to fatigue, the first one under normoxic conditions and the second while breathing hypoxic gas. At least 30 min of rest was allowed between ramp tests. The order of these tests was chosen for two specific reasons: *1*) incremental tests to fatigue under room air conditions demonstrate good reproducibility regarding the parameters of interest ($\dot{V}O_{2\,max}$, LT, efficiency of work, etc) when separated by 30 min or more (24); *2*) we did not, however, know what potential interactive effect previous exposure to hypoxia would have on subsequent exercise tolerance during normoxia. Thus the room air test was administered before the hypoxic one in all subjects. The LT for each inspired oxygen tension was determined from pulmonary gas exchange responses as previously described (24) (Table 1). From the LT and $\dot{V}O_{2\,max}$ the work rates were selected for the constant exercise tests described above.

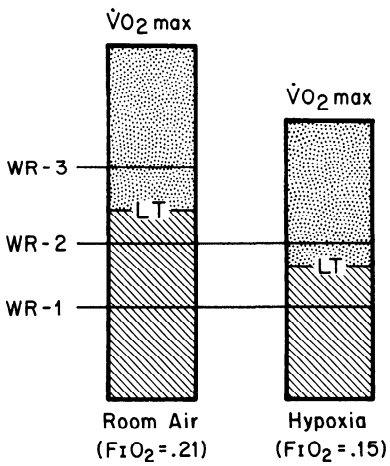

FIG. 1. Description of relative work intensities chosen. Work rate 1 (WR-1) was below LT for both room air and hypoxic tests. WR-2 required an O_2 consumption below LT on air breathing but above LT on hypoxia. WR-3 was only performed on room air conditions and was above room air LT.

TABLE 1. *Subject characterization*

Subj No.	Age, yr	Weight, kg	$\dot{V}O_{2\,max}$, l/min		LT, l/min	
			Room air	Hypoxia	Room air	Hypoxia
1	37	81.6	4.33	2.52	2.94	1.92
2	28	65.3	2.90	2.39	2.58	1.72
3	23	86.5	2.65	2.85	1.77	1.67
4	33	65.8	3.04	2.60	1.84	1.62
5	20	82.5	3.60	3.37	2.97	1.71
6	30	86.2	4.19	3.90	2.05	1.39
7	23	74.8	3.24	3.20	1.90	1.23

Each of the five constant work rate tests was conducted, 1 wk apart, in the morning, with the subject having fasted for 10–12 h. Diet was otherwise not controlled, permitting us to evaluate day-to-day variability in resting E_N. Base-line samples of exhaled gas were collected for determination of E_N as described below. When the study was performed during hypoxia, the subject breathed 0.15 FI_{O_2} for 40 min before exercise. For the last 5 min of the resting base-line period and throughout 40 min of exercise, pulmonary gas exchange and heart rate were measured breath-by-breath. Base-line samples (60 ml) of exhaled gas for determination of $^{13}CO_2$ enrichment were collected at rest, at 20 and 40 min of rest during hypoxia, and at 5, 10, 20, and 40 min of exercise under each condition.

Measurement of pulmonary gas exchange. The subjects breathed through a low-impedance turbine volume transducer and breathing valve, with a combined deadspace of 170 ml. Oxygen and carbon dioxide tensions were determined by mass spectrometry from a sample drawn continuously from the mouthpiece at 1 ml/s. The inspired and expired volume and gas fraction signals underwent analog-to-digital conversion, from which oxygen uptake ($\dot{V}O_2$, STPD), carbon dioxide elimination ($\dot{V}CO_2$, STPD), and minute expired ventilation ($\dot{V}E$, BTPS) were calculated on-line, breath-by-breath, as previously described (3). Heart rate was obtained beat-by-beat using a modified lead V5 electrocardiogram.

Determination of $^{13}C/^{12}C$ of CO_2. $^{13}C/^{12}C$ ratios in CO_2 were determined and reported relative to the $^{13}C/^{12}C$ of the PDB (*Belemnitella americana*) standard (1.1237% ^{13}C). The results are expressed in $\delta^{13}C$ as

$$\delta^{13}C\ (‰) = \left[\frac{(^{13}C/^{12}C)\ \text{sample}}{(^{13}C/^{12}C)\ \text{standard}} - 1.0\right] \times 1000$$

The $\delta^{13}C$ values of the CO_2 samples were determined with a Nier 60° double-collecting mass spectrometer as modified by McKinney et al. (17). An alternative unit sometimes used to report $^{13}CO_2/^{12}CO_2$ is the atom per cent excess (APE) where 1 "per mil" (δ) is equal to 1.123×10^{-3} APE (26).

Data analysis. The effects of work intensity and hypoxia on enrichment of CO_2 during exercise were tested using analysis of variance (one- and two-way with repeated measures). Differences between means were

tested with Duncan's multiple range test. Because of unequal variances among the subjects for resting natural enrichment values, Friedman's nonparametric analysis of variance was used to evaluate differences between the subjects. Student's paired t test was used to compare the value of natural enrichment at 5 min into exercise with the preexercise base line. Significant difference was declared at $P < 0.05$. Regression analysis was used to determine any association between the respiratory exchange ratio R and the natural enrichment E_N.

RESULTS

Rest. There were no significant differences in $\dot{V}O_2$ or R among the rest periods preceding the three room air exercise bouts (mean $\dot{V}O_2$ of 0.422, 0.400, 0.398 liter/min for rest preceding WR-1, WR-2, and WR-3, respectively; corresponding mean R of 0.86, 0.83, and 0.87); likewise, 40 min of breathing hypoxic gas produced no significant changes in either variable (mean $\dot{V}O_2$ of 0.430 and 0.428 l/min for WR-1 and WR-2, respectively, with corresponding R value of 0.85 and 0.82).

Typical natural enrichment values for CO_2 during rest and the five constant work rate protocols are shown for one subject in Fig. 2. As can be seen, there is day-to-day variability in the base-line E_N for this subject, and the magnitude of this variability differed among the seven subjects. Hypoxia did not significantly change the E_N from base-line values at either 20 or 40 min of rest (for rest preceding WR-1: mean base-line $E_N = -21.5$, 20 min $= -21.5$, 40 min $= -21.6$; for rest preceding WR-2: base-line $E_N = -21.7$, 20 min $= -21.8$, 40 min $= -21.9$).

Table 2 details the intra- and intersubject variability in resting E_N in the overnight fasted state determined from the initial base-line values obtained before exercise or exposure to hypoxia. There was a significant subject-to-subject difference for the 5-day average of the resting base line natural enrichment ($P < 0.05$). The coefficients of variation determined for each subject from the resting values for the five protocols ranged from 2.0 to 13.1%, whereas that determined from the means for each of the seven subjects was 5.2%. There was no significant relationship between resting respiratory exchange ratio and resting E_N (not shown).

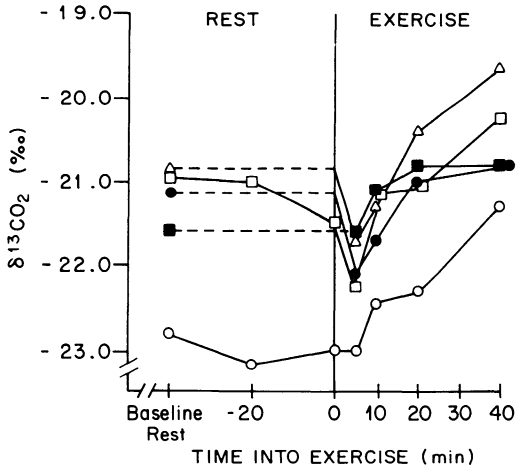

FIG. 2. Natural enrichment values for 1 subject at base-line rest, at hypoxic rest when appropriate, and during exercise. ■, WR-1, room air; □, WR-1, hypoxia ($FI_{O_2} = 0.15$); ●, WR-2, room air; ○, WR-2, hypoxia; △, WR-3, room air. See text for description of work rates. Note fall in E_N at 5 min and rise above resting by 20–40 min.

TABLE 2. *Variability in base-line natural enrichment of breath CO_2*

Subj No.	Base-Line E_N, ‰	Coeff of Variation, %
1	22.4±0.91	4.1
2	20.2±1.69	8.4
3	19.4±2.54	13.1
4	21.7±0.99	4.6
5	22.1±0.44	2.0
6	22.0±0.54	2.5
7	21.5±0.80	3.7
Mean*	21.3±1.12	5.2

Values are means ± SD; $n = 5$. * Determined from mean base-line E_N for each subject.

TABLE 3. *Gas exchange responses to exercise*

	WR-1		WR-2		WR-3
	Room air	Hypoxia	Room air	Hypoxia	Room air
$\dot{V}O_2$, l/min	1.46	1.44	2.01	2.01	2.63
%LT	66	90	89	129	122
R	0.89	0.90	0.91	0.97*	1.01*

Values are group means for individual responses averaged from 5 to 20 min of exercise. $n = 7$ except for where $n = 6$. Hypoxia represented $FI_{O_2} = 0.15$, room air $FI_{O_2} = 0.207$. * Significantly different from both WR-1 conditions and WR-2 in room air ($P < 0.05$). Note that work above LT (WR-2 in hypoxia and WR-3) resulted in a significant increase in R.

Exercise. The average responses of $\dot{V}O_2$ and R over *minutes 5–20* of exercise are summarized in Table 3. $\dot{V}O_2$ increased with each work rate. Hypoxia had no effect on exercise $\dot{V}O_2$, either at work rate 1 or 2. As shown in Table 3, the selection of work rates produced the desired metabolic rates (Fig. 1). Both of the $\dot{V}O_2$ responses for WR-1 were below the respective LTs, the $\dot{V}O_2$ response to WR-2 was below LT on room air but exceeded the LT on hypoxia, whereas WR-3 resulted in a $\dot{V}O_2$ which was clearly above LT. In addition, the average R for the three work conditions below the LT were indistinguishable as shown in Table 3, whereas work performed above the LT resulted in significantly higher R values ($P < 0.05$).

The effect of work intensity during normoxia on group mean values for E_N is shown in Fig. 3*A*, plotted as the change in E_N from resting values. E_N fell significantly 5 min into exercise ($P < 0.05$, paired t test) for all three work rates, and then rose, becoming significantly greater than resting values at 20 and 40 min of exercise for each protocol ($P < 0.05$). There was no difference in the changes in E_N between the two below LT work rates at any time during exercise. However, the changes in E_N at 20 min ($\Delta\delta = 1.79$‰) and 40 min ($\Delta\delta = 2.71$‰) for the above LT work rate were significantly greater ($P < 0.05$) than those observed at the two below LT protocols (work rate 1: $\Delta\delta = 0.66$‰ and 1.16‰; work rate 2; $\Delta\delta = 0.93$‰ and 1.33‰ for 20 and 40 min, respectively).

The effect of work rate relative to the lactate threshold on changes in breath $^{13}CO_2$ during air breathing can be seen clearly in Fig. 4, where the change in $\delta^{13}CO_2$ is

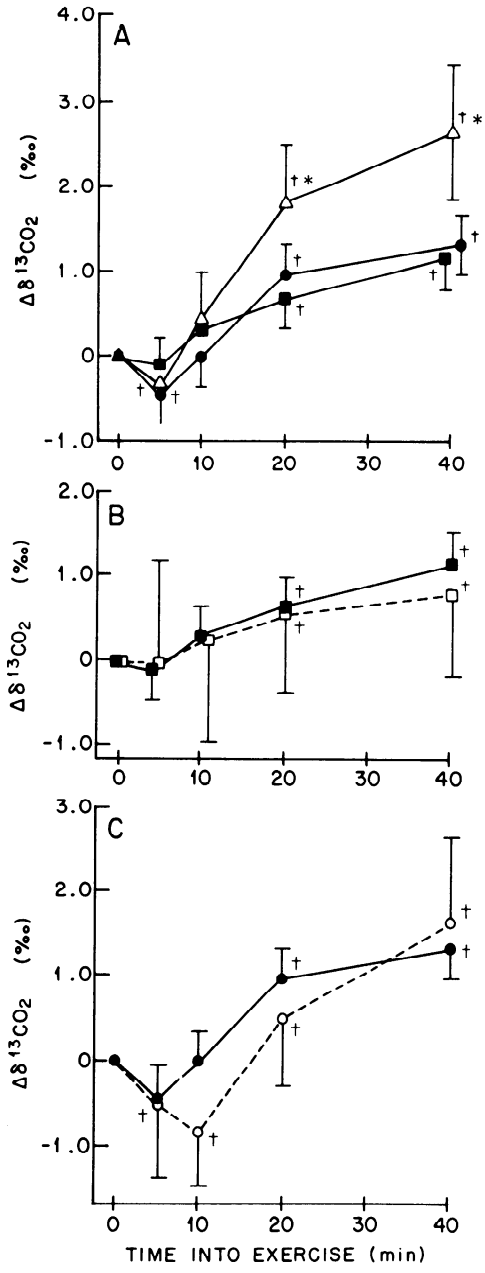

FIG. 3. Effect of work intensity on breath $^{13}CO_2/^{12}CO_2$. Values (±SE of mean difference) represent change from rest ($\Delta\delta^{13}CO_2$). Symbols as in Fig. 2. † Significantly different from resting values ($P < 0.05$). * Significantly different from below LT values ($P < 0.05$). *A*: normoxic response. All 3 protocols resulted in an increase in E_N above resting values at 20 and 40 min. There was no difference between the 2 below LT work rates at any time; WR-3 (above LT) resulted in significantly higher E_N values at 20 and 40 min. *B*: normoxic vs. hypoxic responses at WR-1. There were no differences between responses at any time. *C*: normoxic vs. hypoxic responses for WR-2. Hypoxia depressed the E_N below rest at both 5 and 10 min, but neither value was significantly different from the corresponding normoxic value.

plotted as a function of metabolic rate ($\dot{V}O_2$) at the end of exercise. Although there was a substantial increase in metabolic rate between work rates 1 and 2, there was no concomitant increase in $\delta^{13}CO_2$. However, when a further increase in metabolic rate resulted in a $\dot{V}O_2$ which exceeded the normoxic lactate threshold (WR-3), $\Delta\delta^{13}CO_2$

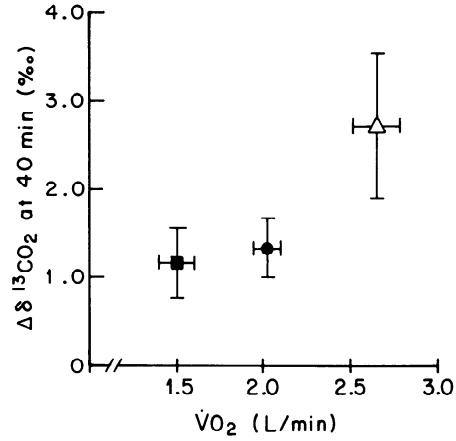

FIG. 4. Effect of metabolic rate during normoxic exercise on change in $^{13}CO_2/^{12}CO_2$ ($\Delta\delta^{13}CO_2$) at end exercise (40 min). Symbols as in Fig. 2. Values are means ± SE for $\dot{V}O_2$ and SE of mean difference for $\Delta\delta^{13}CO_2$. All end exercise values were significantly greater than resting. Despite a large increase in $\dot{V}O_2$ from WR-1 to WR-2, there was no additional increase in end exercise $\Delta\delta^{13}CO_2$. Only when exercise exceeded LT did end exercise $\Delta\delta^{13}CO_2$ rise further.

at the end of exercise was significantly greater than for either below LT work rate.

As shown in Fig. 3*B*, the pattern of change in E_N during hypoxic exercise at the lower work rate (WR-1) was identical with that seen during normoxia, except that E_N was not depressed 5 min into exercise. At the intermediate work rate (WR-2), which was below LT for normoxia but above LT under hypoxic conditions, the fall in E_N during hypoxia was significant at both 5 and 10 min of exercise compared with rest ($P < 0.05$), but was not significantly different from the normoxic response (Fig. 3*C*). By 40 min, E_N had risen significantly above rest ($P < 0.05$) and was indistinguishable from the normoxic value. Thus, hypoxic exercise performed above the hypoxic LT did not result in a significantly higher E_N at the end of exercise compared with below LT exercise, in contrast to the normoxic above LT condition (WR-3). Although the changes in E_N during hypoxic exercise were similar to those found during normoxia, the responses were more variable, as denoted by the larger standard error bars for the hypoxic data in Fig. 3, *B* and *C*.

The time course of change in E_N during exercise (Figs. 2 and 3) did not parallel the change seen in the R (Fig. 5). For clarity, only the R during normoxic exercise is shown; the hypoxic responses showed the same trend over time, and the mean values are given in Table 3. Although both the R and E_N signals decreased initially with the onset of exercise, the R rose more quickly, so that a peak value was obtained by 5–10 min; after this the R fell slowly over the remainder of exercise. In contrast, E_N continued to rise from 5–10 min through 40 min of exercise (Fig. 3). However, the inverse responses of R and E_N with time during exercise were generally not related to each other, as demonstrated in Fig. 6, where group mean responses for $\Delta\delta^{13}CO_2$ are plotted against the corresponding changes in R from base line at 5, 10, 20, and 40 min of exercise for all five exercise protocols. The exception was WR-3, where changes in E_N from rest

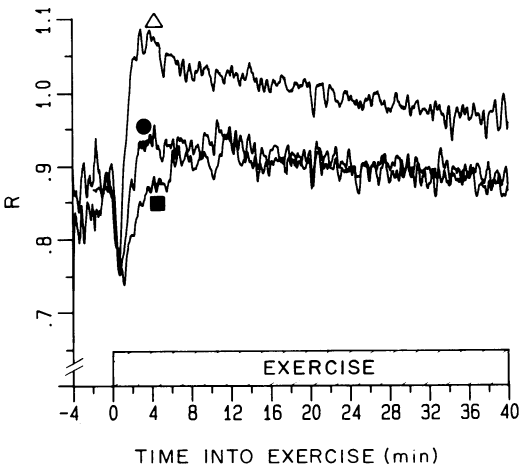

FIG. 5. Time course of R ($\dot{V}CO_2/\dot{V}O_2$) during room air exercise. Symbols as in Fig. 2. Responses of R during hypoxic exercise showed similar time courses (see Table 3). Note downward trend from 10 min to the end of exercise (40 min).

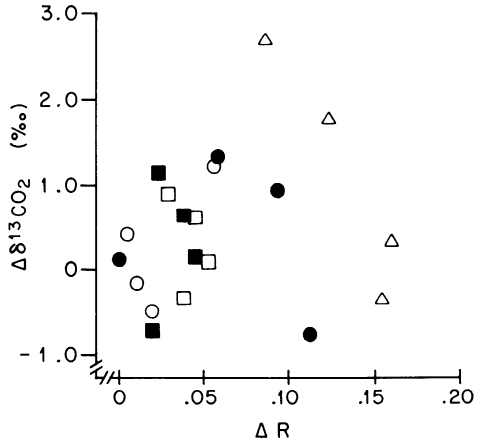

FIG. 6. Changes in breath $^{13}CO_2/^{12}CO_2$ ($\Delta\delta^{13}CO_2$) during exercise as a function of changes in respiratory exchange ratio (ΔR). Symbols as in Fig. 2. Note overall lack of relationship between these 2 potential indicators of oxidative fuel mix. For WR-3, however, $^{13}CO_2$ was significantly negatively correlated ($r = 0.93$, $P < 0.05$) with R.

were negatively correlated with changes in R ($r = -0.93$, $P < 0.05$).

DISCUSSION

This study demonstrated that natural enrichment of breath CO_2 with $^{13}CO_2$ during exercise was consistently higher than that during rest (Figs. 2 and 3). However, our data show that the changes in E_N of breath CO_2 seen with exercise under normoxic conditions are not monotonically related to the intensity of work being performed (Fig. 4). Increases in work below the LT, even when accompanied by a substantial increase in metabolic rate, did not result in a concomitantly higher E_N. In contrast, increases in work which required a metabolic rate that was above the LT resulted in a significantly greater increase in $^{13}CO_2/^{12}CO_2$ in the expired gas. It also appears that hypoxia, even when it transforms below LT work into above LT work, does not have any significant additional effect on the pattern of change in E_N during 40 min of exercise.

The resting background E_N of exhaled CO_2 was significantly different from subject to subject, suggesting that it should be considered when interpreting stable isotope changes under various metabolic conditions. Because resting RQ was not significantly different, this variability was likely caused by variability in the $^{13}CO_2/^{12}CO_2$ of the foodstuffs chosen ad libitum by the subjects. The E_N of exhaled CO_2 appears to reflect the E_N of the oxidative fuel mix at rest, as evidenced by the findings of Schoeller et al. (20), that mean resting E_N of exhaled CO_2 lay between the E_N of plasma glucose and lipid, and shifted toward plasma lipid values during a fast. In addition, both E_N of exhaled CO_2 and the R have been shown to track changes in substrate utilization consequent to exogenous lipid (21) or carbohydrate (14, 18) ingestion. We controlled for potential acute modulators of oxidative fuel mix (e.g., before meal and activity) by the overnight fast and avoidance of strenuous activity 24 h before each test. The lack of correlation between E_N and endogenous fuel mix (RQ) under steady-state rest conditions in the present study may be due to the variability in E_N for a given dietary source of oxidative fuel (21), which would therefore prevent direct association of a particular absolute E_N with a specific carbohydrate-to-lipid oxidation ratio, as can be done for the RQ.

Both R and E_N were elevated above resting levels during exercise, with work above the normoxic LT (WR-3) producing the greatest increase in both variables (Figs. 3 to 5). This is consistent with an apparent increase in carbohydrate utilization occurring above the LT (4, 23). However, several factors may influence the relationship between either R or E_N and the oxidative substrate (i.e., the true RQ). If the various body depots of glycogen (liver and muscle) and triglycerides (intramuscular and adipose) differ in their E_N values, then conceivably any changes in the tissue source of oxidative substrate with exercise could result in a dissociation between changes in E_N and changes in the true oxidative substrate mixture. Thus, as pointed out above for the rest condition, during exercise it may not be possible to associate a specific breath E_N with a specific oxidative fuel mix. We are unaware of any information regarding the variability in E_N for carbohydrate or triglycerides in various tissue. R will reflect substrate oxidation (RQ) under conditions where CO_2 stores are likely to be constant (e.g., rest and steady state exercise below the LT). However, during conditions when CO_2 stores are not constant, such as with hyperventilation, at the onset of exercise, and in response to changing lactic acid concentrations seen for work above the LT, R will not reflect the oxidative fuel mix. This can be seen in Fig. 5, where the R for WR-3 on room air is above 1.0 for approximately the first 20 min of exercise.

Both R and E_N showed a transient fall early in exercise, followed by a slower rise; the time course for the changes in R, however, was much faster than that of E_N (Figs. 3 and 5). This more rapid response of R relative to E_N when the oxidative fuel mix is manipulated during exercise can also be seen for endogenous substrate utilization (16, 20) and after an oral glucose load (19). For neither R nor E_N is the drop early in exercise reported here likely a reflection of transient increased lipid oxidation. Both

R and E_N are ratios of two processes, where the numerators for each [total CO_2 elimination ($\dot{V}CO_2$) for R, $^{13}CO_2$ elimination for E_N] increase more slowly during exercise than the respective processes in the denominator ($\dot{V}O_2$ for R, $\dot{V}CO_2$ for E_N). The consequence of this discrepancy in rates of increase between numerator and denominator leads to an obligatory transient fall in the ratio for both R and E_N. For R, the discrepancy between $\dot{V}O_2$ and $\dot{V}CO_2$ kinetics is due to the relatively greater storage capacity for CO_2 relative to O_2 (2). Regarding E_N, the attenuation in the rate of rise in $^{13}CO_2$ excretion relative to that of total CO_2 elimination ($\dot{V}CO_2$) appears not to be the result of isotopic fractionation across the lungs (8). Although fractionation may occur elsewhere in the body, a more likely explanation is the additional time required for redistribution of $^{13}CO_2$ throughout the body pools of bicarbonate. From compartmental analysis of CO_2 washout kinetics, three or four distinguishable pools within the body have been identified (1, 11, 25). Moreover, these compartments appear to alter their characteristics during exercise (1, 22). The average time for a CO_2 molecule which enters one of these pools to exit the body in the breath (mean residence time) can be as long as 60–90 min at rest (11). $\dot{V}CO_2$, and thus the total CO_2 in these pools, appear to approach a new steady state more rapidly after the onset of exercise (within 4–6 min) than does the enrichment of those pools with $^{13}CO_2$. In summary, $\dot{V}O_2$ adjusts slightly more rapidly from $\dot{V}CO_2$ in the transition from rest to exercise, whereas $^{13}CO_2$ adjusts much more slowly. Therefore, changes in E_N of breath CO_2 will be less likely to reflect the underlying oxidative fuel mix during short duration exercise (up to 40 min) than will the R.

Hypoxia did not alter E_N at rest, consistent with our previous finding of no change in peripheral blood glucose turnover (4). In that study it was also shown that a similar alteration of inspired oxygen concentration at a constant work intensity spanning the lactate threshold produced a marked increase in glucose turnover. Under similar conditions here (WR-2), R, but not E_N, was elevated during hypoxic compared to normoxic work. This suggests that the attenuation in rise in breath E_N with hypoxia may have obscured a true underlying increase in $^{13}CO_2$ production from carbohydrate oxidation. Hypoxia is known to profoundly affect the circulation (7, 10), which may alter the dynamics of bicarbonate flux in the body, and thus alter the appearance of $^{13}CO_2$ at the lungs.

In summary, E_N of expired CO_2 during rest in postabsorptive, healthy subjects can show large intra- and intersubject variability. Both E_N and R increase during exercise, and above LT exercise elicits a significantly greater increase in both variables than does below LT work, but the time course of change in E_N is delayed relative to that of R for both below and above LT exercise. Finally, hypoxia ($F_{I_{O_2}} = 0.15$) does not significantly alter the pattern of change in E_N at rest or during 40 min of exercise. E_N may be a useful, noninvasive means of assessing endogenous fuel mix, so long as a steady state for $^{13}CO_2$ in the body's bicarbonate pools has been achieved.

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REFERENCES

1. BARSTOW, T. J., D. M. COOPER, E. SOBEL, AND S. EPSTEIN. $^{13}CO_2$ washout kinetics can be used to estimate metabolic rate (Abstract). *Physiologist* 30: 230, 1987.
2. BARSTOW, T. J., D. M. COOPER, C. SPRINGER, AND S. EPSTEIN. Estimation of CO_2 stores by tracer dilution and $\dot{V}CO_2$ kinetics (Abstract). *FASEB J.* 2: A306, 1988.
3. BEAVER, W. L., N. LAMARRA, AND K. WASSERMAN. Breath-by-breath measurement of true alveolar gas exchange. *J. Appl. Physiol.* 51: 1662–1675, 1981.
4. COOPER, D. M., D. H. WASSERMAN, M. VRANIC, AND K. WASSERMAN. Glucose turnover in response to exercise during high- and low-$F_{I_{O_2}}$ breathing in man. *Am. J. Physiol.* 251 (*Endocrinol. Metab.* 14): E209–E214, 1986.
5. DENIRO, M. J., AND S. EPSTEIN. Influence of diet on the distribution of carbon isotopes in animals. *Geochim. Cosmochim. Acta* 42: 495–506, 1978.
6. DENIRO, M. J., AND S. EPSTEIN. Mechanism of carbon isotope fractionation associated with lipid synthesis. *Science Wash. DC* 197: 261–263, 1977.
7. DORINSKY, P. M., R. L. HAMLIN, M. E. WHITCOMB, AND J. E. GADEK. Hypoxia results in an increase in total systemic shunt flow in dogs. *Respir. Physiol.* 62: 231–243, 1985.
8. DOWNEY, R. S., A. MELLONE, AND D. E. MATTHEWS. Effect of tracer infusion site on measurement of bicarbonate-carbon dioxide metabolism in dogs. *J. Appl. Physiol.* 60: 1248–1253, 1986.
9. ESSEN, B., L. HAGENFELDT, AND L. KAIJSER. Utilization of blood-borne and intramuscular substrates during continuous and intermittent exercise in man. *J. Physiol. Lond.* 265: 489–506, 1977.
10. FITZGERALD, R. S., R. J. TRAYSTMAN, J. T. SYLVESTER, AND S. PERMUTT. Comparison of the effects of hypoxic hypoxia and carbon monoxide: A review. In: *Morphology and Mechanism of Chemoreceptors*, edited by A. S. Paintal. New Delhi, India: Navchetan, 1976.
11. IRVING, C. S., W. W. WONG, R. J. SHULMAN, E. O'BRIAN SMITH, AND P. D. KLEIN. [^{13}C]Bicarbonate kinetics in humans: intra- vs. interindividual variations. *Am. J. Physiol.* 245 (*Regulatory Integrative Comp. Physiol.* 14): R190–R202, 1983.
12. JANSSON, E. Diet and muscle metabolism in man. *Acta Physiol. Scand.* 487: 1–24, 1980.
13. KRZENTOWSKI, G., B. JANDRAIN, F. PIRNAY, F. MOROSA, M. LACROIX, A. S. LUYCKX, AND P. J. LEFEBVRE. Availability of glucose given orally during exercise. *J. Appl. Physiol.* 56: 315–320, 1984.
14. LACROIX, M., F. MOROSA, M. PONTUS, P. LEFEBVRE, A. LUYCKX, AND G. LOPEZ-HABIB. Glucose naturally labeled with carbon-13: use for metabolic studies in man. *Science Wash. DC* 181: 455–446, 1973.
15. LUSK, G. *The Elements of the Science of Nutrition.* New York: Johnson Reprint, 1976.
16. MASSICOTTE, D., C. HILLAIRE-MARCEL, M. LEDOUX, AND F. PEONNET. The natural isotope tracing with ^{13}C: a noninvasive method for studying the metabolism during exercise. *Can. J. Appl. Sports Sci.* 9: 164, 1984.
17. MCKINNEY, C. R., J. M. MCCREA, S. EPSTEIN, H. A. ALLEN, AND H. C. UREY. Improvements in mass spectrometers for measurement of small differences in isotopic abundance ratios. *Rev. Sci. Instrum.* 21: 724–730, 1950.
18. MOROSA, F., P. LEFEBVRE, F. PIRNAY, M. LACROIX, A. LUYCKX, AND J. DUCHESNE. Quantitative evaluation of the oxidation of an

exogenous glucose load using naturally labeled ^{13}C-glucose. *Metabolism* 25: 1575–1582, 1976.

19. PIRNAY, F., M. LACROIX, F. MOROSA, A. LUYCKX, AND P. LEFEBVRE. Glucose oxidation during prolonged exercise evaluated with naturally labeled [^{13}C]glucose. *J. Appl. Physiol.* 43: 258–261, 1977.
20. SCHOELLER, D. A., C. BROWN, K. NAKAMURA, A. NAKAGAWA, R. S. MAZZEO, G. A. BROOKS, AND T. F. BUDINGER. Influence of metabolic fuel on the $^{13}C/^{12}C$ ratio of breath CO_2. *Biomed. Mass Spectrom.* 11: 557–561, 1984.
21. SCHOELLER, D. A., P. D. KLEIN, J. B. WATKINS, T. HEIM, AND W. C. MACLEAN, JR. ^{13}C abundances of nutrients and the effect of variations in ^{13}C isotopic abundances of text meals formulated for $^{13}CO_2$ breath tests. *Am. J. Clin. Nutr.* 33: 2375–2385, 1980.
22. SLANGER, B. H., N. KUSUBOV, AND H. S. WINCHELL. Effect of exercise on human CO_2-HCO_3 kinetics. *J. Nucl. Med.* 11: 716–718, 1970.
23. WAHREN, J., P. FELIG, G. AHLBORG, AND J. JORFELDT. Glucose metabolism during leg exercise in man. *J. Clin. Invest.* 50: 2715–2725, 1971.
24. WHIPP, B. J., J. A. DAVIS, F. TORRES, AND K. WASSERMAN. A test to determine parameters of aerobic function during exercise. *J. Appl. Physiol.* 50: 217–221, 1981.
25. WINCHELL, H. S., H. STAHELIN, N. KUSUBOV, B. SLANGER, M. FISH, M. POLLYCOVE, AND J. H. LAWRENCE. Kinetics of CO_2-HCO_3 in normal adult males. *J. Nucl. Med.* 11: 711–715, 1970.
26. WOLFE, R. R., J. H. F. SHAW, E. R. NADEL, AND M. H. WOLFE. Effect of substrate intake and physiological state on background $^{13}CO_2$ enrichment. *J. Appl. Physiol.* 56: 230–234, 1984.