Influence of increased metabolic rate on [^{13}C]bicarbonate washout kinetics

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BARSTOW, THOMAS J., DAN M. COOPER, ERIC M. SOBEL, ELLIOT M. LANDAW, AND SAM EPSTEIN. *Influence of increased metabolic rate on [^{13}C]bicarbonate washout kinetics.* Am. J. Physiol. 259 (Regulatory Integrative Comp. Physiol. 28): R163–R171, 1990.—The effect of changes in metabolic rate on the dynamics of CO_2 exchange among its various compartments in the human body is not well understood. We examined CO_2 dynamics in six healthy male subjects using an intravenous bolus of [^{13}C]bicarbonate. Subjects were studied while resting, during light exercise [50% of the lactate threshold (LT), 3–4 times resting O_2 uptake ($\dot{V}O_2$)], and during moderate exercise (95% of the LT, 6 times resting $\dot{V}O_2$). The sum of three exponential terms well described the washout of $^{13}CO_2$ in exhaled breath both at rest and during each exercise level despite substantial increases in metabolic rate accompanying the exercise studies. Average recovery of ^{13}C label rose from 67% during rest to 80% during light and moderate exercise ($P < 0.01$). The estimate of CO_2 elimination ($\dot{V}CO_2$) calculated from the washout parameters and corrected for recovery was in very good agreement with the $\dot{V}CO_2$ directly measured simultaneously breath by breath ($r = 0.993$, SE for $\dot{V}CO_2 = 0.079$ l/min). By use of a three-compartment mammillary model, the quantity of CO_2 in the central pool (Q_1) doubled from rest to light exercise (233 ± 60 to 479 ± 76 mmol, $P < 0.01$) but did not change further with moderate exercise (458 ± 74 mmol). Rate constants for exchange between pools and for irreversible loss from the system tended to increase with metabolic rate, but there was large variation in the responses. We conclude that the compartmental dynamics of CO_2 transport and storage are very sensitive to changes in metabolic rate induced by exercise.

stable isotope; carbon dioxide transport; mammillary model; compartmental analysis; gas exchange

THE STORES of O_2 in the body are relatively small; consequently, changes in O_2 uptake ($\dot{V}O_2$) observed at the mouth parallel closely the simultaneous utilization of O_2 in metabolism by the various tissues of the body (2). In contrast, the body stores of CO_2 are large, so that changes in the metabolic production of CO_2 are not instantaneously translated to changes in CO_2 elimination ($\dot{V}CO_2$) at the mouth (13). These stores of CO_2 and their effect on CO_2 transport in health and disease are not well understood.

CO_2 stores have been studied in the intact organism by the intravenous administration of radioactive ^{11}C-, ^{14}C-, or nonradioactive ^{13}C-labeled bicarbonate (6, 19, 31). The subsequent washout of bicarbonate as labeled CO_2 in the breath during resting conditions typically has been described by the sum of three exponential terms (19, 20, 23, 32, 35), implying the presence of at least three major classes of kinetically distinct processes or pools, which affect the dynamics of $\dot{V}CO_2$ at the mouth. These processes have been interpreted to represent washout of bicarbonate from a central pool in communication with two different tissue pools of bicarbonate with different perfusions (13, 19). Because exercise produces both an increased metabolic production of CO_2 and also significant changes in blood flow to several organs, we predicted that exercise would have a profound effect on the kinetics of bicarbonate washout.

We also wondered how exercise affects the following two aspects of CO_2 dynamics relevant to the evaluation of the oxidation of C-labeled substrates to CO_2: recovery of label and the average time a CO_2 molecule would reside in the exchanging CO_2 pools before being eliminated from the bicarbonate system (mean residence time; MRT). Recovery of injected bicarbonate as labeled CO_2 in the breath during resting conditions has been found to range between 50 and 90% (6, 11, 19–21, 34, 35) and is reported to decrease slightly with very mild exercise (31). No information is currently available regarding the effects of a wide range of metabolic rates on recovery of injected C-labeled bicarbonate or on MRT. To evaluate the influence of exercise on the dynamics of bicarbonate flux in the body and washout in the breath, we measured the washout of intravenously injected [^{13}C]bicarbonate as breath $^{13}CO_2$ during the following three metabolic states: rest, light exercise (three- to fourfold increase in metabolic rate), and moderate exercise (up to sevenfold increase in metabolic rate, but below an intensity that would result in sustained metabolic acidosis from lactic acid accumulation).

METHODS

Subjects. Six male volunteers, aged 21–34 yr, gave informed consent to participate in the study. Each was free from known cardiopulmonary or metabolic disease

at the time of testing. All were physically active but none were undergoing extensive endurance training.

Protocol. Each subject performed a progressive cycle ergometer test to volitional fatigue, from which $\dot{V}O_{2max}$ and the lactate (or anaerobic) threshold (LT, the maximum $\dot{V}O_2$ achieved before lactate begins accumulating significantly in the blood) were determined noninvasively from gas exchange and ventilatory patterns measured on a breath-to-breath basis as described below. Work rate on the cycle ergometer was increased in a ramp fashion by 30 W/min, following a 4-min period of unloaded cycling. Subject characteristics and $\dot{V}O_{2max}$ are given in Table 1.

[^{13}C]bicarbonate washout kinetics were subsequently determined on separate mornings under one of the following three conditions: seated rest or during cycle ergometry at either 50 or 95% of the LT. The washout experiments in each subject were separated by ~1 wk. The experiments were performed in the morning, with the subject fasted for 10–12 h. Within 12 h before each turnover experiment, a 588 mM solution of $NaH^{13}CO_3$ (Merck Sharp & Dohme lot no. 1931-L, 99.0%) in saline was made and sterilized by filtration. Chemical purity of the labeled bicarbonate was determined separately by back-titration with 0.05 N HCl, while the isotopic enrichment was confirmed by independent ^{13}C nuclear magnetic resonance. For the studies at rest, the subject sat in a chair in the lab for 0.5 h before the start of the experiment and for the subsequent 4 h during the experiment. At *time 0* a bolus injection over 5 s, containing 1.176 mmol [^{13}C]bicarbonate was made into an antecubital vein. Aliquots of exhaled gas (60 ml) for subsequent analysis for $^{13}CO_2$ (described below) were drawn from the exhaled port of the breathing valve into plastic syringes and promptly sealed. Samples were drawn over 10 s, with the midpoint corresponding to the sample time, at the following times: ~5 min before the injection and 1, 3, 5, 7, 9, 12, 15, 20, 30, 60, 90, 120, 150, 180, 210, and 240 min after injection. Metabolic rate (as $\dot{V}O_2$ and $\dot{V}CO_2$) was measured breath by breath for a 5-min period every hour during the rest experiments. To avoid any transient hyperventilatory responses, subjects were placed on the mouthpiece at least 1 min before collection of the $^{13}CO_2$ breath samples or measurement of $\dot{V}CO_2$.

In preliminary studies we observed a more rapid washout of $^{13}CO_2$ during exercise, which reduced our ability to describe the components of the washout kinetics using the rest protocol. Therefore, a larger dose of labeled sodium bicarbonate (1.765 mmol) was administered for the exercise studies, and samples were obtained at more frequent intervals. The injection was made 20 min after exercise was begun, when a new metabolic steady state had been reached as judged by stable values for $\dot{V}CO_2$ and $\dot{V}O_2$. Samples of exhaled gas were taken for determination of $^{13}CO_2/^{12}CO_2$ at *time 0* (20 min into exercise) and at 1, 2, 3, 4, 5, 6, 8, 10, 12, 15, 20, 30, 45, 60, 75, 90, 105, and 120 min after injection. $\dot{V}O_2$, $\dot{V}CO_2$, and heart rate were measured during exercise from 20 min before the injection to 20 min after injection and subsequently for 5-min intervals every 30 min after injection.

TABLE 1. *Subject characterization*

Subject	Age, yr	Weight, kg	$\dot{V}O_{2\,max}$, l/min
1	23	86.5	2.37
2	33	65.8	2.73
3	26	73.6	3.48
4	23	74.8	2.91
5	31	68.2	4.02
6	29	79.5	2.96

Measurement of pulmonary gas exchange. The subjects breathed through a low-impedance turbine volume transducer and breathing valve with a combined dead space of 170 ml. A three-way respiratory valve, followed by a respiratory hose with a dead space of ~1 liter, was placed on the expiratory side of the breathing valve for sampling of expired gas for $^{13}CO_2$. Mouth O_2 and CO_2 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 $\dot{V}O_2$ (STPD), $\dot{V}CO_2$ (STPD), and minute expired ventilation ($\dot{V}E$; BTPS) were calculated on line with each breath, as previously described (4). The effect of the breathing valve on these calculations was evaluated by comparison with bag collection. A calibration factor was then used to obtain the final $\dot{V}O_2$ and $\dot{V}CO_2$ reported here. Using this calibration factor, the SE values for $\dot{V}CO_2$ and $\dot{V}O_2$ were 27 and 45 ml, respectively. Heart rate during exercise was measured beat by beat using a modified V5 lead electrocardiogram.

Analysis of exhaled gas for $^{13}CO_2/^{12}CO_2$. The CO_2 was isolated from the breath samples before analysis by ion ratio mass spectrometry by passage through a trap in dry ice (to remove water vapor) and then condensed in a trap in liquid nitrogen, allowing other gases to be evacuated (7). The CO_2 collected from the liquid nitrogen trap was further purified by passage over Cu turnings and MnO_2 powder before isotopic analysis. This combined method of collection, isolation, and analysis led to greater precision (reduced variability) compared with commercial procedures. The ratio of $^{13}CO_2/^{12}CO_2$ in the exhaled gas samples was determined with a Nier 60° double-collecting ion-ratio mass spectrometer, as modified by McKinney et al. (26). The ratio is reported in units of $\delta^{13}CO_2$ relative to the PDB (*Belemnitella americana*) standard (1.1235% ^{13}C) and is defined as

$$\delta^{13}C(‰) = \left[\frac{(^{13}C/^{12}C)\ \text{sample}}{(^{13}C/^{12}C)\ \text{standard}} - 1.0\right] \times 1{,}000 \quad (1)$$

The value of the base line was subtracted from each value collected after injection of the [^{13}C]bicarbonate, yielding a net change in δ (delta over base line; DOB). DOB can be converted to an equivalent excess specific activity (excess $^{13}CO_2$/total CO_2) by multiplying DOB by 1.123×10^{-5}.

General regression analysis of DOB data. For subsequent noncompartmental and compartmental analyses, it was necessary to find an empirical model that best fit the DOB washout data. The empirical models were se-

lected from among the following set

$$\text{DOB} = \sum_{i=1}^{n} A_i e^{\lambda_i t} \quad (2)$$

$$\text{DOB} = \sum_{i=1}^{n} A_i e^{\lambda_i t} + L \cdot t \quad (3)$$

$$\text{DOB} = \sum_{i=1}^{n} A_i e^{\lambda_i t} + C \quad (4)$$

$$\text{DOB} = \sum_{i=1}^{n} A_i e^{\lambda_i t} + L \cdot t + C \quad (5)$$

where $n = 1, 2, 3$, or 4. Thus 16 candidate models were evaluated for each washout experiment. The A_i, λ_i, L, and C are referred to as the macroparameters of the model where A_i is the coefficient and λ_i the rate constant for the exponential process, L is the slope for a linear term, and C is a constant offset. Previous studies (19) included the linear trend (L) and constant offset (C) terms in *Eqs. 3–5*. For a specific candidate model with fixed value of n, the best fit to the washout data was found using the weighted least squares (WLS) programs BMDPAR and BMDP3R (10). An optimal weighting scheme was used, namely weighting each datum inversely proportional to the measurement variance at that time (24). Preliminary analysis of residuals suggested that measurement error variance was approximately proportional to the square root of the observed DOB value. Alternate weighting schemes, including unweighted least squares, gave similar results to those reported below, suggesting robustness of our $1/\sqrt{\text{DOB}}$ weighting scheme. The BMDP programs provide point estimates and asymptotic standard errors for the model parameters and also for desired functions of the model parameters [e.g., area under curve (AUC) and MRT].

Because *Eqs. 2–5* are from a series of nested models, the choice of the best fitting model was made by appropriate comparisons among the WLS fits of the 16 candidate models using an F test (5, 24) and assuming Gaussian errors. When comparing two nested models, we hypothesized that the simpler model is the true model and rejected this hypothesis in favor of the more complex model if the F statistic was sufficiently large (e.g., $P < 0.05$). For confirmation, we also used the Akaike Information Criterion and the Schwartz Criterion (24) to compare all 16 candidate models simultaneously. These results were very similar to the F test.

Noncompartmental analysis of washout kinetics. From the washout curve for $^{13}CO_2$ in the breath, the following three important quantities were estimated: AUC, recovery of injected label, and the MRT. AUC was calculated by integrating to time = ∞, the best fit regression equation for each washout experiment after subtracting out any linear trend or constant offset terms. For purely exponential models (from *Eq. 2*) this was equal to

$$\text{AUC} = \sum_{i=1}^{n} - (A_i/\lambda_i) \quad (6)$$

These regression-based AUC estimates compared well with results calculated directly from the DOB data using the trapezoidal rule with a single exponential extrapolation of the tail.

Recovery was calculated as

$$\text{Recovery (\%)} = \frac{\text{AUC} \times 1.123 \times 10^{-5} \times \dot{V}\text{CO}_2}{D_0} \times 100 \quad (7)$$

where AUC is in units of DOB·min, 1.123×10^{-5} converts DOB to the fractional enrichment of total CO_2 with added $^{13}CO_2$, $\dot{V}\text{CO}_2$ is the measured rate of CO_2 elimination at the mouth in millimoles per minute, and D_0 is the dose in millimoles of [^{13}C]bicarbonate injected at *time 0*.

MRT for the whole bicarbonate system indicates the average time a labeled CO_2 molecule, introduced into the central compartment as in this study, would remain in the exchanging bicarbonate system before being irreversibly lost either into the breath or via unaccounted loss. MRT was estimated from the washout curves as the area under the moment curve (AUMC) divided by the AUC (9), assuming that the system is linear and stationary, that there are no CO_2-bicarbonate traps within the exchanging system, and that CO_2 is eliminated only from the central pool. For the purely exponential model, $\text{AUMC} = \Sigma(A_i/\lambda_i^2)$.

Compartmental analysis. Assuming that the washout data were adequately described by a sum of exponentials, we analyzed the data using a linear, mammillary compartmental system. For example, washout data with three exponentials would correspond to a three-pool model with one central pool (*compartment 1*; e.g., plasma) and two peripheral pools connecting only to *compartment 1*. Assuming tracer entry and CO_2 loss is only via the central pool, this model is diagrammed in Fig. 1, where k_{ij} is the first order rate constant for transfer to *pool i* from *pool j*, Q_i is the steady-state quantity of unlabeled CO_2 stores in *pool i*, and k_{01} (not shown) is the fractional elimination rate for total irreversible loss of CO_2 from the central pool. Because recovery of label as

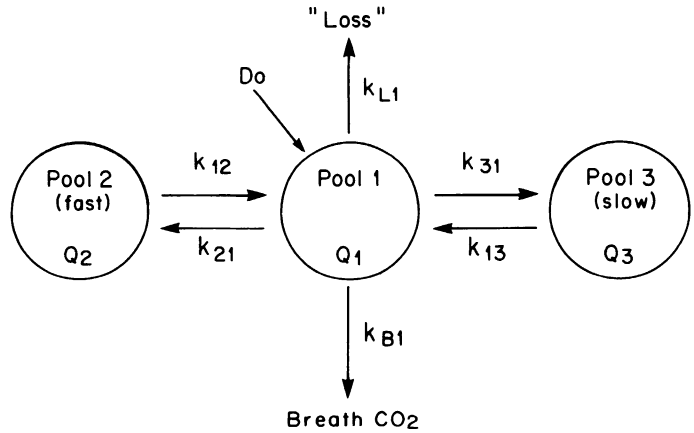

FIG. 1. Representative 3-compartment mammillary model for the washout of [^{13}C]bicarbonate using the sum of 3 exponentials. Q_i is the steady-state quantity of unlabeled CO_2 in each pool, whereas k_{ij} represents 1st order rate constants for exchange of CO_2 from *pool j* to *pool i*. Irreversible loss (clearance) from system through central *pool 1* occurs both by respiratory (measured; k_{B1}) and nonrespiratory (unaccounted loss; k_{L1}) pathways. See text for further details.

$^{13}CO_2$ in the breath was not 100%, this implied loss of ^{13}C label through nonrespiratory pathways as well as in the breath. k_{B1} is thus defined as the rate constant for the measured loss from the central pool in the breath, and k_{L1} is the rate constant for unobserved loss from the central pool via nonrespiratory mechanisms; thus $k_{B1} + k_{L1} = k_{01}$. We assumed that DOB data represented direct measures of $^{13}CO_2$ enrichment (specific activity) in the central pool (11, 19, 20, 23). The peripheral pools were indexed such that $k_{12} > k_{13}$, so that *pool 2* was considered the rapidly exchanging pool and *pool 3* was a slowly exchanging site. The microparameters k_{ij} and central CO_2 stores Q_1 are identifiable from the DOB data and were found explicitly as functions of the macroparameters of the sums of exponentials model (25).

As we do not know the exact site(s) for entry of unlabeled endogenous CO_2 into the system, it is not possible to explicitly estimate the peripheral quantities of CO_2 (Q_2 and Q_3) and thus the total CO_2 in the system (9). However, upper and lower bounds for Q_2 and Q_3 may be derived for the three-pool model. With $k_{01} = \Sigma A_i / \Sigma(A_i/\lambda_i)$ and $Q_1 = D_0/\Sigma A_i$, one obtains the following bounds

$$Q_{min_i} \equiv Q_1 \frac{k_{i1}}{k_{1i}} \leq Q_i \leq Q_1 \frac{k_{i1} + k_{01}}{k_{1i}} \equiv Q_{max_i} \quad (8)$$

These are derived by evaluating the steady-state equations for the Q_i under different assumptions regarding endogenous CO_2 sources. For example, if Qt_i is defined to be the total CO_2 in the system at steady state given that all endogenous CO_2 enters the system only through *pool i*, then

$$Qt_1 = Q_1 + Q_{min_2} + Q_{min_3} \quad (9)$$

$$Qt_2 = Q_1 + Q_{max_2} + Q_{min_3} \quad (10)$$

$$Qt_3 = Q_1 + Q_{min_2} + Q_{max_3} \quad (11)$$

It can be shown that $Qt_1 < Qt < Qt_3$, where Qt is the true value of $Q_1 + Q_2 + Q_3$ (i.e., the total CO_2).

Other statistical analyses. Analyses comparing SE of macroparameters and microparameters within an individual to variability across subjects tended to show that population variability was much greater than estimation variability. Therefore, each summary measure across subjects of parameters, MRT, AUC, etc. is reported by the simple unweighted sample mean ± sample SD. The effect of metabolic rate on the various parameters of the $^{13}CO_2$ decay curves and on the resulting mammillary model parameters was assessed by analysis of variance with repeated measures. Significant differences between the means were further evaluated using paired *t* tests. Significance was declared for $P < 0.05$ after Bonferroni correction for multiple comparisons. Linear regression was used to examine any relationship between recovery of $^{13}CO_2$ in the exhaled breath and total $\dot{V}CO_2$.

RESULTS

Metabolic responses. The group mean for average $\dot{V}O_2$ over 4 h of rest was 281 ± 20 ml/min and for $\dot{V}CO_2$ was 216 ± 15 ml/min (9.7 mmol/min; Table 2). Light exercise caused a three- to fourfold increase in both variables; mean average $\dot{V}O_2$ was 929 ± 133 ml/min, while $\dot{V}CO_2$ increased to 840 ± 113 ml/min (37.7 mmol/min). Moderate exercise increased $\dot{V}O_2$ on average six times over rest (to 1,750 ± 420 ml/min) and $\dot{V}CO_2$ over seven times above resting values (1,639 ± 454 ml/min or 73.6 mmol/min). Light exercise represented 53% of the LT, or 30% of $\dot{V}O_{2max}$, while moderate exercise equated to 95% of the LT, or 57% of $\dot{V}O_{2max}$.

Model identification. The results from a typical washout experiment at each metabolic rate in one subject are shown in Fig. 2. Note that the washout dynamics at different metabolic rates were clearly distinguishable from each other and that even a mild increase in CO_2 production associated with light exercise caused a marked increase in the rate of loss of $^{13}CO_2$ into the breath. All 18 washout curves were well described by the sum of three exponential terms with no linear or constant terms (*Eq. 2*), as found previously by several investigators (19, 20, 23, 30–32, 35). In 12 of these experiments, *Eq. 2* with $n = 3$ was also the statistically best description of the data

$$DOB = A_1 \cdot e^{\lambda_1 t} + A_2 \cdot e^{\lambda_2 t} + A_3 \cdot e^{\lambda_3 t} \quad (12)$$

Whereas *Eq. 12* resulted in excellent fits in the remaining six washout experiments, four of these curves were better fit statistically by the sum of four exponential terms (*Eq. 2* with $n = 4$). These four more complex models were found in only two subjects, *subject 3* (light and moderate exercise) and *subject 6* (rest and moderate exercise). Two other experiments were best described by the sum of three exponentials plus a small constant offset term (*Eq. 4* with $n = 3$). This occurred in *subject 4* (moderate exercise) and *subject 6* (light exercise).

When a three-compartment mammillary model was compared to a four-compartment model for the four data sets for which a sum of four exponentials was the better fit, it appeared that the fast peripheral pool of the three-compartment model had split into two intermediate pools, with little change associated either with the central pool or with the slowest peripheral pool. In addition, the dynamic characteristics and quantity of CO_2 in the entire exchanging system were similar between the three- and four-compartment models for the same data sets. For purposes of comparison, therefore, we chose to utilize the three-exponential description of the washout (*Eq. 12*) for all data sets in order to estimate noncompartmental parameters (e.g., AUC and MRT) and for deriving compartmental information. In all cases, the effect of increased metabolic rate with exercise on the washout and model parameters was much greater than any changes in the parameter estimates due to an extra constant or exponential term.

Washout characteristics and noncompartmental analysis. Table 2 gives the work rate performed by each subject, the resulting $\dot{V}CO_2$, and the parameter estimates for *Eq. 12* for each of the three metabolic conditions. Asymptotic standard errors of the parameter estimates were generally much smaller than the standard deviation across subjects. Increased $\dot{V}CO_2$ associated with exercise resulted in significant reductions in A_1, A_3, and all three

TABLE 2. *Work rate, metabolic rate, and washout parameters from three exponential functions (Eq. 12)*

Subject	Work Rate, W	$\dot{V}CO_2$, mmol/min	A_1, DOB	λ_1, min^{-1}	A_2, DOB	λ_2, min^{-1}	A_3, DOB	λ_3, min^{-1}
				Rest				
1		10.54	437.0±10	−0.439±0.022	85.3±9.1	−0.068±0.014	49.9±7.2	−0.012±0.001
2		10.36	257.9±6.8	−0.367±0.019	77.9±7.1	−0.053±0.011	58.7±10	−0.013±0.001
3		9.61	331.7±13	−0.526±0.038	172.5±15	−0.108±0.010	55.7±3.1	−0.010±0.001
4[a]		8.95	201.9±6.9	−0.400±0.029	79.6±6.3	−0.053±0.010	52.7±7.7	−0.011±0.001
5		9.69	184.8±12	−0.465±0.047	115.5±13	−0.110±0.013	72.8±2.6	−0.012±0.000
6		9.07	370.0±11	−0.540±0.036	169.3±14	−0.110±0.009	65.0±2.7	−0.010±0.000
Mean		9.70	297.2	−0.456	116.7	−0.084	59.1	−0.012
±SD		±0.65	±99.3	±0.068	±44.2	±0.029	±8.5	±0.001
				Light exercise				
1	31	41.50	146.4±5.3	−0.430±0.022	117.1±4.6	−0.097±0.006	40.3±3.5	−0.022±0.001
2	46	42.69	222.0±16	−0.752±0.120	125.7±14	−0.135±0.025	46.4±9.1	−0.025±0.003
3	44	40.08	220.2±6.2	−0.684±0.043	150.2±5.8	−0.124±0.008	49.7±4.6	−0.026±0.003
4	28	29.81	153.3±2.0	−0.461±0.010	83.9±1.8	−0.084±0.004	53.2±2.5	−0.023±0.001
5	59	33.39	146.2±7.4	−0.475±0.039	94.0±6.5	−0.095±0.014	42.1±8.1	−0.026±0.003
6	28	39.06	176.4±7.1	−0.489±0.032	100.2±6.3	−0.100±0.012	48.0±6.4	−0.024±0.002
Mean		37.76	177.4[d]	−0.549	111.9	−0.106	46.6[b]	−0.024
±SD		±5.05	±35.6	±0.134	±24.2	±0.019	±4.8	±0.002
				Moderate exercise				
1	94	64.70	135.8±8.4	−0.893±0.117	127.0±8.7	−0.209±0.021	60.1±5.5	−0.051±0.002
2	139	86.40	219.5±12	−1.226±0.142	161.8±11	−0.288±0.020	44.0±3.4	−0.057±0.003
3	131	79.76	230.8±5.3	−0.728±0.038	114.3±5.9	−0.155±0.010	12.5±2.2	−0.027±0.003
4	82	51.42	223.3±6.4	−0.619±0.035	117.3±6.4	−0.127±0.011	34.6±4.2	−0.027±0.002
5	170	104.55	124.8±5.7	−0.993±0.102	119.0±6.5	−0.238±0.014	24.2±2.0	−0.046±0.002
6	60	54.96	201.1±3.7	−0.658±0.026	122.3±3.8	−0.128±0.006	28.0±2.2	−0.025±0.001
Mean		73.63	189.2[d]	−0.853[de]	127.0	−0.191[de]	33.9[d]	−0.039[cd]
±SD		±20.39	±46.8	±0.212	±17.6	±0.065	±16.6	±0.014

Values are means ± SE unless otherwise indicated. $\dot{V}CO_2$, CO_2 elimination; A_i and λ_i, washout parameters DOB, δ over base line. Asymptotic SE values of estimate were generally much smaller than SD values across subjects. [a] Actual dose was 1.76 mmol, not 1.176; A_i divided by 1.5. [b] $P < 0.05$ vs. rest; [c] $P < 0.05$ vs. light exercise; [d] $P < 0.01$ vs. rest; [e] $P < 0.01$ vs. light exercise.

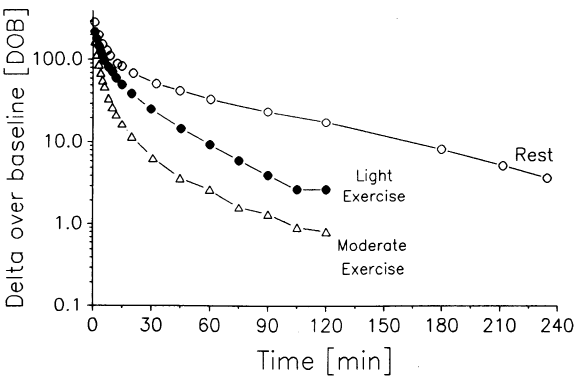

FIG. 2. Semilog plot of washout of breath $^{13}CO_2$ after intravenous injection of [^{13}C]bicarbonate at rest and during light and moderate exercise. Enrichment of breath CO_2 with ^{13}C expressed in units of δ over base line.

rate constants (λ_1, λ_2, λ_3) compared with rest.

The AUC, using *Eq. 6* and normalized for a standard injection of 1.76 mmol [^{13}C]bicarbonate, fell dramatically for light exercise compared with rest conditions (10,787 ± 1,195 DOB·t for rest vs. 3,308 ± 267 DOB·t at light exercise, $P < 0.01$). Moderate exercise caused a further significant decrease in AUC (1,761 ± 445 DOB·t, $P < 0.01$ compared with light exercise and rest).

MRT fell significantly from 65 ± 7 min (mean ± SD) at rest to 27 ± 2 min with light exercise ($P < 0.01$) and fell significantly lower still during moderate exercise (16 ± 5 min, $P < 0.01$ compared with rest and light exercise).

Recovery of $^{13}CO_2$ in the exhaled breath for all of the 18 individual experiments ranged from 59 to 90% (Fig. 3). Recovery rose significantly from a mean of 66.8 ± 5.3% at rest to 79.6 ± 10.9% for light exercise ($P < 0.01$), with no further change during moderate exercise (80.8 ± 3.3%). Coefficients of variation across subjects for recovery were 7.9% at rest, 13.7% for light exercise, and 4.1% during moderate exercise.

Compartmental analysis. The interindividual means and standard deviations for the estimates of rate constants for exchange of CO_2 among the three compartments in the mammillary model and for loss of CO_2 into the breath and via unaccounted routes are presented in

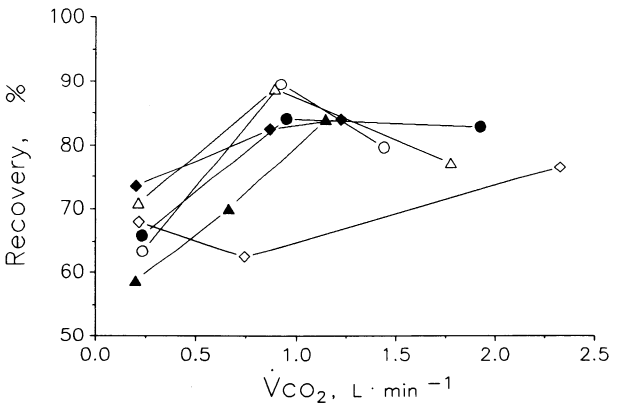

FIG. 3. Effect of metabolic rate ($\dot{V}CO_2$) on recovery of [^{13}C]bicarbonate as breath $^{13}CO_2$. Each symbol represents results for 3 metabolic rates in same subject.

Table 3. Interestingly, while there was a tendency for the rate constants to increase with light exercise over rest, these differences were not statistically significant; only moderate exercise resulted in significant speeding of the exchange dynamics. All of the rate constants except k_{31} were significantly greater during moderate exercise than during rest, whereas all but k_{31} and k_{13} were also greater than the corresponding values for light exercise (Table 3).

In contrast to the changes in rate constants with exercise, the quantity of exchangeable CO_2 in *compartment 1* (Q_1) rose dramatically with light exercise (from 234 ± 60 to 479 ± 76 mmol, on average, $P < 0.01$) but did not change further with additional increases in metabolic rate (458 ± 74 mmol)(Table 3). As discussed in METHODS, the quantities in *pools 2* and *3* can only be bounded by lower and upper limits (Q_{min} and Q_{max} for each pool). Mean estimates for CO_2 in the "fast" *pool 2* ranged from 176 to 247 mmol at rest, from 267 to 428 mmol during light exercise, and from 226 to 421 mmol for moderate exercise. Ranges for CO_2 in the "slow" *pool 3* were 496–947 mmol, 557–1,616 mmol, and 674–2,261 mmol for rest, light exercise, and moderate exercise, respectively. Table 3 also lists estimates for the total exchangeable CO_2 in all three compartments under three possible conditions; all of the natural metabolic production of CO_2 occurs either in *pool 1* (Qt_1), *pool 2* (Qt_2), or *pool 3* (Qt_3). If endogenous CO_2 production is spread among the pools (e.g., one-third in each), then total exchangeable CO_2 at steady state equals the appropriate weighted average of the Qt_i (e.g., $\Sigma Qt_i/3$). If the fractional distribution of endogenous CO_2 production among the three pools remains constant with increasing metabolic rate, then two important observations can be made from Table 3: *1*) irrespective of the compartmental source of endogenous CO_2, total exchangeable CO_2 increases dramatically with exercise and *2*) the preponderance of change is from rest to light exercise, with a slight, nonsignificant further increase in total CO_2 from light to moderate exercise. This is shown graphically in Fig. 4*A*, where the mean values for Qt_1, Qt_2, and Qt_3 are plotted at the three average metabolic rates.

TABLE 3. *Mean parameter estimates and average estimation errors from three-compartment mammillary model*

Parameter	Estimation Error, %	Rest	Light Exercise	Moderate Exercise
k_{01}	2.9	0.066±0.016	0.102±0.019	0.201±0.056‡§
k_{B1}		0.058±0.015	0.089±0.024	0.170±0.045
k_{L1}		0.008±0.005	0.013±0.010	0.032±0.015
k_{12}	12.7	0.215±0.062	0.304±0.064	0.494±0.175†‡
k_{21}	10.3	0.163±0.017	0.173±0.052	0.232±0.047*†
k_{13}	12.6	0.032±0.007	0.045±0.004	0.066±0.029*
k_{31}	13.7	0.077±0.034	0.055±0.019	0.091±0.029
Q_1	2.2	233±60	479±76‡	458±74‡
Q_{min_2}	10.7	193±82	267±44	226±46
Q_{max_2}	10.5	268±107	428±66	421±96
Q_{min_3}	6.7	521±120	557±77	674±164
Q_{max_3}	9.4	1004±115	1616±136	2261±863
Qt_1	3.6	948±65	1303±93‡	1358±234‡
Qt_2	3.7	1023±67	1464±115‡	1643±234‡
Qt_3	7.7	1431±146	2361±174‡	2996±867‡

Values are means ± SD. k_{ij}, rate constants for transfer to *pool i* from *pool j* in min^{-1}; Q_i, quantity of CO_2 in *pool i* in mmol; Q_{max_i} and Q_{min_i}, upper and lower limits of quantities of CO_2 in *pool i*; Qt_i, total quantity of CO_2 in *pool i* in mmol given endogenous CO_2 production only in *pool i*. Estimation error is average across all 3 metabolic rates of the asymptotic SE values expressed as % of the estimate (i.e., coefficient of variation). * $P < 0.05$ vs. rest; † $P < 0.05$ vs. light exercise; ‡ $P < 0.01$ vs. rest; § $P < 0.01$ vs. light exercise.

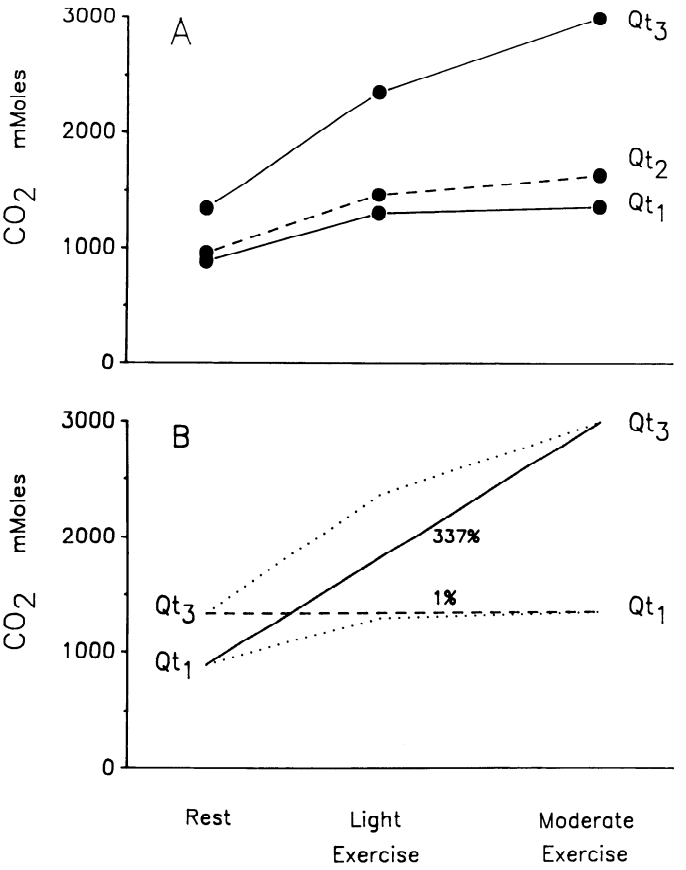

FIG. 4. *A*: range of possible values for total exchangeable CO_2 at each metabolic rate. Qt_1, Qt_2, and Qt_3 represent estimates for total CO_2 assuming that metabolic production of CO_2 occurs only in central compartment (Qt_1), in fast peripheral compartment (Qt_2), or only in slow peripheral compartment (Qt_3). *B*: extremes in possible changes in CO_2 stores with exercise. If endogenous source of CO_2 production changes from *compartment 3* at rest to *compartment 1* during moderate exercise (dashed line), there is virtually no change in total CO_2 stores (1%). Conversely, if the endogenous source of CO_2 changes from *compartment 1* at rest to *compartment 3* with moderate exercise, total CO_2 increases over 17 liters (337%; solid line). Note, however, that no negative changes (i.e., loss of CO_2) are predicted.

Estimation of $\dot{V}CO_2$. The rate of total $\dot{V}CO_2$ ($k_{01}Q_1$) is analogous to clearance and can be estimated noncompartmentally from the washout data (dose divided by AUC)(9). However, clearance will be greater than the measured $\dot{V}CO_2$ because recovery is less than 100% [i.e., $k_{01}Q_1$ estimates all loss of label, both in the breath and any nonrespiratory (unmeasured) loss]. Figure 5 shows the clearance data, corrected for the average recovery at rest (0.67) or exercise (0.80), as a function of the measured $\dot{V}CO_2$ at the mouth, both in units of liters CO_2 excreted per minute. The dashed line is the line of identity. Correction by the average recovery led to a very good prediction of the measured $\dot{V}CO_2$ from the washout characteristics ($r = 0.993$, SE for $\dot{V}CO_2$ = 79 ml/min).

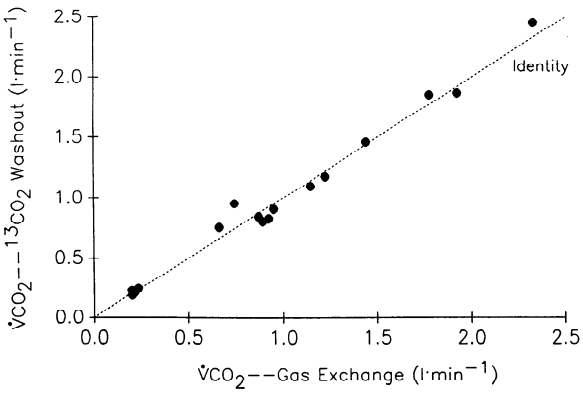

FIG. 5. Estimation of $\dot{V}CO_2$ calculated from [^{13}C]bicarbonate washout as breath $^{13}CO_2$ and corrected for average recovery at rest (0.67) or exercise (0.80), compared with $\dot{V}CO_2$ measured directly breath by breath. Dotted line is line of identity. Regression analysis: intercept = -0.009, slope = 1.017, r = 0.993, SE for $\dot{V}CO_2$ = 79 ml/min.

DISCUSSION

In the present study, washout of labeled bicarbonate was consistently and sufficiently well described by the sum of three exponential terms across a wide range of metabolic rates. In contrast, Irving et al. (19) found a small linear term suggesting a base-line drift, in addition to the three exponential terms, in their study of subjects at rest, which they attributed to changes in oxidative substrate mix, since carbohydrate and lipid differ slightly but measurably in $^{13}C/^{12}C$ (29). However, this term was only detectable between 4 and 6 h after the bolus injection of labeled bicarbonate. In the present study, respiratory quotient (RQ)($\dot{V}CO_2/\dot{V}O_2$) fell slightly on average from 0.88 to 0.72 over the first 2 h of rest experiments and from 0.91 to 0.85 during the 2 h of moderate exercise, with no consistent change during the light exercise protocols. This small change in RQ would suggest a change in background $^{13}CO_2/^{12}CO_2$ of only 1–2‰ (3, 29). However, no significant linear drift components to the washout characteristics were found in any of the 18 individual experiments in the present study. In addition, the infrequent occurrence (2 of 18) and small size (1–2 DOB) of the constant term suggest that it is most likely not a fundamental component of the washout characteristics.

There remains uncertainty regarding the physiological correlates of the three-compartment mammillary model derived from the washout of labeled CO_2. The earliest observers (15, 23, 30, 32) speculated that the capillary and cellular membranes represented a significant diffusion barrier for CO_2 equilibration between extra- and intracellular spaces. Thus the central compartment represented vascular and extracellular bicarbonate, the fast peripheral compartment was intracellular bicarbonate of soft tissues, and the slower peripheral compartment, when observed, was bone carbonate.

In contrast, other investigators have suggested a perfusion-limited, organ-based model (13, 19, 31, 35), in which the central compartment represents vascular and possibly some interstitial bicarbonate and the two peripheral compartments are composed of tissues distinguishable by different perfusions. The tissue compartment with relatively fast exchange with the central compartment under resting conditions is assumed to represent metabolically active tissue with high perfusion (heart, brain, kidney, etc.), while the slower equilibrating pool is hypothesized to consist primarily of resting skeletal muscle, which has a relatively low perfusion at rest. Exchange of labeled bicarbonate with bone carbonate was envisioned to represent an essentially unidirectional loss of label over the course of a typical experiment (several hours). This interpretation is based on the electrical analog model of Farhi and Rahn (13) and is consistent with the observation that the initial ability of the body to store CO_2 with rebreathing is greater when perfusion to resting skeletal muscle is increased either by vascular denervation or increased metabolic rate (16). It is interesting to note, however, that washout of solutes (28) or inert gases (27) from isolated, resting skeletal muscle demonstrates multiexponentiality, which would argue against the simple electric analog model of Farhi and Rahn (13). Thus, the precise physiological location of the three compartments remains unresolved.

The increase in Q_1 seen with mild exercise may reflect an increase in total CO_2 content within the bicarbonate system or a shift of bicarbonate from one of the two peripheral pools into the central pool. This large increase in Q_1 is not consistent with the hypothesis that membrane transport of CO_2-bicarbonate is the rate-limiting process in CO_2 exchange. Rather, these findings support the notion that perfusion is an important determinant of exchange, especially if the additional bicarbonate in the central pool is associated with the contracting skeletal muscles. However, if tissue perfusion, per se, was the sole limiting factor governing CO_2 exchange, one would predict a linear increase in Q_1 with increasing metabolic rate, which would parallel the known linear rise in blood flow which accompanies increases in metabolism (14, 22). This, in fact, did not occur. An alternative interpretation is that with even small increases in metabolic rate above rest, most or all of the muscle capillaries [known to be partially closed under resting conditions (18)] are "recruited" (i.e., opened), so that the diffusion surface approaches its maximum and the resistance to exchange for CO_2 between the intracellular pool and the vascular compartment is minimized (16, 18).

In contrast to the shift from rest to mild exercise, the bicarbonate model predicts that the greater rate of $\dot{V}CO_2$ elimination with moderate relative to light exercise is associated solely with an increased rate of fractional elimination from the central pool (as k_{01}) with no further increase in the amount of CO_2 in that pool (Q_1). This suggests that convective processes (e.g., blood flow and pulmonary ventilation) may play a greater role in facilitating removal of CO_2 from the blood to the environment at this level of exercise.

If it were known in which pool(s) the endogenous production of CO_2 occurred in vivo, then the total mass of exchangeable CO_2 in the three pools could be explicitly defined. However, this information can not be derived from the washout data alone (9); hence, only lower and upper bounds could be estimated for CO_2 in the two peripheral pools and, thus, for total exchangeable CO_2. If all of the metabolic production of CO_2 occurs in the central compartment, then the total quantity of CO_2 is

explicitly defined by Qt_1 (*Eq. 9*), can be estimated noncompartmentally as the product of MRT and $\dot{V}CO_2$, and represents the minimum possible total CO_2 content. If endogenous production of CO_2 occurs in one of the peripheral compartments, with clearance remaining only from the central compartment, then the enrichment of the various CO_2 pools will not be uniform. In this case, the total CO_2 in the system is greater than Qt_1, with production of CO_2 entirely in the slow pool (*compartment 3*) yielding the largest estimate of exchangeable CO_2 (Qt_3). This is reflected by a 50% range in the estimates of total CO_2 between Qt_1 and Qt_3 for the rest condition and by a range of 121% for moderate exercise (Table 3 and Fig. 4).

Similarly, the effect on the total CO_2 content of increased CO_2 production with exercise can be bounded but not calculated precisely. This is shown diagrammatically in Fig. 4*B*, where Qt_1 and Qt_3 have been plotted as functions of metabolic rate. For each metabolic rate, Qt_1 represents the minimum and Qt_3 the maximum estimate of total exchangeable CO_2 in the three pools. Depending on the hypothesized source of endogenous CO_2, increases of 1% (Qt_3 at rest to Qt_1 for moderate exercise, dashed horizontal line in Fig. 4*B*) up to 337% (Qt_1 rest to Qt_3 for moderate exercise, solid vertical line) can be derived for the increase in body CO_2 with exercise. Independent estimates of the increase in CO_2 stores with exercise would shed considerable light on this current uncertainty.

Recovery of injected or infused labeled bicarbonate as CO_2 in the breath has been reported to range from 50 to 90%, whether the species is man (1, 6, 19–21, 31, 34, 35), dog (11), or cat (23). We found that recovery increased during mild exercise but did not rise further when metabolic rate was increased with moderate exercise. Similarly, Van Aerde et al. (33) found in newborn infants a variable ($r = 0.64$) but significant ($P < 0.01$) increase in recovery of $^{13}CO_2$ (from 70 to 84%), which was proportional to resting metabolic rate over a small range, from 5 to 7.5 $ml \cdot kg^{-1} \cdot min^{-1}$. Slanger et al. (31) found that recovery fell, on average, from 83% at rest to 73% with very mild exercise (60% increase in $\dot{V}CO_2$), but this was not statistically significant (our analysis).

Unaccounted loss of labeled bicarbonate (k_{L1}) was assumed by us and others (19, 31, 35) to occur from the central pool; this meant that all of the model rate constants (k_{ij} values) were identifiable. If loss occurred from *compartments 2* and/or *3* only upper and lower bounds rather than explicit values for the rate constants associated with that pool can be found (8, 25). Physiological processes that may represent effective loss of labeled bicarbonate over the course of an experiment include: *1*) loss of labeled CO_2 in the breath with the first pass of venous blood through the lungs (12), *2*) true, irreversible loss of bicarbonate directly into the urine, sweat, or urea (23), and/or *3*) transfer into alternate pools whose turnover is so slow as to represent effectively unidirectional flux over the course of the experiment, such as incorporation into bone (23) or macromolecules (17). Loss of labeled CO_2 with the first pass through the lungs is equivalent to a reduction in the injected dose by that amount of label. Kornberg et al. (23) found in the resting, anesthetized cat that 6% of injected [^{14}C]bicarbonate label was found in bone after 5 h, while 3% remained as urea. Irving et al. (19) estimated that bone sequestering of bicarbonate label could be as high as 13% over 4 h of rest in humans. While incorporation of labeled bicarbonate specifically into blood glucose is low (1%; 17), accumulation of label by other moieties of intermediary metabolism could be quantitatively important.

We have shown here that across subjects and a wide range of metabolic rates without metabolic acidosis, labeled CO_2 washout dynamics generally exhibit triexponential decay. In addition, recovery of label increased with a modest increase in metabolic rate during mild exercise but did not change further during moderate exercise. We also found that metabolic rate as $\dot{V}CO_2$ could be accurately predicted from the washout curve for $^{13}CO_2$. Finally, the three-compartment mammillary model constructed from the washout curve allowed us to evaluate the effects of increased metabolic rate with exercise on CO_2 pool dynamics within the body. Characterization of the washout of labeled bicarbonate thus yields substantial information regarding the bicarbonate storage/transport system.

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