Increase in bicarbonate stores with exercise

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Abstract. We previously described bicarbonate exchange dynamics in humans at rest and during exercise using a three-compartment model. In the present study we tested the effect of certain assumptions of this model on the prediction of the change in exchangeable bicarbonate with the increased metabolic rate of exercise. We compared this prediction with a measurement of CO_2 retention after exercise onset determined from gas exchange data. The change in tissue bicarbonate stores was estimated from differences in the kinetics of adjustment of $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$, and this was added to an estimate of the changes in venous blood gas stores to estimate the total change in bicarbonate. When the commonly held assumption that endogenous CO_2 production, thought to occur in a rapidly equilibrating peripheral compartment at rest, was also applied to the exercise condition, the three-compartment bicarbonate model predicted an unphysiologically large increase in bicarbonate stores (700 mmol, or over 15 L). In contrast, the 'gas exchange' approach predicted a relatively small increase in bicarbonate (26 mmol), consistent with other reports. The incompatibility of these findings with the assumption about the source of endogenous CO_2 production in the bicarbonate model requires that the underlying physiological correlates of the three compartments change from rest to exercise.

Bicarbonate, stores, rest, exercise; CO_2, stores, rest, exercise; Kinetics, bicarbonate exchange, rest, exercise; Model, bicarbonate stores, rest, exercise

Regulation of cell pH requires that changes in CO_2 concentrations which accompany changes in metabolism be minimized; thus, CO_2 elimination at the mouth must quickly adjust to match tissue CO_2 production. However, this adjustment is not instantaneous, and CO_2 stores in the body fluctuate as a function of metabolic rate, blood flow, pH, *etc.* (Farhi and Rahn, 1960).

In order to evaluate the effects of increased metabolic rate with exercise on CO_2 storage dynamics, we have previously analyzed the washout of $^{13}CO_2$ in the breath following injection of [^{13}C]bicarbonate (Barstow *et al.*, 1990a). The washout kinetics

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were well described by the sum of three decreasing exponential terms at rest (eq. (1)), as shown by others (Barstow *et al.*, 1990a; Irving *et al.*, 1983; Issekutz *et al.*, 1968; Kornberg *et al.*, 1951; Shipley *et al.*, 1959; Slanger *et al.*, 1970; Winchell *et al.*, 1970)

$$DOB = A_1 \cdot e^{-\lambda_1 t} + A_2 \cdot e^{-\lambda_2 t} + A_3 \cdot e^{-\lambda_3 t}$$ (1)

where DOB is the excess $^{13}CO_2/^{12}CO_2$ in exhaled breath above the preinjection baseline, t is time after the bolus injection, A_i the coefficients and λ_i the rate constants. Exercise, which resulted in an increased metabolic rate and CO_2 elimination in the breath, increased the rate of washout. These kinetics during exercise were also well described by a three-exponential function, although the rate constants (λ_i) and coefficients (A_i) varied with different metabolic rates.

From the washout data, a three-compartment mammillary model was constructed (Fig. 1) (Landaw *et al.*, 1984), with a central compartment in communication with two peripheral compartments, one termed fast which exchanged CO_2 with the central compartment rapidly, and another compartment (termed slow) which exchanged more slowly with the central compartment. We also assumed that irreversible loss of CO_2 occurred only from the central compartment. The traditional interpretation of the three-compartment model for resting conditions has been that the central compartment represented vascular bicarbonate, the fast compartment represented tissues which at rest had a high metabolic rate (such as heart, brain, kidney, *etc.*), while the slow compartment was thought to represent tissue with a low metabolic rate at rest, such as resting skeletal muscle and/or possibly bone (Irving *et al.*, 1983; Issekutz *et al.*, 1968; Kornberg *et al.*, 1951; Shipley *et al.*, 1959; Slanger *et al.*, 1970; Winchell *et al.* 1970). However, the underlying physiological correlates of these three virtual pools remain undefined.
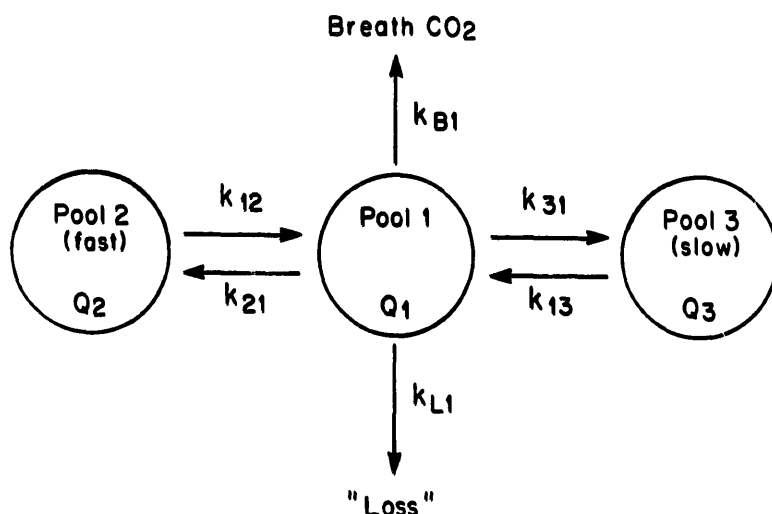
Breath CO2

Fig. 1. Three-compartment mammillary model derived from the three-exponential regression of the washout curves [from Barstow *et al.* (1990a)]. k_{ij} are rate constants; Q_i are the quantities of exchangeable CO_2 in each compartment.

Following the onset of exercise, a certain amount of increased CO_2 production is not excreted but is stored (Hughson and Inman, 1985; Yano, 1986). The three-compartment model can be used to predict the magnitude of the increase in whole-body exchangeable CO_2 stores. However, the estimated increase in CO_2 stores depends on the assumption as to which compartment(s) represent the metabolically active tissue where endogenous CO_2 is produced and enters the bicarbonate system (Barstow et al., 1990a; DiStefano and Landaw, 1984). The goal of this study was to test the hypothesis that the entry point for the endogenous CO_2 production (as pool 1, 2 or 3) remained constant from rest to exercise. We initially assumed that all of the metabolic production of CO_2 occurred in the fast peripheral compartment, both at rest [as assumed by others (Irving et al., 1983; Slanger et al., 1970; Winchell et al., 1970)] and during exercise. We compared these results with an independent measurement of the increase in total CO_2 stores with exercise. With this second technique we estimated, in the transition from rest to exercise, the amount of CO_2 which was metabolically produced but not excreted at the mouth.

We assumed this increase in CO_2 stores to be comprised of two components: (1) increased tissue stores, and (2) changes in the venous content of CO_2 (Hughson and Inman, 1985; Yano, 1986). We then compared this quantity of increase in CO_2 stores with that predicted from the three-compartment model. We found a large discrepancy in the predicted increase in CO_2 stores by the two different methods which required reinterpretation of the underlying physiology represented by the three-compartment model. Specifically, the assumption that the compartment(s) in which endogenous CO_2 was metabolically produced was the same for rest and during exercise may not be correct.

Methods

Subjects. Five subjects, in whom we had previously determined bicarbonate washout kinetics, both at rest and during exercise (Barstow et al., 1990a), participated in the study. The protocol was approved by the institutional Human Subjects Committee and informed consent was obtained. Their weight, height, and maximal aerobic capacity (as $\dot{V}_{O_2,max}$) are given in Table 1.

TABLE 1

Subject characteristics.

Subject	Age (yr)	Body weight (kg)	$\dot{V}_{O_2,max}$ (L/min)
1	23	86.5	2.37
2	33	65.8	2.73
3	23	74.8	2.91
4	31	68.2	4.02
5	29	79.5	2.96

Protocol. The subjects came to the laboratory in the morning following an overnight fast. Four to six rest-to-exercise transitions were performed by each subject. The work rate was chosen to correspond to that performed during the bicarbonate washout experiment performed earlier, and represented moderate exercise. Each exercise bout was continued until a new steady state for respiration had been reached (4–6 min), and the exercise periods were separated by at least 10 min of rest. Gas exchange, ventilation and heart rate were measured with each breath, as described below. The separate transition responses for $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ were time aligned to the start of exercise and averaged for each subject.

Measurement of gas exchange. The subjects breathed through a low-impedance turbine volume transducer with a deadspace of 90 ml. Oxygen and carbon dioxide tensions were determined by mass spectrometry from a sample drawn continuously from the mouthpiece at $1 \, \text{ml} \cdot \text{sec}^{-1}$. 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 with each breath, as previously described (Beaver *et al.*, 1981). Heart rate was obtained beat-by-beat using a modified lead V5 EKG.

Calculation of the increase in CO_2 stores from gas exchange. In the steady state of rest or exercise, where CO_2 stores are constant, the rate of CO_2 production by the tissue will equal the rate of elimination at the mouth as $\dot{V}_{CO_2}$. However, tissue and venous blood CO_2 content predictably will rise, because the increase in blood flow with exercise does not exactly match the increase in metabolic rate. The total increase in CO_2 stores from rest to the steady state of exercise can be estimated as the difference between the predicted amount of CO_2 produced by substrate oxidation in the tissues during the transition period and that amount which was irreversibly lost at the mouth as measured by integrating $\dot{V}_{CO_2}$ over the same period. Assuming lung and arterial CO_2 stores remained constant during the transition to the steady state of exercise, the increase in CO_2 stores with exercise was partitioned into that occurring in the tissue and that associated with increased venous blood CO_2 content. These were calculated using the following reasoning.

Tissue O_2 stores as oxymyoglobin change little if any for work of the intensity performed in this study. Therefore, the rate of rise of $\dot{V}_{O_2}$ at the mouth likely reflects the rate of tissue O_2 extraction from the blood, and the decreasing venous O_2 content (Barstow *et al.*, 1990b). Likewise, the rate of rise of $\dot{V}_{CO_2}$ will be determined by both the time course of tissue release of CO_2 into the blood and the increase in venous CO_2 stores [$\Delta CO_2(v)$]. The latter can be estimated in the steady state from the Fick equation as:

$$\Delta CO_2(v) = [(\dot{V}_{CO_2}/\dot{Q})_{ex} - (\dot{V}_{CO_2}/\dot{Q})_{rest}] \cdot V_{venous} \tag{2}$$

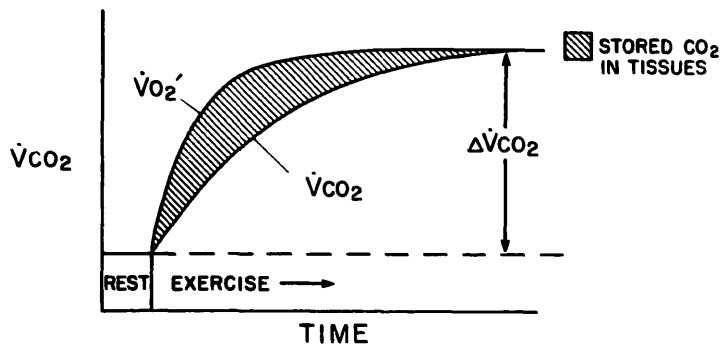where V_{venous} is venous volume [calculated as 5% of body weight (Yano, 1986), and

Fig. 2. Schematic showing time course of rise in $\dot{V}'_{O_2}$ and $\dot{V}_{CO_2}$ after onset of exercise. $\dot{V}'_{O_2}$ represents the $\dot{V}_{O_2}$ response which has been normalized to the rest and steady-state exercise $\dot{V}_{CO_2}$ values. Difference between two curves (shaded area) represents increase in tissue CO_2 content.

$\dot{Q}$ is cardiac output, estimated as a function of $\dot{V}_{O_2}$ from the regression of Faulkner et al. (1977).

Tissue CO_2 production from oxidative metabolism was assumed to parallel the tissue utilization of O_2, with any change in RQ assumed to occur instantaneously at the start of exercise. In addition, the pure transport delay between the tissues and the lungs was assumed to be the same for both O_2 and CO_2. Since changes in venous stores of O_2 and CO_2 are similar in magnitude, differing primarily in sign and secondarily by any change in RQ, any discrepancy between the $\dot{V}_{O_2}$ kinetics and those of $\dot{V}_{CO_2}$ will reflect differences between the quantity of O_2 extracted by the tissues and the quantity of CO_2 released into the blood. This difference thus represents a change in tissue CO_2 stores, [ΔCO_2(tis)], and is calculated by normalizing the $\dot{V}_{O_2}$ response to the steady-state change in $\dot{V}_{CO_2}$, integrating both responses, and taking the difference (Fig. 2). The $\dot{V}_{O_2}$ response, normalized in this way, reflects what the time course of rise in $\dot{V}_{CO_2}$ would be if tissue stores of CO_2 did not increase with exercise. This approach to calculating the increase in CO_2 stores following the onset of exercise is similar to that used by others (Hughson and Inman, 1985; Yano, 1986).

To ensure that the $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ responses had reached a new steady state of exercise, monoexponential fits (eq. (3)) were also made to those responses using nonlinear regression techniques (Dixon, 1983) using eq. (3):

$$\text{net } \dot{V}_x(t) = \Delta\dot{V}_x \cdot (1 - e^{-t/\tau}) \tag{3}$$

where net $\dot{V}_x(t)$ is the rate of exchange of gas x above baseline at any time t after the start of exercise, $\Delta\dot{V}_x$ is the net change from rest to steady-state exercise, and τ is the time constant for the response. Since four time constants equals 98% of the response, a steady state was assumed if the resulting time constants were less than 60 sec (so that the steady-state exercise level could be defined as the average response during min 4–6 of exercise). For moderate exercise (i.e., where there is very little if any sustained lactic acidosis), $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ kinetics have been repeatedly shown to be well described by monoexponential functions with no slow drift-like components (e.g., Linnarsson 1974).

TABLE 2

Steady-state responses.

Subject	Work rate (W)	$\dot{V}_{O_2}$ (L/min)		$\dot{V}_{CO_2}$ (L/min)		Cardiac output[a] (L/min)	
		Rest	Exercise	Rest	Exercise	Rest	Exercise
1	94	0.31	1.58	0.24	1.44	7.3	13.9
2	110	0.28	2.07	0.23	1.92	5.8	15.1
3	82	0.25	1.25	0.20	1.15	6.2	11.4
4	175	0.27	2.31	0.22	2.33	5.9	16.5
5	60	0.26	1.34	0.20	1.22	6.6	12.2

[a] Estimated from regression of Faulkner *et al.* (1977).

Results

The mean values for $\dot{V}_{CO_2}$ and $\dot{V}_{O_2}$ for rest and moderate exercise are listed in Table 2, along with the corresponding estimated cardiac outputs. $\dot{V}_{O_2}$ increased from a mean $\pm$ standard deviation of $0.27 \pm 0.02 \, L \cdot min^{-1}$ during rest to $1.71 \pm 0.46 \, L \cdot min^{-1}$ during exercise. $\dot{V}_{CO_2}$ showed a similar increase from $0.22 \pm 0.02 \, L \cdot min^{-1}$ to $1.61 \pm 0.50 \, L \cdot min^{-1}$ on average, while the mean estimated cardiac output increased from 6.4 ± 0.6 to $13.8 \pm 2.1 \, L \cdot min^{-1}$. The time constants for $\Delta \dot{V}_{O_2}$ determined in the present study ranged from 22.9 to 32.2 sec (mean 29.5 sec), and for $\Delta \dot{V}_{CO_2}$ from 29.6 to 56.4 sec (mean 41.3 sec). Note that all time constants were less than 60 sec, demonstrating the appropriateness of using the average of the responses from 4 to 6 min into exercise to estimate the steady state.
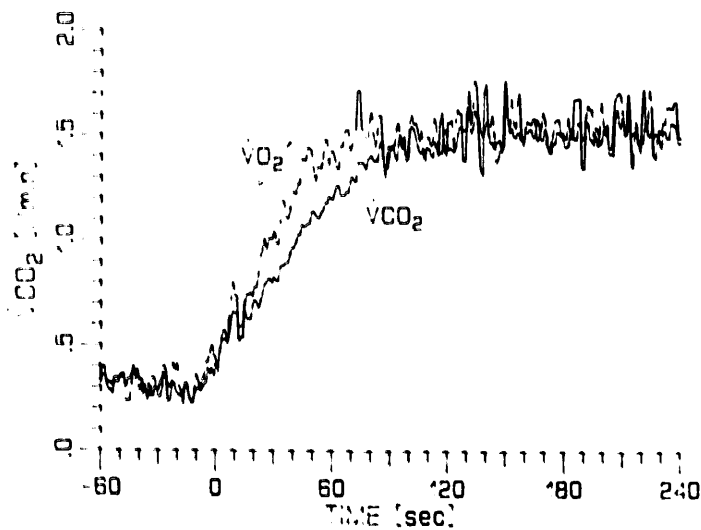

Fig. 3. Averaged responses of $\dot{V}_{CO_2}$ and $\dot{V}_{O_2}$ measured breath-by-breath in one subject, showing discrepancy between time courses.

TABLE 3

Increase in CO_2 stores with exercise.

Subject	Change in CO_2 stores (mmol)		
	$\Delta CO_2 (tis)$[a]	$\Delta CO_2 (v)$[b]	Total
1	15.2	13.9	29.1
2	12.2	12.9	25.1
3	17.1	11.4	28.5
4	6.8	16.1	22.9
5	9.6	12.4	22.1
Mean	12.2	13.3	25.6
SD	4.2	1.8	3.2

[a] Change in CO_2 stores in tissues.
[b] Change in venous CO_2 stores.

The time-aligned, averaged responses of $\dot{V}_{CO_2}$ for the transition from rest to moderate exercise is shown in Fig. 3 for one subject (No. 1). The $\dot{V}_{O_2}$ response, normalized to the rest and steady state exercise $\dot{V}_{CO_2}$ values, is also shown. The area between these two curves during exercise represents the volume of CO_2 stored in the tissues. These volumes for each subject are presented in Table 3, along with the estimates for the change in venous CO_2 content. Tissue CO_2 stores rose on average 12.2 ± 4.2 mmol (271 ± 93 ml). Venous CO_2 content increased by 13.3 ± 1.8 mmol (297 ± 39 ml), yielding a total increase in CO_2 stores of 25.6 ± 3.2 mmol, with a range of 22.1 to 29.1 mmol.

Discussion

Comparison of results with predictions from three-compartment model. Early interpretations of the nature of the three bicarbonate compartments hypothesized that CO_2 exchange between extra- and intracellular spaces was limited by diffusion through the capillary and cellular membranes (Kornberg *et al.*, 1951; Shipley *et al.*, 1959). The central, fast peripheral and slow peripheral compartments were therefore thought to represent blood and extracellular bicarbonate, intracellular bicarbonate, and carbonate of bone, respectively. More recently it has been suggested that bicarbonate exchange dynamics were determined by perfusion (Farhi and Rahn, 1960; Irving *et al.*, 1983; Slanger *et al.*, 1970; Winchell *et al.*, 1970). According to this perspective, the central pool represented blood bicarbonate, the fast pool represents tissue(s) with a high metabolic rate and blood flow (*e.g.*, brain, heart, kidney, splanchnic region), while the slow pool was composed of tissue(s) with a low metabolic rate and blood flow at rest (primarily skeletal muscle). With either interpretation, if the bicarbonate pools represent

TABLE 4

Estimates of total CO_2 stores from three-compartment bicarbonate model [Barstow *et al.* (1990a)].

	Qt_1[a] (mmol)	Qt_2[a] (mmol)	Qt_3[a] (mmol)
Rest	941[b] ± 71	1021 ± 75	1429 ± 163
Moderate exercise	1320 ± 241	1604 ± 238	2717 ± 593
Predicted increase	379 ± 235	583 ± 281	1288 ± 714

[a] Assumes all endogenous CO_2 production originates in compartment 1, 2 or 3.
[b] Mean ± S.D.

discrete anatomical sites at rest, it seemed appropriate to believe these same sites would be differentiable during exercise, with the primary effect of exercise being to increase the rates of exchange of the peripheral (intracellular or organ) compartments with the central (extracellular or vascular) compartment, as reflected by increased intercompartment rate constants.

To evaluate the predictions of the three-compartment model regarding increases in CO_2 stores with exercise, group mean estimates for total exchangeable CO_2 as previously described (Barstow *et al.*, 1990a) are listed in Table 4. Qt_i represents the total exchangeable CO_2 in the body, assuming that all of the metabolic production of CO_2 occurs in pool i. Also listed are the resulting predicted increases in total exchangeable CO_2 assuming the source pool for CO_2 production within the body remained constant from rest to moderate exercise, either as compartment 1 (Qt_1), compartment 2 (Qt_2) or compartment 3 (Qt_3). Note that with this assumption, irrespective of the compartment, each of the predicted increases in total CO_2 is 16–61 times greater than that measured by gas exchange (mean 25.6 mmol). Using a reasonable estimate for the CO_2 dissociation curve for the whole body of approximately 1 ml · $mmHg^{-1}$ · kg^{-1} (Clode *et al.*, 1967), these increases in predicted CO_2 content represent an average increase in whole body P_{CO_2} for the subjects in the present study of 113, 174 and 385 mmHg, clearly unphysiological values. These results effectively eliminate the possibility that the site(s) of endogenous production of CO_2 associated with the three bicarbonate compartments remains constant across the wide range of metabolic rates examined in our previous study (Barstow et al., 1990a). In addition, these results are inconsistent with the hypothesis that the determinants of bicarbonate exchange between compartments (*e.g.*, organ perfusion or body water compartments) are the same for exercise as for rest.

Alternatively, CO_2 production can be distributed among the pools in the three-compartment model. The resulting total exchangeable CO_2 then can be calculated from a linear combination of the fractional contribution of CO_2 production originating in each pool as:

$$Qt_s = f_1 \cdot Qt_1 + f_2 \cdot Qt_2 + f_3 \cdot Qt_3$$

where Qt_s is the total exchangeable CO_2, f_i is the fractional contribution to the total CO_2 production entering pool i, and $f_1 + f_2 + f_3 = 1$. From this, possible combinations for the distribution of CO_2 production among the three pools which would predict the small increase in total CO_2 with exercise, as determined by gas exchange in this study, can be examined using the following equation

$$Qt_{s,exercise} = Qt_{s,rest} + 25.6 \text{ mmol}$$

Examination of Table 4 reveals that the only combinations which would predict such a small increase in total CO_2 are those where the majority of CO_2 production occurs in compartment 3 (the slow compartment) at rest, and virtually all of the production occurs in the central and fast-equilibrating peripheral compartments during moderate exercise. Specifically, if *during exercise* all CO_2 production occurs in the central pool, then *at rest*, CO_2 production originating in compartment 3 must be 67% of the total if the remainder is in compartment 2, or 74% if the remainder originates in compartment 1.

Further evidence that the underlying nature of the three compartments of bicarbonate changes with exercise is provided by the observation that the total increase in bicarbonate stores calculated by gas exchange (26 mmol) is much less than the increase in quantity of bicarbonate in the central compartment (225 mmol) previously determined from the bicarbonate washout experiments (increase in Q_1 from 233 $\pm$ 60 mmol at rest to 458 $\pm$ 74 during moderate exercise) (Barstow *et al.*, 1990a). Thus, the increase in bicarbonate in the central pool cannot simply denote the additional CO_2 retained following the start of exercise, but must represent a redefinition of the total bicarbonate space. Rather than this reflecting actual physical movement of bicarbonate from one anatomical site to another, it seems more likely that this reflects inclusion of tissue bicarbonate associated with one or both of the peripheral compartments into the central compartment. As we have previously speculated (Barstow *et al.*, 1990a), this increase in bicarbonate in the central compartment may result from recruitment of skeletal muscle capillaries with exercise, which would reduce the diffusion distance for CO_2 exchange between blood and tissue. This, coupled with the dramatic increase in muscle capillary blood flow with exercise, could result in such rapid exchange of bicarbonate between the blood and intramuscular stores as to appear to represent a single central compartment during the washout experiment.

Assumptions of the gas exchange approach. CO_2 stores in the body can be partitioned in several ways. A useful approach for the gas exchange method is to lump the stores into four anatomical locations: (1) pulmonary air space, (2) arterial blood, (3) all of the tissues, and (4) venous circulation. We assumed that the stores in locations 1 and 2 remained constant between rest and moderate exercise. In fact, changes in CO_2 stores in the lung which accompany exercise can be approximated in the same way that alveolar-blood gas exchange is estimated from gas exchange measured at the mouth (Beaver *et al.*, 1981). Using the data of Linnarsson (1974) for 80 W and 160 W exercise [similar to the exercise performed in our previous bicarbonate washout experiments

(Barstow *et al.*, 1990a) and in the present study], changes in lung stores of CO_2 for this range of exercise estimate to less than 5 ml of CO_2. Thus, any steady-state changes in lung stores of CO_2 are small in comparison to those in the tissues and venous blood.

Changes in arterial P_{CO_2} will alter CO_2 stores. Estimates of the 'immediate' CO_2 storage capacity with exercise, determined by rebreathing or hyperventilation, range from 0.57 (Fowle and Campbell, 1964) to 1.8 (Jones and Jurkowski, 1979) ml · $mmHg^{-1}$ · kg^{-1} change in Pa_{CO_2}. This represents a potential average change in CO_2 stores in the present study of up to 128 ml (5.7 mmol) per mmHg of sustained change in Pa_{CO_2}, or 24% of the total increase in CO_2 stores calculated from gas exchange. However, for the relative intensity of exercise examined here (moderate exercise below that which results in sustained metabolic acidosis), Pa_{CO_2} during steady-state exercise appears tightly regulated at rest levels (Wasserman *et al.*, 1967). Thus, no sustained changes in CO_2 stores due to arterial loading or unloading would be expected. Any transient hyper- or hypocapnia, due to discrepancies between the rate at which ventilation $\dot{V}_{CO_2}$ and adjust after the onset of exercise, would lead to a small transient change in arterial stores at best, which would correct themselves in the steady state.

The increase in CO_2 stored in the venous circulation was calculated from eq. (2) using estimations of cardiac output derived from the regression of Faulkner *et al.* (1977). As found by these authors, the inter-subject variance in estimating cardiac output (slope and intercept) was not different from the intra-subject variance. This suggests that cardiac output could be as accurately predicted from the group regression equation as it could from determinations of the individual regression for any given subject.

We have made three assumptions regarding the cellular production of CO_2: (a) CO_2 production was only due to oxidative metabolism in the transition from rest to exercise, (b) the time course of oxidative metabolism could be estimated by that of $\dot{V}_{O_2}$, and (c) any steady-state changes in tissue RQ occurred with the same time course as the rise in $\dot{V}_{O_2}$. Regarding the first assumption, tissue CO_2 production, in fact, represents the net summation of both oxidative metabolism and changes in acid-base homeostasis. The latter is a balance between alkalizing (splitting of phosphocreatine) and acidifying (production of lactic acid) processes (Dawson *et al.*, 1980), each of which are small and transient, if present at all for the moderate intensity of exercise examined here. With regard to the second assumption, O_2 stores in the body are small and change little with moderate exercise so that $\dot{V}_{O_2}$ kinetics at the mouth likely reflect changes in venous O_2 stores and muscle O_2 utilization (Barstow *et al.*, 1990b). Since changes in venous CO_2 stores are similar to those of O_2, differing only in sign and any second-order corrections for changes in steady-state RQ (Yano, 1986), the difference between $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ kinetics at the mouth will reflect CO_2 which was produced but not released into the venous circulation (*i.e.*, stored in the tissues). Regarding the last assumption, Hughson and Inman (1985) calculated that the pattern of RQ change following exercise onset would have a very small effect on the calculation of stored CO_2 (maximal effect 12%, or 0.04 ml CO_2 · $mmHg^{-1}$ · kg^{-1}), so small as to be virtually negligible for the purposes of our analysis.

All of the above considerations would only slightly affect the accuracy of the calcula-

tion of the increase in CO_2 stores with exercise from gas exchange. They do not, however, significantly diminish the dramatic discrepancy of this measurement with the prediction derived from the assumption of constancy of the source pool of endogenous CO_2 production, using the three-compartment bicarbonate model. Rather, the discrepancy that we found indicates that the assumption of the unchanging character of the bicarbonate compartments with exercise must be re-examined.

The apparent discrepancy between the time constant for adjustment of total CO_2 output (as $\dot{V}_{CO_2}$) following the onset of exercise in the present study (mean of 41 sec) and the overall time constant (as mean residence time) for the washout of $^{13}CO_2$ observed in the same subjects during the bicarbonate experiments previously reported (mean of 16 min) (Barstow et al., 1990a) requires a word of explanation. Since arterial P_{CO_2} is well regulated at resting levels during moderate exercise, any increase in total CO_2-bicarbonate content with exercise is confined to the tissues and venous circulations where the increased metabolic rate occurred. The time constant for the rise in $\dot{V}_{CO_2}$ reported here reflects the rate at which the steady state CO_2 content of these specific tissues and circulations adjust during the transition from a steady state of rest to one of exercise. Conversely, the labeled CO_2, injected as labeled bicarbonate intravenously, is not confined to the venous circulation; some traverses the pulmonary circulation without being excreted into the alveolar spaces and becomes distributed throughout the body. The mean transit time reported previously (Barstow et al., 1990a) describes the average time a labeled CO_2 molecule will remain circulating throughout the entire bicarbonate space, defined for a specific steady state and metabolic rate, before being excreted at the mouth or lost as unidentified irreversible loss. Thus, the two time constants for CO_2 loss at the lungs reflect entirely different processes, and are not comparable.

In conclusion, we have shown that the increase in exchangeable bicarbonate stores with exercise are small, and inconsistent with current interpretations about the underlying physiology of the three compartments of bicarbonate observed following bolus labeled bicarbonate injections. These results require that the nature of the three compartments change from the resting condition to that of exercise. Finally, interpretation of substrate oxidation studies, where rates of oxidation are calculated from appearance in the breath of labeled CO_2, must consider the effect of bicarbonate pool exchange dynamics, and the consequences of a change in the site of oxidation of the labeled substrate within the bicarbonate model, on the rate of appearance of labeled CO_2 at the mouth.

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