J Appl Physiol
90: 2181–2187, 2001.

First-pass effect of an intravenous bolus of [^{13}C]bicarbonate displayed breath-by-breath

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Received 25 April 2000; accepted in final form 17 January 2001

Roecker, K., E. Landaw, H. Striegel, F. Mayer, and H.-H. Dickhuth. First-pass effect of an intravenous bolus of [^{13}C]bicarbonate displayed breath-by-breath. *J Appl Physiol* 90: 2181–2187, 2001.—The dilution of an intravenous bolus dose of [^{13}C]bicarbonate is used as an estimate for the metabolic rate under certain conditions. It is a consistent finding in all studies that the total amount of intravenous [^{13}C]bicarbonate cannot be recovered as breath $^{13}CO_2$. In this study, we used a breath-by-breath analysis of $^{13}CO_2$ to depict the washout of $^{13}CO_2$ at a high temporal resolution to analyze the extent to which a probable first-pass effect is responsible for the reduced recovery. Eight healthy men were tested at seated rest and with bicycle exercise at a constant load relative to 40 and 75% maximal O_2 consumption ($\dot{V}O_{2\,max}$). [^{13}C]bicarbonate (0.0125 g/kg body wt) was administered as an intravenous bolus in each test. Respiratory mass spectrometry was used to derive the course of the end-tidal $^{13}CO_2$-to-$^{12}CO_2$ ratio from the breath-by-breath data. Approximately 2 min after ^{13}C administration, the washout curve could be fitted well by a two-exponential curve describing a two-compartment mammillary model. Immediately after administration of the bolus dose, an excess peak in the end-tidal $^{13}CO_2$-to-$^{12}CO_2$ ratio appeared. This peak could not be included in the two-exponential fitting. The area under the first peak resulted in 3.8 ± 1.3% of the total [^{13}C]bicarbonate dose at rest, 11.5 ± 2.9% at moderate exercise (40% $\dot{V}O_{2\,max}$), and 16.9 ± 4.0% at intensive exercise (75% $\dot{V}O_{2\,max}$). The first-pass effect had an increasing impact of up to about two-thirds of the lacking bicarbonate with higher exercise intensity. The "loss" of tracer via this first-pass effect must be considered when the results of studies with parenteral administration of [^{13}C]bicarbonate are considered, especially when it is given as a bolus dose and during exercise.

$^{13}CO_2$; stable isotopes; respiration; intravenous administration; tracer recovery

BREATH STUDIES HAVE BEEN PERFORMED with a wide spectrum of ^{13}C-labeled substances. The metabolism of virtually any organic substance in the human organism can be estimated with this technique. After a substance is labeled with ^{13}C, the expiration of $^{13}CO_2$ correlates to the oxidation or turnover of the substance. Common experiments include estimation of the oxidation of exogenous glucose (20, 24, 28) or fatty acids (26, 31). One difficulty in these metabolic studies consists of the body's stores of CO_2, which are relatively large. It has been reported that the production of $^{13}CO_2$ is delayed by the CO_2 pools in various tissues before CO_2 is expired from the mouth (21, 27, 33). Studying the compartmental distribution of exogenous $^{13}CO_2$ is a prerequisite for the estimation of this retardation.

Other investigations using ^{13}C labeling have focused on the physiology of the bicarbonate pools. The compartmental distribution of CO_2 in the body at rest and during exercise could be described using intravenous bolus injections of [^{13}C]bicarbonate with subsequent measurement of breath enrichment (3, 37). In other applications, the metabolic rate has been estimated by the [^{13}C]bicarbonate washout characteristics (2, 3, 6, 19, 37).

A consistent finding in all these studies is an incomplete occurrence of a given ^{13}C dose in the expired air. Recovery of administered [^{13}C]bicarbonate has been calculated to be 50–90% (1, 3, 22, 27, 32, 36). It is likely that this phenomenon affects studies of substrate oxidation in the same way. The physiological reasons for this irreversible "loss" of $^{13}CO_2$ are described as via urine, sweat, or urea (3, 16) and the transfer into bone (15, 16) or macromolecules (4, 14).

A first-pass effect of the venous blood through the lungs was supposed by Armon et al. (2), Barstow et al. (3), and Drury et al. (8) as an additional effect in the reduction of the administered ^{13}C dose. Nevertheless, this effect could not be shown directly. A study with [^{13}C]bicarbonate infusions administered to dogs could not show any effect of the passage of the substance through the lungs (7). In this study, a previously described breath-by-breath measurement of $^{13}CO_2$ in the expired air (30) is used to depict this first-pass effect. The high temporal resolution of a breath-by-breath system provides a direct observation of the washout curve instantaneously after injection of the [^{13}C]bicarbonate. To estimate the exactness of the breath-by-breath method, compartmental parameters from the washout curves were calculated as described previously (3, 17).

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METHODS

Eight healthy men were examined. All subjects were free of metabolic or cardiovascular abnormalities, lean (9.2 ± 3.4% body fat by skinfold measurements), and nonsmokers (Table 1). The subjects granted written consent to participate in the study. Review by the Ethics Commission of the University of Tuebingen brought no objections to the performance of the study.

Experimental procedure. An intravenous bolus of 0.0125 g/kg body wt [^{13}C]bicarbonate ($NaH^{13}CO_3$; CIL, Andover, MA; purity ≥99% ^{13}C) in an 8.4% water solution was administered in each test. The solutions were prepared and sterile filtered within 5 h before each test. To prevent evaporation of bicarbonate, gastight bottles with sealed plugs were used. The bolus injection was given into an antecubital vein of each subject's right arm with an automated volumetric infusion pump (model 591, Ivac) within 1.0 s. Gas analysis was started 5 min before the [^{13}C]bicarbonate was administered. The measurement before bicarbonate administration served to record the baselines of the $^{13}CO_2$-to-$^{12}CO_2$ ratio and spirometry. The moment of bolus injection was defined as *time 0*. Gas analysis ended 30 min after the bicarbonate injection. Seated rest and two different exercise intensities on a cycle ergometer (Excalibur, LODE) were tested. The bicarbonate washout was determined at a constant workload relative to 40 and 75% of each subject's maximal O_2 consumption ($\dot{V}O_{2\,max}$). Exercise began 5 min before the bicarbonate injection. The subjects rested ≥2 days between the tests to allow for tracer washout.

Measurement of gas volumes. The total respired gas volume was determined using a light-weight low-impedance turbine flowmeter (Triple V transducer, Mijnhardt, Nijmwegen, The Netherlands) in BTPS conditions. The dead space of this flowmeter unit was 125 ml. The gas volume was integrated via software from the flow signal. The device has a linear flow-to-signal characteristic, which was checked before the tests by application of low to supraphysiological airflows with the calibration pump. The momentum of the turbine had no impact on the gas volume results. The flow/volume-measuring unit was calibrated before each individual test with a 3-liter calibration pump.

Respiratory gas fraction analysis. The respiratory gas fractions were determined using respiratory mass spectrometry (model AMIS 2000, Innovision, Odense, Denmark, with a Quadrupole QMA 20, Balzers, Balzers, Liechtenstein). The mass spectrometer was calibrated before each individual test by means of a two-point calibration with a zero offset with all valves closed. The calibration gas should contain 1% $^{13}CO_2$, 1% Ar, 5% $^{12}CO_2$, 16% O_2, and 77% N_2. The $^{13}CO_2$-to-$^{12}CO_2$ ratio in the calibration gas (0.2) corresponds to the expected maximum values of the isotope ratio in the expired air during the tests. The actual concentration of each gas was validated gravimetrically with a relative accuracy of ±0.01%. We used these validated values in the calibration procedure. The recording frequency was 11.1 Hz, corresponding to a cycle duration of 90 ms. The respired gas fractions for O_2, $^{12}CO_2$, $^{13}CO_2$, N_2, and Ar were measured in each cycle. A delay of 0.28 s between the gas concentration signal and the signal for gas flow was taken into consideration for the breath-by-breath calculations.

Table 1. *Anthropometric characteristics of subjects*

Age, yr	27.5 ± 4.5
Height, cm	181.2 ± 8.4
Weight, kg	70.0 ± 9.8

Values are means ± SD.

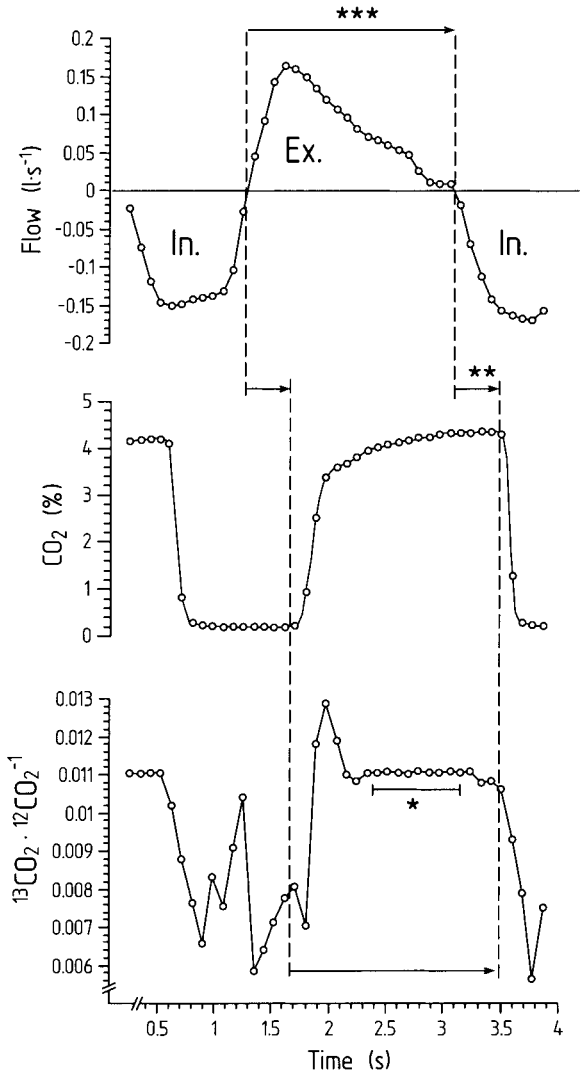

Fig. 1. $^{13}CO_2$-to-$^{12}CO_2$ ratio in the course of one breath cycle without previous administration of [^{13}C]bicarbonate. *Top*: flow signal for inspiration (In) and expiration (Ex). With advancing duration of expiration, analyzed gas comes from deeper in the lung and will be less diluted than gas from the functional dead space (***). *Middle*: course of the CO_2 concentration. *Bottom*: course of the $^{13}CO_2$-to-$^{12}CO_2$ ratio. The average values of the plateau phase during the expiration were used as end tidal (*). The time delay between the flow and gas concentration signals (**) was taken into consideration for the breath-by-breath calculations.

The curve of the end-tidal $^{13}CO_2$-to-$^{12}CO_2$ ratio within one breath is presented in Fig. 1. This example was recorded without previous administration of [^{13}C]bicarbonate. The values for further calculations in each case were taken from the arithmetic mean of the plateau phase during expiration to prevent the dead space gas portion from influencing the isotope ratio. The breath-by-breath data of all measured gases and the data for the $^{13}CO_2$-to-$^{12}CO_2$ ratio were calculated on-line with the recording personal computer under STPD conditions.

Determination of the detection limit. To check the stability and reliability of the gas-measuring method, the detection limit (DL) was calculated as follows

$$DL = \left(\frac{U_{noise} \cdot 3}{U_{calibration\ gas}}\right) \cdot c_{gas} \qquad (1)$$

where U is the voltage signal at the mass spectrometer unit and c_{gas} is the gas concentration. The quantization limit (QL) is three times the DL. The results of these calculations are presented in Table 2, whereby the result of the DL for the gases $^{13}CO_2$ and $^{12}CO_2$ can be considered adequate for our purposes.

The $^{13}CO_2$-to-$^{12}CO_2$ ratio is usually given in the PDB-(Belemnitella americana) standard (1.1235% ^{13}C), in so-called $\delta^{13}C$ (‰) units. The dimension of this unit is calculated as described by Armon et al. (2) and Barstow et al. (3).

Background concentration of $^{13}CO_2$. ^{13}C can be found in various concentrations in the natural environment (13, 35). The source of daily nutrition determines the $^{13}CO_2$-to-$^{12}CO_2$ ratio without additional ^{13}C enrichment. This individual baseline is therefore subtracted from the measured isotope ratio to obtain the net changes in this value [delta over baseline (DOB)]. DOB is equivalent to the increase in the specific concentration of exogenous [^{13}C]bicarbonate in the system (2, 3).

The amount of an expired gas can be calculated from the concentration in relation to the total expiratory volume. The expired amount of $^{13}CO_2$ from exogenous sources can be calculated analogously from the product of the DOB and CO_2 output ($\dot{V}_{CO_2}$). *Equation 2* describes the expiratory flow of $^{13}CO_2$ ($ex\dot{V}^{13}_{CO_2}$) from exogenous sources at *time t*

$$ex\dot{V}^{13}_{CO_2}(t) = \dot{V}_{CO_2}(t) \cdot DOB \cdot 1.1235 \cdot 10^{-5} \quad (2)$$

Recovery. Only a part of the exogenous amount of [^{13}C]bicarbonate can be found in the expired air (2, 10, 15, 29). This is due to an unobserved loss of $^{13}CO_2$ from the central compartment (k_{v1}; Fig. 2). *Equation 3* gives recovery as the relationship between the cumulative volume of the expired $^{13}CO_2$ ($exV^{13}_{CO_2}$) and the total dose of [^{13}C]bicarbonate (D_0)

$$\text{recovery} = (exV^{13}_{CO_2} \cdot K) \cdot D_0^{-1} \quad (3)$$

where $exV^{13}_{CO_2}$ is in liters and D_0 is in moles. The constant K (=0.0446) is for the conversion between moles and liters. As described in earlier studies, the values for recovery can be used for a correction of the calculations for $^{13}CO_2$ distribution (2, 9, 25).

An alternative method for calculating recovery is the use of the area under the extrapolated curve (AUC; see below) and the mean $\dot{V}_{CO_2}$ (*Eq. 4*)

$$\text{recovery} = \frac{AUC \cdot 1.1235 \cdot 10^{-5} \cdot \dot{V}_{CO_2}}{D_0} \quad (4)$$

Compartmental kinetics of bicarbonate. The distribution of $^{13}CO_2$ and [^{13}C]bicarbonate can be described with a linear multicompartmental mammillary model (3, 17, 27). Figure 2 shows the two-compartment model that was used for bicarbonate distribution in this study. It is presumed that bicarbonate entry and irreversible loss of CO_2 are only via the central *compartment 1*. The steady-state quantity of CO_2 in *compartment i* is described by Q_i. The rate constant for the transfer from *compartment j* to *compartment i* is k_{ij}. For instance, k_{21} is the rate constant for the transport from the central *compartment 1* to the peripheral *compartment 2;* k_{A1} is the transfer rate from *compartment 1* to environmental air. The rate constant for unobserved loss of bicarbonate from *compartment 1* is k_{L1}, which has been described as "nonrespiratory" in earlier studies (3). The total rate constant for the CO_2 flow from the central compartment (k_{01}) is $k_{B1} + k_{L1}$. DOB stands directly for the $^{13}CO_2$ enrichment in the central compartment (3, 7, 15). Models with more than two compartments are built analogously (18). After administration of D_0 at *time 0*, the course of the concentration in *compartment 1* is measured by the $^{13}CO_2$-to-$^{12}CO_2$ ratio or DOB, respectively.

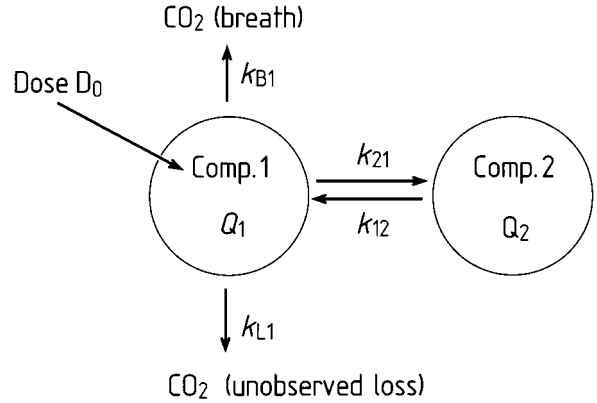

Fig. 2. Schematic illustration of a mammillary 2-compartment model for distribution of [^{13}C]bicarbonate given in a dose D_0. k_{ij}, Rate constant for transfer from *compartment j* to *compartment i*. Clearance of [^{13}C]bicarbonate occurs only from the central *compartment 1* (k_{01}; not shown). Although some of this loss can be measured via the washout curves at the mouth (k_{B1}), another amount remains unobserved (k_{L1}). [Modified from Barstow et al. (3).]

Washout curves. Bicarbonate and CO_2 are distributed quickly and homogenously after administration into the central compartment. The impulse of D_0 is answered by the washout course of DOB. With two compartments, the sum of $n = 2$ exponentials describes this washout curve (15, 19). An empirical model without additional constants was used for this study. This model has been evaluated as best fitting for bicarbonate washout kinetics (3) (*Eq. 5*)

$$DOB(t) = \sum_{i=1}^{n} A_i e^{\lambda_i t} \quad (5)$$

The macroparameters of the exponential function, A_i and λ_i, are calculated from the course of the discrete data pairs by way of an iterative procedure. The parameter of each iteration was evaluated by using the Levenberg-Marquardt maximum neighborhood algorithm (12, 23).

AUC. With the use of the macroparameters, the course of the washout curves can be extrapolated to infinity. Subsequently, the total amount of expired $^{13}CO_2$ can be estimated from this extrapolated curve as the AUC (*Eq. 6*)

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

Identification of the microparameters. The steady-state constants k_{ij} and Q_i can be calculated from the washout curve parameters A_i and λ_i (3, 17). *Compartment 2* does not have a substance leak ($k_{02} = 0$); thus all microparameters can be

Table 2. *Detection characteristics for the measured gas ions*

	$^{12}CO_2^+$	$^{13}CO_2^+$
Signal, V	1.20 ± 0.01	0.24 ± 0.002
Noise, mV	119 ± 2.9	2.11 ± 0.3
S/N	219.3 ± 29.6	217.8 ± 27.0
DL, ‰	0.015 ± 0.0006	0.25 ± 0.0001
QL, ‰	0.44 ± 0.0002	0.76 ± 0.0003

Values (means ± SD) are derived from 9 single calibrations. S/N, signal-to-noise ratio; DL, detection limit (*Eq. 1*); QL, quantization limit (for further description see *Determination of the detection limit*).

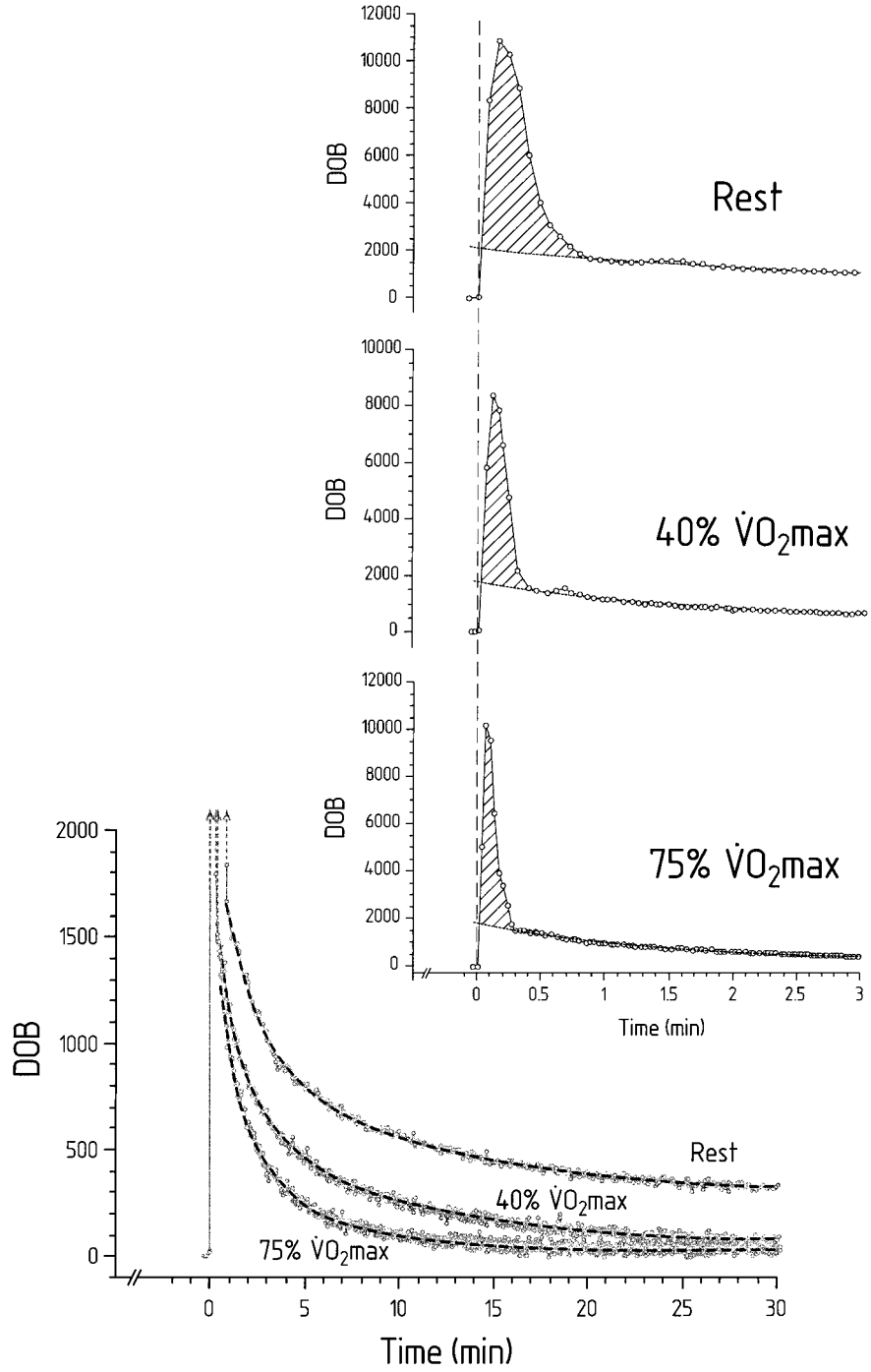

Fig. 3. Washout curves after administration of a venous bolus of [^{13}C]bicarbonate shown as specific $^{13}CO_2$ enrichment [delta over baseline (DOB)] of the exhaled air breath-by-breath. Each data point represents the DOB of one single breath. The bicarbonate was administered at *time 0* at rest and during exercise at 40 and 75% of maximal O_2 consumption ($\dot{V}O_{2max}$). *Bottom*: total measurement duration. *Top*: same curves at shown at *bottom* for first 3 min. Hatched areas under the excessive peaks are interpreted as first-pass effect in the lungs. Dashed lines are the result of the best fit for the sum of 2 exponentials (see *Eq. 6*).

explicitly determined for the two-compartment model used in this study.

A noncompartmental calculation of Q_1 is possible for the steady state (*Eq. 7*)

$$Q_1 = \frac{D_0}{(A_1 + A_2) \cdot 1.1235 \cdot 10^{-5}} \quad (7)$$

The average $\dot{V}CO_2$ is analogous to the clearance of exogenous [^{13}C]bicarbonate from D_0. The $\dot{V}CO_2$ from the compartmental data is given by *Eq. 8* in units of volume per time

$$\dot{V}CO_2 = k_{01}Q_1 \quad (8)$$

First-pass effect. Some [^{13}C]bicarbonate cannot be measured via the DOB washout curves after bolus injection. A first-pass effect is considered to be one of the factors for this unaccounted loss of [^{13}C]bicarbonate. We progressively excluded the data at the beginning of the washout curves to make this effect apparent. The resulting two-exponential fitting was evaluated by an *F* test with every step (18). The best of these fits was used as the reference washout curve. This curve was extrapolated to *time 0* and subtracted from the original measured washout curve. The area under the peak (FP_{area}) was taken as substance loss via the first-pass effect (hatched areas in Fig. 3). The first-pass effect as a percentage of the total D_0 was calculated as FP% analogously

Table 3. *Percentage of given [^{13}C]bicarbonate lost via the first peak after intravenous administration*

	FP, % of total [^{13}C]bicarbonate administered		
Subject No.	Rest	40% $\dot{V}_{O_2max}$	75% $\dot{V}_{O_2max}$
1	4.5	12.3	14.5
2	2.9	11.1	12.4
3	6.0	18.1	18.1
4	1.9	14.1	15.4
5	2.6	16.7	16.2
6	3.6	16.8	13.5
7	3.4	19.2	26.2
8	4.7	14.2	18.7
Mean ± SD	3.8 ± 1.3	11.5 ± 2.9	16.9 ± 4.0

Area under the peak was determined from the difference between the fitted washout curve and the measured values (see Fig. 3). All means are significantly different ($P < 0.001$). FP, first peak; $\dot{V}_{O_2max}$, maximum O_2 consumption.

to *Eq. 4* by using FP_{area} instead of AUC. D_0 was corrected by FP% in the compartmental calculations. Finally, the fraction of FP% in the tracer loss was calculated using the recovery values.

Statistics. Statistical calculations and fittings were performed using JMP (SAS Institute, Cary, NC) and KaleidaGraph (Abelbeck) software on a personal computer (Apple Macintosh).

Values are means ± SD. Simple linear regressions were used in the comparative statistics, whereby the precision of these estimates is shown as 95% confidence interval. A Fisher's *z*-transformation was used to calculate the confidence limits from correlation coefficients. As a test for differences in the means, a nonparametric rank analysis in the one-way ANOVA (Wilcoxon test) was applied. $P < 0.001$ was considered statistically significant.

RESULTS

The measuring characteristics for the mass spectrometry unit are listed in Table 2.

An excessive first peak in the isotope ratio could be observed instantly after [^{13}C]bicarbonate administration in all subjects, at rest and during exercise. FP_{area} became smaller with increasing workload (Fig. 3) and was 2,373.5 ± 819.2 DOB at rest, 1,208.4 ± 281.3 DOB during moderate exercise (40% $\dot{V}_{O_2max}$), and 963.3 ± 301.1 DOB during intensive exercise (75% $\dot{V}_{O_2max}$). All means were significantly different ($P < 0.001$). However, the higher the workload, the higher the relative loss of [^{13}C]bicarbonate was via this first peak. At rest, the first-pass peak accounted for the loss of 4.01 ± 1.1% of the total administered bicarbonate dose. During moderate and intensive exercise, significantly higher values were reached (Table 3).

With the use of *Eq. 3*, an average recovery of 61.4 ± 14.6% was calculated for resting conditions, but with a tendency to higher values during moderate (64.0 ± 8.9% at 40% $\dot{V}_{O_2max}$) and intensive exercise (71.0 ± 10.1% at 75% $\dot{V}_{O_2max}$). These recovery values show that the first-pass effect was responsible for 11.32 ± 6.1% of the unaccounted loss of bicarbonate at rest, 33.9 ± 9.4% during moderate exercise, and 58.2 ± 14.0% during intensive exercise. All means were significantly different ($P < 0.001$).

Beyond the first peak, the course of the washout curves could be fitted with the sum of two exponentials (*Eq. 6*). The fit resulted in a mean correlation coefficient of 0.997 ± 0.002 for all tested subjects. The use of a three-exponential model did not lead to a better fit at rest or with exercise ($r = 0.980 \pm 0.027$ for all subjects). Table 4 shows the individual macroparameters for these curves.

The rate constants k_{ij} for the transfer of the substance between the compartments and the related quantities Q_1 and Q_2 are shown in Table 5. The CO_2 clearance could be derived from *Eq. 8* ($k_{01}Q_1$) and is given in Table 6 compared with the spirometrically measured $\dot{V}_{CO_2}$. There were no significant mean differences between $\dot{V}_{CO_2}$ and compartmental CO_2 clearance at rest or during moderate exercise. During intensive exercise (75% $\dot{V}_{O_2max}$), the spirometric values for the $\dot{V}_{CO_2}$ were significantly higher than the calculated values.

DISCUSSION

The main finding of these experiments is the steep peak of the breath ^{13}C enrichment directly after intravenous administration of [^{13}C]bicarbonate. This early phase of the washout curves cannot be included satisfactorily into the established multiexponential distribution model for bicarbonate. We assume that this "nonfitting" segment corresponds to a first-pass effect of the ^{13}C substance in the lungs. This results from a high bolus concentration of the substance through the pulmonary system for the first subsequent circulation cycle after administration.

The resulting transfer constants and quantities of the bicarbonate distribution are within the range of other studies (2, 3, 11, 15, 27, 36). In addition, the CO_2 clearance rate from the compartmental calculation does not differ from the values measured by our spirometric system. This indicates that the chosen breath-by-breath method shows the course of the ^{13}C enrichment correctly. Admittedly, the fitting does not apply to a third exponential, as in the study of Barstow et al. (3). The shorter time span of our experiment could

Table 4. *Macroparameters of the two-exponential fitting (Eq. 6) at rest and during bicycle ergometry*

	A_1, DOB	λ_1, min^{-1}	A_2, DOB	λ_2, min^{-1}
Rest	2734 ± 1251	0.359 ± 0.04	760.5 ± 125.8	0.0295 ± 0.004
Exercise				
40% $\dot{V}_{O_2max}$	562.9 ± 120.2	0.085 ± 0.01	1,040 ± 155.2	0.551 ± 0.21
75% $\dot{V}_{O_2max}$	452.3 ± 98.1	0.166 ± 0.03	1,317 ± 210.4	0.794 ± 0.17

Values are means ± SD. *A* and λ, macroparameters of exponential function; DOB, delta over baseline.

Table 5. *Rate constants for transfer between compartments and quantities for mammillary distribution model using two exponentials analogous to two compartments*

	k_{01}, min^{-1}	k_{12}, min^{-1}	k_{21}, min^{-1}	Q_1, liters	Q_2, liters
Rest	0.103 ± 0.031	0.108 ± 0.0301	0.176 ± 0.022	6.79 ± 1.93	11.24 ± 3.14
Exercise					
40% $\dot{V}O_{2max}$	0.188 ± 0.082	0.248 ± 0.15	0.199 ± 0.11	11.76 ± 2.56	9.41 ± 2.88
75% $\dot{V}O_{2max}$	0.403 ± 0.12	0.340 ± 0.14	0.230 ± 0.14	10.66 ± 2.97	7.51 ± 1.49

Values are means ± SD. k_{01}, Rate constant for CO_2 flow from central compartment; k_{12} and k_{21}, rate constants for flow between central *compartment 1* and peripheral *compartment 2;* Q_1 and Q_2, quantity of CO_2 in *compartments 1* and *2.* Mammillary distribution model is shown in Fig. 2.

prevent the transfer to the third compartment from becoming evident.

A first-pass effect through the lungs is supposed as one factor for the unaccounted loss of the labeled substance in ^{13}C studies (2, 3, 8). However, such an effect could not be shown directly in the past because of the low temporal resolution of the methods. Single-breath sample entrapment is very time consuming because of the need to dry and purify the individual gas samples before measurement.

The detection characteristics of $^{13}CO_2$ (Table 2) meet the requirements for the performed breath-by-breath application. This is especially true during the first minutes of the test procedure, when the first-pass effect occurs. The scattering of the $^{13}CO_2$-to-$^{12}CO_2$ ratio increases slightly with advancing test duration and decreasing activity of the labeled substance in the system. This scattering is presumably caused by the biological variability and the increase in the signal-to-noise ratio. However, the results of the fitting procedure are not influenced by the scattering, as with a systematic error caused by the equipment and the experimental environment.

FP_{area} could be estimated and compared with the given total dose of the labeled substance. The result of ~4% of the total bicarbonate dose illustrates the low impact of the first-pass effect on isotope recovery at rest. Earlier studies report an isotope recovery of 50–90% (1–3, 5, 22, 36). Given an average value of 61%, the first-pass effect determines only about one-tenth of the total tracer loss at rest.

As reported in other studies (3, 34), bicarbonate recovery increased with exercise. This may be due to a shortening of slower CO_2 distribution processes to deeper compartments in favor of CO_2 expiration. At higher exercise intensity, an additional amount of CO_2 is exhaled as "nonmetabolic CO_2" via bicarbonate buffering. This contributes to a larger tracer recovery. Furthermore, the proton buffering by bicarbonate might be the cause of the higher $\dot{V}CO_2$ measured with the spirometer than with the dilution calculation during intensive exercise (Table 6). This indirectly confirms that tracer dilution is the result of only the "metabolic" portion of $\dot{V}CO_2$ from substrate oxidation (6).

A higher cardiac output during exercise with shortened lung transit time is assumed to negatively impact the first-pass effect. Indeed, as shown in Fig. 3, FP_{area} decreases with increasing work intensity. However, although the "unobserved loss" of tracer decreases in total, the relative influence of the first-pass effect increases markedly with exercise. With our recovery values taken into account, a mean of ~30% of the given bicarbonate was not exhaled during the intensive exercise. This means that the first-pass effect could be responsible for up to two-thirds, or even more, of the lacking bicarbonate under these conditions.

Interestingly, the application of an infusion (and not a bolus) of labeled bicarbonate failed to show a significant first-pass effect in another study (7). Downey et al. (7) found no difference in the pulmonary expiration between venous and arterial application of bicarbonate. Consequently, the higher substance concentration leads to the mentioned first-pass effect only due to a bolus administration. This is confirmed by a meta-analysis on 34 human bicarbonate studies, which showed statistically significant higher recovery values for infusion than for bolus administration (22). This difference might be partly caused by the described first-pass effect.

In conclusion, the illustrated first-pass effect must be considered particularly in kinetic impulse studies with venous bolus injection of [^{13}C]bicarbonate. Because of the much slower metabolism and distribution, other ^{13}C-labeled substances, such as [^{13}C]glucose, will not be considerably affected, even if given intravenously. Studies of substrate oxidation, as with labeled glucose or amino acids, may not be affected by any first-pass effect, especially after "priming" of the bicarbonate pool (1).

Nevertheless, some studies have been performed that relate the influence of the bicarbonate distribution to other ^{13}C experiments with regard to the oxidation of labeled substances (27). Our results suggest that bicarbonate characteristics cannot be applied directly for corrections in glucose studies, leaving a first-pass effect unconsidered. Especially during exercise, the first-pass effect leads to an overestimation of D_0 for the following washout calculations. With a first-pass loss of ~20%, for

Table 6. *Average $\dot{V}CO_2$ from spirometric measurements and from compartmental analysis*

	$\dot{V}CO_2$, l/min	$\dot{V}CO_{2Dil}$, l/min
Rest	0.325 ± 0.056	0.308 ± 0.052
Exercise		
40% $\dot{V}O_{2max}$	1.39 ± 0.31	1.30 ± 0.29
75% $\dot{V}O_{2max}$	3.12 ± 0.56	2.53 ± 0.46*

Values are means ± SD. $\dot{V}CO_2$, CO_2 output; $\dot{V}CO_{2Dil}$, $\dot{V}CO_2$ calculated by dilution. *Significantly different from zero ($P < 0.001$, by Wilcoxon rank analysis).

instance, the related dilution calculations for $\dot{V}_{CO_2}$ and the size of the compartments (Q_n) will be overestimated by this 20%. Other pathways for losing $^{13}CO_2$ become rather insignificant with increasing exercise intensity. On the other hand, this allows for an initial correction of the tracer dose with the use of the absolute recovery values as performed in previous bicarbonate kinetic calculations (3). However, in resting conditions, only a small amount of the initial tracer dose should be corrected corresponding to the first pass (Table 3). It could be that the other part of the unobserved tracer loss acts as a function over time. Thus it is likely that the calculation of kinetic distribution parameters is more uncertain at rest than during exercise.

REFERENCES

1. **Allsop JR, Wolfe RR, and Burke JF.** Tracer priming the bicarbonate pool. *J Appl Physiol* 45: 137–139, 1978.
2. **Armon Y, Cooper DM, Springer C, Barstow TJ, Rahimizadeh H, Landaw E, and Epstein S.** Oral [^{13}C]bicarbonate measurement of CO_2 stores and dynamics in children and adults. *J Appl Physiol* 69: 1754–1760, 1990.
3. **Barstow TJ, Cooper DM, Sobel EM, Landaw EM, and Epstein S.** Influence of increased metabolic rate on [^{13}C]bicarbonate washout kinetics. *Am J Physiol Regulatory Integrative Comp Physiol* 259: R163–R171, 1990.
4. **Beckers EJ, Halliday D, and Wagenmakers A.** Glucose metabolism and radioactive labeling—what are the real dangers. *Med Sci Sports Exerc* 26: 1316–1318, 1994.
5. **Clugston GA and Garlick PJ.** Recovery of infused [^{14}C]bicarbonate as respiratory $^{14}CO_2$ in man. *Clin Sci (Colch)* 64: 231–233, 1983.
6. **Coggan AR, Habash DL, Mendenhall LA, Swanson SC, and Kien CL.** Isotopic estimation of CO_2 production during exercise before and after endurance training. *J Appl Physiol* 75: 70–75, 1993.
7. **Downey RS, Mellone A, and Matthews DE.** Effect of tracer infusion site on measurement of bicarbonate-carbon dioxide metabolism in dogs. *J Appl Physiol* 60: 1248–1253, 1986.
8. **Drury DR, Wick AN, and Almen MC.** Rate of elimination of labeled carbon dioxide from the body. *Am J Physiol* 186: 361–364, 1956.
9. **Elia M, Fuller NJ, and Murgatroyd PR.** Measurement of bicarbonate turnover in humans: applicability to estimation of energy expenditure. *Am J Physiol Endocrinol Metab* 263: E676–E687, 1992.
10. **Elia M, Jones MG, Jennings G, Poppitt SD, Fuller NJ, Murgatroyd PR, and Jebb SA.** Estimating energy expenditure from specific activity of urine urea during lengthy subcutaneous $NaH^{14}CO_3$ infusion. *Am J Physiol Endocrinol Metab* 269: E172–E182, 1995.
11. **Farhi L and Rahn H.** Dynamics of changes in carbon dioxide stores. *Anesthesiology* 21: 604–614, 1960.
12. **Garfinkel D and Fegley K.** Fitting physiological models to data. *Am J Physiol Regulatory Integrative Comp Physiol* 246: R641–R650, 1984.
13. **Hawley J, Dennis S, and Noakes T.** Oxidation of carbohydrate ingested during prolonged endurance exercise. *Sports Med* 14: 27–42, 1992.
14. **Hetenyi G Jr.** Correction for the metabolic exchange of ^{14}C for ^{12}C atoms in the pathway of gluconeogenesis in vivo. *Federation Proc* 41: 104–109, 1982.
15. **Irving C, Wong W, Shulman R, O'Brian-Smith E, and Klein P.** [^{13}C]bicarbonate kinetics in humans: intra- vs. interindividual variations. *Am J Physiol Regulatory Integrative Comp Physiol* 245: R190–R202, 1983.
16. **Kornberg HL, Davues RE, and Wood DR.** The metabolism of ^{14}C-labelled bicarbonate in the cat. *Biochem J* 51: 351–357, 1951.
17. **Landaw EM, Chen BC-N, and DiStefano JJ.** An algorithm for the identifiable parameter combination of the general mammillary compartmental model. *Math Biosci* 72: 199–212, 1984.
18. **Landaw EM and DiStefano JJ.** Multiexponential, multicompartmental, and noncompartmental modeling. II. Data analysis and statistical considerations. *Am J Physiol Regulatory Integrative Comp Physiol* 246: R665–R677, 1984.
19. **Leese GP, Nicoll AE, Varnier M, Thompson J, Scrimgeour CM, and Rennie MJ.** Kinetics of $^{13}CO_2$ elimination after ingestion of ^{13}C bicarbonate: the effects of exercise and acid-base balance. *Eur J Clin Invest* 24: 818–823, 1994.
20. **Lefèbvre PJ, Morosa F, Lacroix M, Luyckx A, and Lopez-Habib G.** Naturally labelled ^{13}C-glucose: metabolic studies in human diabetes and obesity. *Diabetes* 24: 185–189, 1975.
21. **Lefèbvre PJ, Mosora F, Lacroix M, Pirnay F, Scheen A, Krzentowski G, Jandrain B, Gautier JF, Pallikarakis N, and Riou JP.** Use of ^{13}C substrates for metabolic studies in exercise: methodological considerations. *J Appl Physiol* 71: 2059–2061, 1991.
22. **Leijssen DP and Elia M.** Recovery of $^{13}CO_2$ and $^{14}CO_2$ in human bicarbonate studies: a critical review with original data. *Clin Sci (Colch)* 91: 665–677, 1996.
23. **Marquardt D.** An algorithm for least-squares estimation of nonlinear parameters. *J Soc Indust Appl Math* 11: 431–441, 1963.
24. **Massicotte D, Péronnet F, Brisson G, Boivin L, and Hillaire-Marcel C.** Oxidation of exogenous carbohydrate during prolonged exercise in fed and fasted conditions. *Int J Sports Med* 11: 253–258, 1990.
25. **Murgatroyd PR, Shetty PS, and Prentice AM.** Techniques for the measurement of human energy expenditure: a practical guide. *Int J Obes Relat Metab Disord* 17: 549–568, 1993.
26. **Murphy JL, Jones A, Brookes S, and Wootton SA.** The gastrointestinal handling and metabolism of [1-^{13}C]palmitic acid in healthy women. *Lipids* 30: 291–298, 1995.
27. **Pallikarakis N, Sphiris N, and Lefebvre P.** Influence of the bicarbonate pool and on the occurrence of $^{13}CO_2$ in exhaled air. *Eur J Appl Physiol* 63: 179–183, 1991.
28. **Pirnay F, Lacroix M, Mosora F, Luyckx A, and Lefèbvre P.** Glucose oxidation during prolonged exercise evaluated with naturally labeled [^{13}C]glucose. *J Appl Physiol* 43: 258–261, 1977.
29. **Poyart CF, Freminet A, and Bursaux E.** The exchange of bone CO_2 in vivo. *Respir Physiol* 25: 101–107, 1975.
30. **Röcker K, Krieg B, Niess A, and Dickhuth HH.** Breath-by-breath measurements for the analysis of exogenous glucose oxidation during intense endurance exercise using [C-13] isotopes. *Int J Sports Med* 17: 480–486, 1996.
31. **Romijn JA, Coyle EF, Sidossis LS, Gastaldelli A, Horowitz JF, Endert E, and Wolfe RR.** Regulation of endogenous fat and carbohydrate metabolism in relation to exercise intensity and duration. *Am J Physiol Endocrinol Metab* 265: E380–E391, 1993.
32. **Spear ML, Darmaun D, Sager BK, Parsons WR, and Haymond MW.** Use of [^{13}C]bicarbonate infusion for measurement of CO_2 production. *Am J Physiol Endocrinol Metab* 268: E1123–E1127, 1995.
33. **Sphiris N and Pallikarakis N.** A computer program for estimating the influence of the body bicarbonate pool during CO_2 breath tests. *Comput Methods Programs Biomed* 46: 225–232, 1995.
34. **Van Aerde JE, Sauer PJ, Pencharz PB, Canagarayar U, Beesley J, Smith JM, and Swyer PR.** The effect of energy intake and expenditure on the recovery of $^{13}CO_2$ in the parenterally fed neonate during a 4-hour primed constant infusion of $NaH^{13}CO_3$. *Pediatr Res* 19: 806–810, 1985.
35. **Wagenmakers AJ, Brouns F, Saris WH, and Halliday D.** Oxidation rates of orally ingested carbohydrates during prolonged exercise in men. *J Appl Physiol* 75: 2774–2780, 1993.
36. **Winchell H, Staehlin H, Kusubov N, Slanger B, Fish M, Pollycove M, and Lawrence J.** Kinetics of CO_2-HCO_3 in normal adult males. *J Nucl Med* 11: 711–715, 1970.
37. **Zanconato S, Cooper DM, Barstow TJ, and Landaw E.** $^{13}CO_2$ washout dynamics during intermittent exercise in children and adults. *J Appl Physiol* 73: 2476–2482, 1992.