Eur J Appl Physiol (1991) 63:179-183

European Journal of Applied Physiology and Occupational Physiology

Influence of the bicarbonate pool and on the occurrence of $^{13}CO_2$ in exhaled air

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Accepted May 17, 1991

Summary. In $^{13}CO_2$ breath tests, based on $^{13}C:^{12}C$ ratio measurements, the appearance of ^{13}C in exhaled CO_2 was monitored after the administration of a ^{13}C-labelled compound. Independently of the substrate used, the existence of a bicarbonate pool into which the CO_2 produced enters before being exhaled, imposes a delay on the appearance of changes in the $^{13}C:^{12}C$ ratio. To estimate the nature and magnitude of this delay, we applied a two-compartment model to describe the kinetics of the body bicarbonate pool and we evaluated the $^{13}C:^{12}C$ ratio of CO_2 entering that pool from the measured $^{13}C:^{12}C$ ratio in the exhaled CO_2 after an oral intake of "naturally labelled" ^{13}C-glucose. Our results demonstrated that discrepancies between total and exogenous glucose oxidation in relation to the peak occurrence time, as well as the absolute quantities, could be adequately explained by the interference of the bicarbonate stores.

Key words: Carbon isotopes - CO_2 breath test - Bicarbonate pool - Compartmental model

Introduction

The exhalation of labelled CO_2 derived from artificially or naturally labelled compounds has been shown to be a useful means for the diagnosis of various metabolic disorders or the quantitative estimation of their oxidation rate. This method, generally known as "CO_2 breath test", has several methodological advantages:
1. The labelled material has almost identical chemical and physical properties as its natural homologue
2. The procedure is non-invasive
3. All organic compounds can, in principle, be labelled and already an impressive number of either radioactive (^{14}C) or stable (^{13}C) labelled compounds are available.

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Most CO_2 breath tests involve the oral administration of a carbon labelled substance, which releases the labelled carbon isotope in its metabolic pathway. The rate of appearance of the label in the exhaled CO_2 can yield information about factors affecting the rate-limiting step of the overall metabolic route (Schoeller et al. 1977; Halliday and Rennie 1982).

By definition, CO_2 is an end-product in the catabolism of the administered compound and several factors affecting different steps in the route followed by the substrate, or its metabolites, may influence the rate of appearance of the label in the exhaled CO_2. Of all factors such as gastric emptying, rate of intestinal absorption, endogenous substrate pools or the bicarbonate pool, only the latter has the same degree of influence on the results of all tests, irrespective of the compound used.

Previous studies on bicarbonate kinetics (Young et al. 1967; Waterhouse et al. 1969; Slanger et al. 1970; Winchell et al. 1970; Irving et al. 1983) have led to compartmental models which can be employed in the study of this influence.

The aim of this investigation was to provide a quantitative estimation of the influence of the bicarbonate pool on the appearance of the labelled CO_2 in breath, at rest or during exercise, with application to experimental data from previous studies of our own, using naturally enriched ^{13}C-glucose (Pallikarakis 1980).

Methods

Experimental protocol and data base. In "naturally labelled" glucose studies, exogenous glucose has been used as a tracer for metabolic studies in man for almost two decades. The isotopic ratio $^{13}C:^{12}C$ of the expired CO_2, is measured by means of a double collector mass spectrometer and enables the calculation of the proportion of CO_2 yielded by the ingested "naturally labelled" (exogenous) glucose. As has been described previously (Pallikarakis et al. 1980; Mosora et al. 1981) the quantity of the exogenous glucose metabolized by the subject is determined on the basis of the ^{13}C appearance in breath and of the total CO_2 production. The enrichment of expired CO_2 with ^{13}C is compared to the maximal theoretical enrichment which would have been obtained if all

expired CO_2 was derived from the exogenous glucose load. Results reported here have been derived from two different protocols. The first one was applied to five subjects at rest, who fasted overnight, then received orally 100 g of exogenous glucose, dissolved in 400 ml of water. These subjects were monitored for the following 7 h. The second protocol was applied to three subjects during low intensity exercise. The subjects, fasted overnight, walked on a motor-driven treadmill at 21.7% (SEM 2.5%) of their maximal oxygen uptake. After a 15-min period of adaptation to exercise, they received orally the same dose and were subsequently tested over a 90-min period.

The CO_2 produced and the O_2 consumed, were measured every 30 min for the subjects at rest and every 15 min during exercise and these figures were used for the calculation of the nonprotein respiratory quotient (RQ_{NP}) taking into account the total nitrogen excreted in the urine collected at the end of the tests. Thus, indirect calorimetry provided an estimation of the total amount of carbohydrates and lipids consumed, at any time of the experiment and their respective contribution to the energy requirements.

The ^{13}C enrichment is expressed in terms of $\delta^{13}C$, according to the formula:

$$\delta^{13}C(‰) = \left(\frac{(^{13}C/^{12}C)_{sam}}{(^{13}C/^{12}C)_{ref}} - 1\right) \cdot 1000 \quad (1)$$

where $(^{13}C/^{12}C)_{ref}$ is our own laboratory CO_2 reference and its $\delta^{13}C(‰)$ value is -28.50 relative to the usual PDB standard. Isotopic measurements were performed using a CH5 mass spectrometer, Variant MAT GmbH, Bremen, FRG, with pure CO_2 samples, derived from expired air collected every 30 min for the protocol at rest and every 15 min for the one during exercise. Details on subjects, protocols, analytical procedures and calculations are described elsewhere (Pallikarakis 1980; Mosora et al. 1981).

Fitting the experimental data. The discrete values of $\delta^{13}C$ were fitted using Eq. 2, which consists of a constant term (a_1), expressing initial (and final) $\delta^{13}C$ values, and a nonlinear term, expressing its change due to the administration of the naturally labelled glucose.

$$\delta^{13}C(t) = a_1 + a_2 \cdot t \cdot e^{a_3 t} \cdot \sin(a_4 t) \quad (2)$$

This equation was found to fit well our experimental results and was in good agreement with initial and final conditions. An iterative technique was used to estimate the coefficients a_i for $i = 1,2,3,4$ of this non-linear equation. The estimates at each iteration were obtained by Marquard's maximum neighbourhood algorithm, which combines the Gauss (Taylor series) method and the method of the steepest descent (Marquardt 1963; Bevington 1969). The curve fitting programme was developed and run on an Macintosh II personal computer, Apple Computer Inc., Cupertino, CA, USA.

Description of the general model used. Of the different models given in the literature which describe the kinetics of CO_2 in the human body, we selected the one proposed by Winchell and coworkers (Slanger et al. 1970; Winchell et al. 1970) and shown in Fig. 1. The selection was based on the fact that the particular model is considered to be a reliable one (Irving et al. 1983) and provides data for both rest and exercise conditions.

The first of these compartments included the bicarbonate contained, within the blood and organs with high vascular perfusion, such as heart, liver, kidneys and the intestinal tract. The second compartment included the bicarbonate contained in organs with low vascular perfusion such as muscles, skin and fat. Bicarbonate rate constants and pool sizes for the two different protocols are given in Table 1 and were considered to be constant for the whole duration of the test. Despite the fact that this assumption does not hold in nonsteady-state conditions, under our experimental protocol these changes were small enough not to influence the results.

The $\delta^{13}C$, measured in breath, was considered to be the output of compartment 1 at predetermined time intervals. Our aim was to calculate the corresponding $\delta^{13}C$ of the CO_2 entering the system.

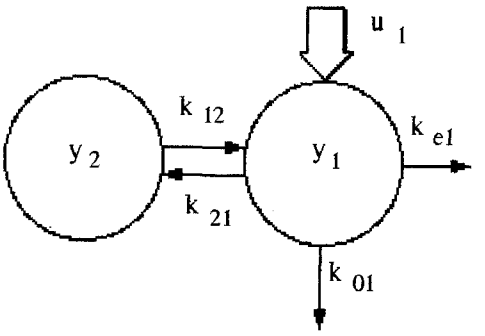

Fig. 1. The two compartment model of CO_2 kinetics used to study its influence on the appearance of labelled carbon in expired air

Table 1. Fractional turnover rates and pool sizes of the bicarbonate model used. Values for the two compartments are expressed in litres taking into account the mean bodymass of the subjects. Values of constant rate k_{01} for both cases were adapted to the individual data for expired CO_2 ($l \cdot min^{-1}$) as they have been derived from respiratory measurements

	k_{12} (min^{-1})	k_{21} (min^{-1})	k_{01} (min^{-1})	k_{e1} (min^{-1})	y_1 (l)	y_2 (l)
Rest	0.047	0.067	0.022	0.005	9.0	12.9
Exercise	0.052	0.028	0.043	0.009	15.7	8.5

The constants of the transfer rate between the compartments, from compartment i to compartment j, are denoted by k_{ji} for $i = 1,2$ and $j = 1,2$ $(i \neq j)$, with subscript 0 denoting the environment and with subscript e representing the labelled bicarbonate leaving compartment 1 with no significant recycling (bone biosynthesis).

The dynamics of the system may be described mathematically by the linear time-invariant differential equations:

$$\dot{x}_1(t) = -(k_{21} + k_{01} + k_{e1}) \cdot x_1(t) + k_{12} \cdot x_2(t) + v_1(t) \quad (3a)$$

$$\dot{x}_2(t) = k_{21} \cdot x_1(t) - k_{12} x_2(t) \quad (3b)$$

where x_1, x_2 are the amounts of ^{13}C in the two compartments, v_1 is the input of ^{13}C into compartment 1 and $\dot{x}_1$, $\dot{x}_2$ are the derivatives of x_1, x_2 with respect to time. Let y_1, y_2 represent the total amount of carbon in compartments 1 and 2 respectively and u_1 be the total input of carbon into the compartment 1. Let R_{out1}, R_{out2} be the corresponding $^{13}C:^{12}C$ ratios in compartments 1 and 2 and R_{in1} the $^{13}C:^{12}C$ ratio in the input of the model. The values x_i, $i = 1,2$ are equal to the product $y_i \cdot R_{outi}$, $i = 1,2$ and $v_1 = u_1 \cdot R_{in1}$. After substituting the values of x_i, $i = 1,2$ and v_1 from the last equalities, the Eqs. (3) become:

$$\dot{R}_{out1}(t) = -(k_{21} + k_{01} + k_{e1}) \cdot R_{out1}(t) + \frac{k_{12} \cdot y_2}{y_1} \cdot R_{out2}(t) + \frac{u_1}{y_1} \cdot R_{in1}(t) \quad (4a)$$

$$\dot{R}_{out2}(t) = \frac{k_{21} \cdot y_1}{y_2} \cdot R_{out1}(t) - k_{12} \cdot R_{out2}(t) \quad (4b)$$

The value of $R_{sam} = (^{13}C:^{12}C)_{sam}$ from the Eq. 1 is:

$$R_{sam} = \left(\frac{\delta^{13}C(‰)}{1000} + 1\right) \cdot R_{ref} \quad (5)$$

After substituting the values of R_{outi}, $i = 1,2$ and R_{in1} from (5), the Eqs. (4) become:

$$\dot{\delta}^{13}_{out1}C(t) = -(k_{21}+k_{01}+k_{e1})\cdot\delta^{13}_{out1}C(t) + \frac{k_{12}\cdot y_2}{y_1}\cdot\delta^{13}_{out2}C(t) + \frac{u_1}{y_1}\cdot\delta^{13}_{in1}C(t) \quad (6a)$$

$$\dot{\delta}^{13}_{out2}C(t) = \frac{k_{21}\cdot y_1}{y_2}\cdot\delta^{13}_{out1}C(t) - k_{12}\cdot\delta^{13}_{out2}C(t) \quad (6b)$$

From Eq. 6a we obtain $\delta^{13}_{in1}C(t)$:

$$\delta^{13}_{in1}C(t) = [\dot{\delta}^{13}_{out1}C(t) + (k_{21}+k_{e1}+k_{01}) \cdot\delta^{13}_{out1}C(t)]\cdot\frac{y_1}{u_1} - \frac{k_{12}\cdot y_2}{y_1}\cdot\delta^{13}_{out2}C(t) \quad (7)$$

The value of $\delta^{13}_{out2}C(t)$ is calculated by integrating Eq. 6b.

Results

To evaluate the influence of the body CO_2 stores in the expired $^{13}CO_2$, we have applied the previously described model to the original data. Table 2 contains the calculated values of $\delta^{13}C$ corresponding to the two compartments as well as the quantities of glucose calculated according (a) to the directly acquired data (DAD) and (b) to the model modified values (MMV).

The introduction of the model led to a peak value of calculated exogenous glucose oxidation, which was greater than that calculated directly from the DAD. It was also clear that both total carbohydrate consumption and the exogenous glucose oxidation reached their maxima at the same time.

Figure 2 shows the mean values and SEM obtained from all five subjects for exogenous glucose oxidation, calculated with and without taking into account the body bicarbonate stores. It also includes the values of the total carbohydrate consumed during the tests according to the indirect calorimetry measurements. It should also be pointed out that the crossing-over of the total carbohydrate consumed curve with the exogenous glucose oxidation curve disappears in the case of corrected exogenous glucose oxidation values.

Figure 3 shows the mean values and SEM of exogenous glucose oxidation obtained from the second protocol (exercise), both with and without taking into account the body bicarbonate stores. It also includes the values of total carbohydrate consumed during the tests according to the indirect calorimetry measurements. Despite the significant differences observed between the exogenous glucose oxidation calculated from DAD and MMV, the relative differences are much smaller compared to those obtained at rest.

Table 2. Changes in $\delta^{13}C$ values corresponding to the CO_2 entering model modified values (MMV) and leaving directly acquired data (DAD) compartment 1 and the calculated corresponding values of exogenous glucose oxidation

Time (min)	$\delta^{13}C_{DAD}$ (‰)	Exogenous glucose oxidation-DAD ($g\cdot30\ min^{-1}$)	$\delta^{13}C_{MMV}$ (‰)	Exogenous glucose oxidation-MMV ($g\cdot30\ min^{-1}$)
0	3.46		3.46	
30	3.62	0.12	4.23	0.61
60	4.12	0.57	5.77	2.02
90	4.85	1.26	7.06	3.26
120	5.60	1.89	7.85	3.88
150	6.24	2.50	8.18	4.24
180	6.71	2.85	8.14	4.10
210	6.99	3.01	7.86	3.74
240	7.09	3.14	7.42	3.42
270	7.03	3.00	6.88	2.88
300	6.84	2.65	6.32	2.25
330	6.56	2.39	5.77	1.78
360	6.23	2.20	5.26	1.43
390	5.87	1.88	4.81	1.05
420	5.49	1.55	4.41	0.72
Total		29.01		35.38

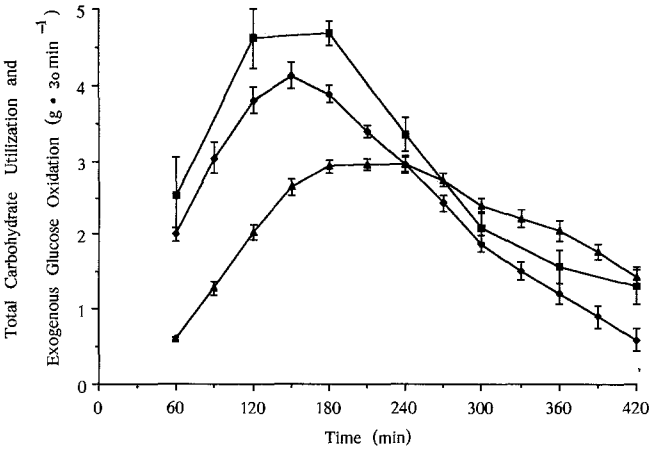

Fig. 2. Total carbohydrate utilization (— ■ —) and exogenous glucose oxidation calculated from carbon dioxide production measured every 30 min and either from directly aquired data (— ▲ —) or model modified values (— ● —) at rest

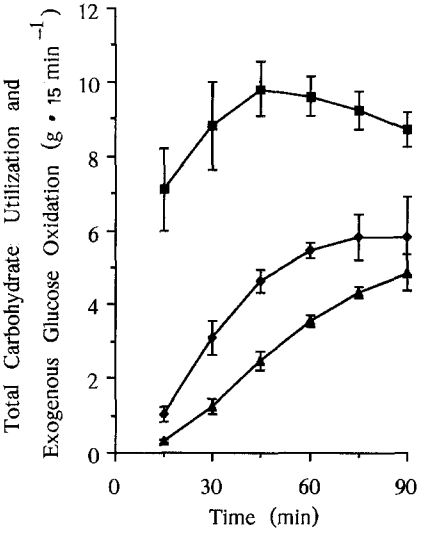

Fig. 3. Total carbohydrate utilization (— ■ —) and exogenous glucose oxidation calculated from carbon dioxide production measured every 15 min either from directly aquired data (— ▲ —) or model modified values (— ◆ —) during exercise

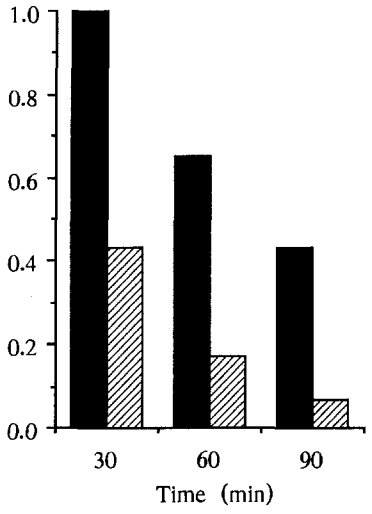

Fig. 4. Relative differences of exogenous glucose oxidation between values calculated with and without taking into account the bicarbonate pool and normalized to one, at rest (■) or during exercise (□)

This becomes apparent in Fig. 4, where the relative differences between the exogenous glucose oxidation calculated from MMV and DAD are normalized to one. As can be seen, during low intensity exercise, this difference is almost 40% of that observed at rest, after 0.5 h. During the following hour this difference falls continuously and becomes less than 1 $g \cdot 15 min^{-1}$ corresponding to an underestimation of about 20% at the end of the exercise (90 min).

Discussion

Examination of the curve depicting the exogenous glucose oxidation, obtained from DAD, indicated a delay in the occurrence of the peak value compared to the corresponding peak value of the total carbohydrate utilization. In fact, the oxidation of the exogenous glucose reached its maximum 1 h later than that of the total carbohydrate oxidation. Previously reported results (Ferrannini et al. 1980) similarly have shown such a delay in exogenous glucose oxidation, measured by mass spectrometry, when compared to the total carbohydrate utilization, estimated by indirect calorimetry. This delay has been attributed to the influence of the body CO_2 stores (Pallikarakis 1980). Our calculations demonstrated that this could be the case, since when the bicarbonate pool is taken into account, the shift in the peak position is eliminated.

It is evident that after intestinal absorption, the orally administered exogenous glucose enters the endogenous glucose pools where it becomes diluted. This dilution influences, of course, the exogenous glucose oxidation rate without, however, any effect on the absolute quantities oxidized. In fact, their evaluation does not concern the kinetics of the exogenous glucose in the carbohydrate compartments of the body but only its complete oxidation to CO_2.

The glucose absorption period, after the administration of 100 g, has been estimated to last about 2–3 h at rest (Bjorntorp and Sjostrom 1978). It can thus be assumed that the total carbohydrate stores are partially labelled which would be reflected in the total carbohydrate and exogenous glucose oxidation rate curves. In other words, after the third hour exogenous glucose oxidation should represent a part of the total carbohydrate consumption. However, the corresponding curves representing total carbohydrate oxidation and exogenous glucose oxidation, calculated from DAD, showed an obvious crossover. In fact, during the fifth hour the exogenous glucose oxidation appeared to be greater than the total carbohydrate consumption while in fact the former was part of the latter. The same crossover phenomenon has also been observed in other relevant studies that use naturally labelled glucose as the exogenous load at rest (Ferrannini et al. 1980; Acheson et al. 1985). The proposed model eliminates this crossover and can fully account for its appearance. We showed here that our results also supported by the previously suggested hypothesis, that the existence of the body bicarbonate pool can by itself be the most plausibe explanation of the crossover phenomenon. This fact should be kept in mind when interpreting directly derived quantitative results concerning the oxidation of a ^{13}C-labelled substrate at rest. However, simple comparison of results under the same metabolic conditions could be used at a qualitative level.

On the other hand, the relative difference between the exogenous glucose oxidation calculated from MMV and from DAD was lower during exercise because of the acceleration of metabolic turnover and of the increased volumes of expired CO_2. Despite the significant reduction, this difference was quite large at low intensity effort and the bicarbonate pool must also be taken into account.

Quantitative results can only be considered reliable when the studies are performed during moderate or high intensity muscular exercise (Pirnay et al. 1982) since the CO_2 production rate is then much higher, leading to an analogous turnover rate. In this case the influence of the bicarbonate pool on the results obtained is limited and can be ignored without introduction of significant error.

Acknowledgements. The primary data used in this work have been obtained in the University of Liege in collaboration with Professors F. Mosora-Stan, F. Pirnay and M. Lacroix to whom we express our thanks.

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