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$^{13}CO_2$ recovery fraction in expired air of septic patients under mechanical ventilation

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The continuous intravenous administration of isotopic bicarbonate ($NaH^{13}CO_2$) has been used for the determination of the retention of the $^{13}CO_2$ fraction or the $^{13}CO_2$ recovered in expired air. This determination is important for the calculation of substrate oxidation. The aim of the present study was to evaluate, in critically ill patients with sepsis under mechanical ventilation, the $^{13}CO_2$ recovery fraction in expired air after continuous intravenous infusion of $NaH^{13}CO_2$ (3.8 µmol/kg diluted in 0.9% saline in ddH_2O). A prospective study was conducted on 10 patients with septic shock between the second and fifth day of sepsis evolution (APACHE II, 25.9 ± 7.4). Initially, baseline CO_2 was collected and indirect calorimetry was also performed. A primer of 5 mL $NaH^{13}CO_2$ was administered followed by continuous infusion of 5 mL/h for 6 h. Six CO_2 production (VCO_2) measurements (30 min each) were made with a portable metabolic cart connected to a respirator and hourly samples of expired air were obtained using a 750-mL gas collecting bag attached to the outlet of the respirator. $^{13}CO_2$ enrichment in expired air was determined with a mass spectrometer. The patients presented a mean value of VCO_2 of 182 ± 52 mL/min during the steady-state phase. The mean recovery fraction was 0.68 ± 0.06%, which is less than that reported in the literature (0.82 ± 0.03%). This suggests that the $^{13}CO_2$ recovery fraction in septic patients following enteral feeding is incomplete, indicating retention of $^{13}CO_2$ in the organism. The severity of septic shock in terms of the prognostic index APACHE II and the sepsis score was not associated with the $^{13}CO_2$ recovery fraction in expired air.

Key words: Recovery fraction; $^{13}CO_2$; Stable isotope; Septic shock; Intensive care setting

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Introduction

Sepsis, septic shock and multiple organ dysfunctions are the main causes of death in intensive care units despite the recent great technological and scientific advances and efforts devoted to improving outcome (1-3).

Several aspects deserve special attention regarding septic patients. In addition to hemodynamic, infectious and immune response evaluations, metabolic parameters are also relevant. Organic metabolism is a dynamic process, especially in pathological situations, with protein synthesis and degradation continuing to occur. Carbon dioxide (CO_2) is produced mostly due to the oxidation of substrates, which can be quantified by using stable isotopes. The endocrine-metabolic response triggered by sepsis cannot be avoided but can be treated by preventing greater tissue injury on the basis of a detailed knowledge of metabolism. One of the most important metabolic changes occurring during sepsis/septic shock is the predominance of the catabolic response of skeletal muscle. This response is multifactorial and reflects the inhibition of amino acid uptake and the reduction of protein synthesis, with acceler-

ated protein breakdown (4). Protein degradation exceeds protein synthesis leading to increased O_2 consumption (VO_2), which, if not corrected, can be associated with multiple organ dysfunctions.

The measurement of nutrient oxidation rate in critically ill patients receiving nutritional therapy is of great interest (5-7). There are few reports using stable isotopes such as labeled carbon (^{13}C) in sepsis/septic shock. It is possible to obtain the CO_2 recovery fraction in expired air by administering labeled bicarbonate intravenously ($NaH^{13}CO_2$) (8). Since the CO_2 produced from the labeled substrate interacts with the bicarbonate pool before being expired, it is necessary to determine this $^{13}CO_2$ recovery fraction in expired air in order to study the oxidation rate for a given substrate (for example, protein). This recovery fraction can be used as a correction factor in the calculation of protein oxidation in subsequent studies.

The objective of the present study was to measure the $^{13}CO_2$ recovery fraction in expired air of critically ill patients with sepsis or septic shock on mechanical ventilation who were receiving enteral or parenteral nutritional treatment, by continuous intravenous infusion of $NaH^{13}CO_2$.

Subjects and Methods

Subjects

A prospective clinical study was conducted in the Intensive Care Center (Campus) of the University Hospital, Faculty of Medicine of Ribeirão Preto. The study was approved by the Research Ethics Committee of the University Hospital, Faculty of Medicine of Ribeirão Preto and written informed consent was obtained from the patients or persons responsible for them (Process #4989/99, HCRP). The investigation involved 10 patients (4 men and 6 women aged 15 to 85 years with sepsis of any origin or septic shock after blood volume restoration and hemodynamic stabilization. We included patients in the period between the 2nd and 5th day of evolution of the clinical picture, with an indication for invasive mechanical ventilation, with oxygen fraction in inspired air (FiO_2) <0.6, mean arterial pressure >50 mmHg, hourly diuresis >50 mL/h, and requiring the use of nutritional therapy. Exclusion criteria were: oliguric renal insufficiency of any etiology, spontaneous ventilation, brain death and refusal to participate by the patient or person responsible. The criteria used for the diagnosis of sepsis and septic shock were those established by the consensus conference of the American College of Chest Physicians/Society of Critical Care Medicine (9). The patients were divided into two groups according to severity as determined by the prognostic index APACHE II (Acute Physiologic and Chronic Health Evaluation) (10) and by sepsis score (11). All patients were submitted to blood volume restoration, mechanical ventilatory support with a microprocessor-controlled ventilator (Bird 8400 STi Bird Prod Corp., USA; Servo 900C Siemens, Sweden; Savina Draegger, Germany), appropriate antibiotic therapy, and the use of vasoactive drugs and nutritional therapy according to the real energy expenditure calculated by indirect calorimetry (70% carbohydrates, 30% lipids in relation to total calorie value, and approximately 1 $g \cdot kg^{-1} \cdot day^{-1}$ protein) according to the diet standardized by HCFMRP-USP. The patients were sedated with benzodiazepines and/or opiates, and a muscle blocker was used when necessary. Indirect calorimetry was performed on all patients using a portable Deltatrac II Metabolic Monitor (Datex-Ohmeda, Finland) coupled to the mechanical ventilator. Barometric and gas pressure were calibrated before each set of measurements. Calorimetry was performed during the 6 h of the protocol, and mean VCO_2 was calculated for each hour together with expired air collection. The patients were weighed using a portable scale (Slingscale 2002, Instrucom/Hill-Rom series, Hillenbrand Industries, USA).

Patient age ranged from 28-78 years (median 56 years; mean 55.1 ± 19 years). The APACHE II prognostic index ranged from 17-37 (median 24.5; mean 25.9 ± 7.4), with a calculated death risk with a median of 45 (29-88) and a mean value of 60 ± 20%. Sepsis score showed a median value of 18 and ranged from 11-26 with a mean of 19.1 ± 4.2. The intravenous use of the stable isotope ($NaH^{13}CO_2$) did not alter the acid-base status of the patients, as confirmed by arterial gas measurement before and after the study. Of the 10 patients studied, 8 (80%) died within 5 days.

Experimental design

The protocol lasted 6 h. Basal expired CO_2 was determined before starting the infusion of bicarbonate with labeled carbon (3.8 µmol/kg $NaH^{13}CO_2$ diluted in 0.9% NaCl in ddH_2O) and indirect calorimetry was used throughout the study. After basal gas collection, 5 mL $NaH^{13}CO_2$ was infused in bolus, followed by continuous infusion at 5 mL/h for 6 h (T1 to T6). The mean value obtained for each hour (time) of study was used as the basis for the calculation of $^{13}CO_2$ recovery fraction in expired air. For the calculation of the recovery fraction, we used VCO_2 (converted from mL/min to $mmol \cdot kg^{-1} \cdot h^{-1}$) obtained by indirect calorimetry and the $^{13}CO_2$ value in expired air.

The dose of $NaH^{13}CO_2$ was calculated according to the weight of the patient on the day when the protocol was applied. The dose used for bolus infusion (prime) was 3.8 µmol/kg and the dose for continuous infusion was 3.8 $\mu mol \cdot kg^{-1} \cdot h^{-1}$, based on the study of Tissot et al. (12). The stable isotope used was $NaH^{13}CO_2$ (99 atm%; Mass Trace

Technologies, USA). The isotope was prepared in the Pharmacy Division of the University Hospital of Ribeirão Preto under a laminar flow hood and under aseptic and antiseptic conditions.

Arterial gases were measured before and after the study to determine possible interferences of the amount of $NaH^{13}CO_2$ with the metabolic state of the patient.

Expired air was initially collected to establish a basal reference value at times -40, -25, and -15 min before the beginning of isotope infusion. Two samples per hour were then collected throughout the procedure (6 h of isotope infusion). Collection was performed with a 750-mL gas collecting bag (Quintron, USA) connected to the expiratory outlet of the mechanical ventilator. The samples were analyzed by mass spectrometry using isotope ratio mass spectrometry (Europa Scientific, England).

Equations used for the calculation of VCO_2, the $^{13}CO_2$ enrichment as well as $^{13}CO_2$ recovery fraction in expired air samples were previously described in the literature (13,14). The value for Enr $^{13}CO_2$ APE (enrichment of expired air in percent of excess atoms) x 1000 refers to the arithmetic difference between the $^{13}CO_2$ values obtained for expired air samples and the basal values before $NaH^{13}CO_3$ administration.

Statistical analysis

Data sets were first evaluated by Minitab 13.20 software (USA) to test three hypotheses: independence, normality and variance in order to assess their parametric or non-parametric nature. Considering the non-parametric nature of all data sets, comparison between the two groups studied according to severity was performed using the non-parametric Wilcoxon sign post-test for two correlated samples, with the level of significance set at $P < 0.05$. Data are reported as median (min-max) as well as mean ± SD. Additionally, non-parametric Spearman correlation coefficients were also determined between $^{13}CO_2$ recovery fraction and the minute volume and the VCO_2.

Results

The median energy expenditure for the patients was calculated to be 1493 ranging from 1131-2353 and mean value of 1587 ± 430 kcal/min, with a value of 1440 ± 121 kcal/min when the Harris-Benedict equation (14) without correction factors was used, avoiding the super-estimation that the correction factor yields in the energy expenditure values. Using this approach, the difference between the two methods (146 kcal/min) was non-significant ($P = 0.43$). The median value of minute volume was 7.6 (5.2-11.1) with a mean of 7.7 ± 1.8 L/min, indicating that the alveolar minute ventilation was within normal limits. This suggested a low possibility of interference of CO_2 elimination with the recovery fraction since partial CO_2 pressure ($paCO_2$) remained stable. Mean CO_2 production was 182 ± 52 mL/min, also corresponding to values within normal limits (Table 1).

Median O_2 consumption was 216 (140-343) with a mean of 229 ± 67 mL/min, which remained stable throughout the study. This was expected because the patients, despite their septic shock condition, were stable due to the hemodynamic support provided. The median respiratory quotients of 0.8 ranging from 0.57-0.90 with a mean of 0.79 ± 0.10 characterized an adequate metabolic state, with no predominance of lipolysis, of gluconeogenesis or of lipogenesis (Table 1).

Mean $^{13}CO_2$ enrichment in expired air was 4.01 ± 0.76 (APE x 1000) during the period from 120 to 360 min.

Table 1. Real energy expenditure, minute volume, CO_2 production (VCO_2), oxygen consumption (VO_2), respiratory quotient, and $^{13}CO_2$ recovery fraction in the patients studied.

Patient	Energy expenditure (kcal/min)	Minute volume (L/min)	VCO_2 (mL/min)	VO_2 (mL/min)	Respiratory quotient	$^{13}CO_2$ recovery fraction
1	1191	6.0	149	174	0.85	0.71
2	1155	7.9	145	140	0.9	0.68
3	2258	10.0	277	327	0.84	0.74
4	1455	8.5	168	215	0.78	0.70
5	1458	6.4	165	217	0.76	0.70
6	1565	6.9	165	211	0.78	0.60
7	1773	7.5	158	277	0.57	0.70
8	1131	5.2	146	163	0.89	0.55
9	2353	11.1	281	343	0.82	0.72
10	1528	7.6	162	225	0.72	0.72
Median (min-max)	1493 (1131-2353)	7.6 (5.2-11.1)	163.5 (145-281)	216 (140-343)	0.80 (0.57-0.90)	0.70 (0.55-0.74)
Mean ± SD	1587 ± 430	7.7 ± 1.8	182 ± 2	229 ± 67	0.79 ± 0.10	0.68 ± 0.06

Dynamic evaluation throughout the study demonstrated the presence of a steady state at about 2 h after the beginning of isotope infusion, an expected result due to the administration of the initial priming dose. A gradual increase in enrichment occurred thereafter, reaching a steady state in 120 min. No significant difference in enrichment was observed between groups of different septic severity (P = 0.99) (data not shown).

There was a positive, although weak, correlation between enrichment and minute volume and real energy expenditure, suggesting that alveolar ventilation and the diet offered somehow affected the percentage of $^{13}CO_2$ enrichment in expired air.

The median $^{13}CO_2$ recovery fraction in expired air was 0.70 (range 0.55-0.74) with a mean of 0.68 ± 0.06, differing from those reported for non-septic critically ill patients, which suggests that at some time during the course of septic shock there may have been a shift of CO_2 to other metabolic pathways. Comparison of the $^{13}CO_2$ recovery fraction between different groups according to age (P = 0.81), sex (P = 0.25) and severity of disease (P = 0.62) did

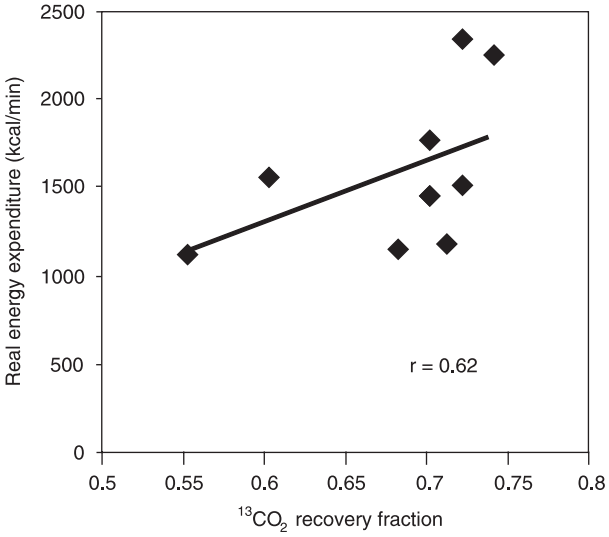

Figure 1. Correlation of the $^{13}CO_2$ recovery fraction in expired air and the real energy expenditure for the 10 patients studied. Spearman correlation test was used for statistical analysis as described in Methods (P < 0.05).

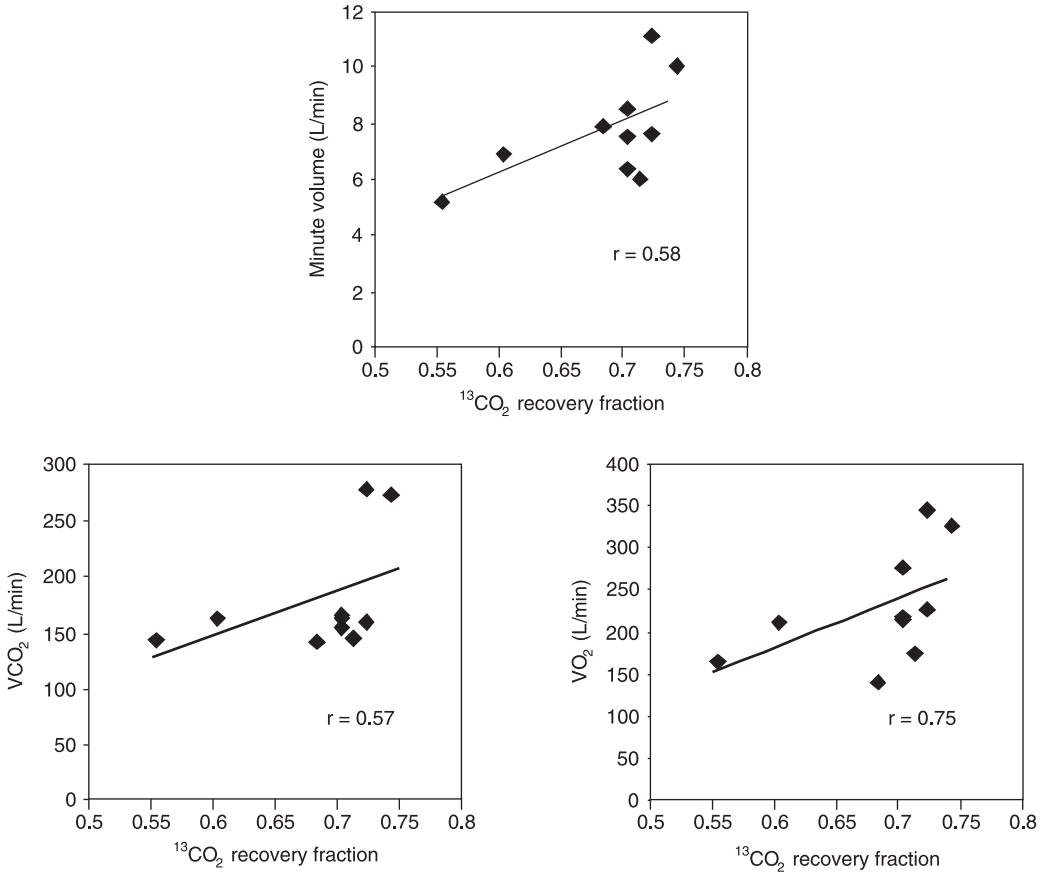

Figure 2. Correlation of the $^{13}CO_2$ recovery fraction in expired air and the minute volume, VCO_2 and VO_2 values for the 10 patients studied. Spearman correlation test was used for statistical analysis as described in Methods (P < 0.05).

not identify statistically significant differences (data not shown). Again, comparison of patient groups selected according to serum urea and bicarbonate levels did not reveal statistically significant differences (P = 0.99 and P = 0.25, respectively) (data not shown).

When groups were compared according to VCO_2 values, we observed that 2 patients had VCO_2 above normal (>200 mL/min) and 8 patients had VCO_2 <200 mL/min. When groups categorized according to VCO_2 values and APACHE II above and below the mean were compared, 7 patients were found to present APACHE II above the mean and 3 were found to present APACHE II below the mean. Among the 7 patients with APACHE II above the mean, 5 presented VCO_2 above the mean, and all 3 patients with APACHE II below the mean presented VCO_2 below the mean.

Regarding the route used for nutritional therapy, no significant difference was observed between the 3 patients receiving parenteral nutrition and the 7 patients receiving enteral nutrition (P = 0.5) (data not shown).

There was a positive correlation between recovery fraction and real energy expenditure (r = 0.62; Figure 1). There was also a positive, although weak, correlation between recovery fraction and minute volume (r = 0.58) as well as VCO_2 and VO_2 (r = 0.75, r = 0.57; Figure 2).

Discussion

In the present study, we evaluated for the first time the $^{13}CO_2$ recovery fraction in expired air from septic patients divided into groups according to severity. One of the initial objectives was also to correlate the $^{13}CO_2$ recovery fraction in expired air with the metabolic variable obtained by indirect calorimetry, i.e., VO_2, VCO_2, minute volume, and real energy expenditure.

The use of $^{13}CO_2$ permits the study of CO_2 metabolism and of its pathways of action. The technique is based on the principle of injection of a substance enriched with ^{13}C into blood circulation and evaluation of the recovery fraction of this substance in collected expired air. Furthermore, the $^{13}CO_2$ estimation is required for whole-body protein turnover, using the ^{13}C-leucine technique (13). Indeed, protein kinetics can be quantified from the rate at which labeled leucine is released from protein and the amount of leucine per gram protein. The leucine oxidation is calculated from the ratio of its first step metabolite α-ketoisocaproic acid (^{13}C-KIC measured in blood) and $^{13}CO_2$ production, corrected for the retention of $^{13}CO_2$ in the bicarbonate pool (fractional recovery). Moreover, several studies have assessed the $^{13}CO_2$ recovery fraction in experimental animals and in humans by infusing $NaH^{13}CO_2$, with results ranging from 0.51 to 0.95 (Table 2) (8,12,15-22).

Several studies have reported variation in the $^{13}CO_2$ recovery fraction in adults (17,20,22,23). In resting adults, this rate may range from 0.52 to 0.94 during fasting and from 0.72 to 0.90 during feeding. Several factors may explain the discrepancies detected in these studies, such as patient age, metabolic or nutritional status, the presence of underlying pathological conditions, and, especially, the conditions under which the experimental protocol was carried out.

The few studies available about critically ill patients under mechanical ventilation did not distinguish between specific diseases and this cannot be extrapolated to any one specific disease. According to Tissot et al. (12), $^{13}CO_2$ recovery fraction in expired air of stable critically ill patients under mechanical ventilation was 0.89 ± 0.26, suggesting that a percentage is retained in the organism, with possible utilization in other metabolic pathways that have not been fully clarified.

The principle behind the use of stable isotopes assumes that the element (carbon 13) will be recognized by the organism as the natural element which already exists, since the two elements share the same chemical properties. The isotope will have to reach equilibrium within preexisting metabolic pathways. The quantity of the isotope that enters the pool is equal to the amount that leaves the pool. In this case, the entry is represented by the administration of the substance and the exit by its oxidation. In order to reach stability more rapidly, in the present study a priming dose of the isotope that led to steady state within approximately 120 min was administered. Without this priming dose, this equilibrium would have been reached

Table 2. Measurement of $^{13}CO_2$ recovery fraction in expired air during $NaH^{13}CO_2$ infusion in different situations.

Patients	Nutritional therapy	Recovery fraction	Reference
Adults	Fasting	0.81	8
Adults	Fasting	0.51	15
Adults	Fasting	0.74	16
Adults	Postprandial and physical exercise	0.78 to 0.98	17
Child	Postprandial	0.57	18
Adults	Fasting	0.73	19
Adults	Feeding	0.82	19
Healthy	Fasting	0.52 to 0.95	20
Newborn	Postprandial	0.96	21
Critically ill	Postprandial	0.9	12
Trauma	Fasting and postprandial	1.0	22

within an interval as long as 24 h (8). The duration of the present study was 360 min in order to provide a safe and detailed evaluation of the kinetics of $^{13}CO_2$. When $NaH^{13}CO_2$ was administered intravenously and its recovery fraction in expired air was determined, we noted that the isotope was not fully recovered. Clinical studies evaluating the $^{13}CO_2$ recovery fraction in expired air have reported values ranging from 0.52 to 0.95. Values below 1 indicate that the injected isotope fraction is not fully eliminated as $^{13}CO_2$ in expired air. Three main destinations have been identified thus far for the retention of this isotope in the organism. First, $^{13}CO_2$ may be incorporated into the synthesis of urea (15). Second, $^{13}CO_2$ may be incorporated into intermediate metabolites such as oxaloacetate or malate (15,24). Finally, $^{13}CO_2$ may participate in an exchange in the system of bone bicarbonate, contributing to increase its concentration in the osseous pool, with a consequent reduction in plasma flow. An experimental study on rats (25) demonstrated that, after 120 min of infusion, 7 to 10% of $NaH^{14}CO_2$ is recovered in the skeleton. Considering the ^{13}C incorporation into urea, we evaluated seric urea levels and detected no significant difference in $^{13}CO_2$ recovery fraction in expired air between groups with high and normal urea levels.

Since $^{13}CO_2$ is diluted in a pre-existing bicarbonate pool, we can rule out the possible interference of the basal serum bicarbonate level with the $^{13}CO_2$ recovery fraction in expired air. If the basal bicarbonate value is elevated, ^{13}C will be diluted in a larger pool and therefore the chance of its elimination in expired air may be lower than in cases with lower bicarbonate levels. No significant difference was detected between patients with low and high bicarbonate levels.

Other factors may interfere with $^{13}CO_2$ retention in the organism, such as moderate and prolonged physical exercise, obesity (26), critical patient status (13), and fasting (8,16,17,20,21) or postprandial (18,22,23) condition.

CO_2 production should also be considered to be a factor that may interfere with the $^{13}CO_2$ recovery fraction in expired air. Since this variable may be affected by several factors, especially alveolar ventilation and oxidative stress, we evaluated selected patients in terms of their VCO_2 values. Assuming that $^{13}CO_2$ is eliminated by the lungs, the alveolar minute ventilation may interfere with its elimination and consequently affect the $^{13}CO_2$ recovery fraction in expired air. Alveolar minute ventilation probably did not interfere with VCO_2 values since the ventilatory pattern remained stable and physiological throughout the study protocol. On the other hand, the body weight factor, which determines the amount of bicarbonate infused and the individual metabolism, may alter the VCO_2 value. On this basis, a lower amount of $^{13}CO_2$ elimination would be expected by patients with higher VCO_2. However, 2 patients presented VCO_2 above normal values (>200 mL/min), while 7 patients presented VCO_2 below normal values and the $^{13}CO_2$ recovery fraction in expired air was similar for both groups.

In the present study, the $^{13}CO_2$ recovery fraction was 0.68 ± 0.06 in septic patients receiving nutritional therapy. There was no statistically significant difference between $^{13}CO_2$ recovery fraction in patients with sepsis of different severity, according to the APACHE II index and sepsis score. The same occurred when we evaluated different groups selected according to age and gender. The variation (0.68 ± 0.06%) among the patients was 8.8%, demonstrating that the result was homogeneous for the population studied. This result is important because the population of septic patients is intrinsically heterogeneous but, in this context, the heterogeneity did not interfere with the present measurements.

The factor that probably contributed most to the $^{13}CO_2$ recovery fraction in expired air was the metabolic rate. Wolfe (17) reported an increase in recovery fraction from 0.78 to 0.98 during intense and prolonged exercise. The same increase was detected by Hoerr et al. (20) in response to diet, with a positive correlation between VO_2, VCO_2, real energy expenditure and $^{13}CO_2$ recovery fraction in expired air in adults. However, Tissot et al. (12) detected a positive, although weak, correlation between the recovery fraction and VO_2 and real energy expenditure ($r = 0.55$ for both, with $P < 0.05$) and found no correlation between the recovery fraction and VCO_2. On this basis, care should be taken in interpreting these data because of the risk of mathematical coupling between parameters that share common variables (27,28). This risk is higher for the correlation between the recovery fraction (RF) and CO_2 production (VCO_2): [$RF = (VCO_2 \times {}^{13}CO_2\ Enr)/(F \times 0.99)$]. On the other hand, minute volume is a variable used to calculate both recovery fraction and VO_2. In the present study, there was a positive, although weak, correlation between minute volume and $^{13}CO_2$ recovery fraction in expired air ($r = 0.58$) and a better correlation between real energy expenditure and $^{13}CO_2$ recovery fraction in expired air ($r = 0.62$) and between VO_2 and $^{13}CO_2$ recovery fraction in expired air ($r = 0.75$). These findings suggest that the correlation between these variables (VO_2 and $^{13}CO_2$ recovery fraction in expired air) was not the result of simple mathematical coupling since the variable shared by the two parameters (minute volume) did not correlate with $^{13}CO_2$ recovery fraction in expired air. Since VO_2 is a variable related to survival (29), the $^{13}CO_2$ recovery fraction in expired air might play a similar role, a fact that was

not demonstrated in the present study, perhaps owing to the small number of patients evaluated. This line of reasoning is particularly relevant if we assume that a lower $^{13}CO_2$ recovery fraction in expired air may be related to greater severity, i.e., that a lower $^{13}CO_2$ recovery may mean that $^{13}CO_2$ is being shifted to alternative metabolic pathways that may be activated in order to resolve the injury in question.

However, the occurrence of incomplete $^{13}CO_2$ recovery in expired air has not been fully explained because there are no consistent data documenting in which organic compartment the isotope is lost. It is possible that a significant portion of the isotope is incorporated through alternative metabolic pathways that may function in different manners according to the pathological condition involved. Studies are needed to elucidate the values of the recovery fraction in different groups according to age, nutritional therapy and specific pathological conditions in order to better interpret the oxidation of substrates. On this basis, we can use an additional path, little explored until now, to alter the prognosis, to validate new treatment modalities and perhaps to improve the survival of patients with sepsis and septic shock.

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