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Use of [^{13}C]Bicarbonate for Metabolic Studies in Preterm Infants: Intragastric *versus* Intravenous Administration

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ABSTRACT

The metabolic fate of substrates in humans can be examined by the use of stable isotopes, one of which, [^{13}C]bicarbonate, may serve to estimate CO_2 production rate. In view of minimizing the burden of metabolic studies for preterm infants, the authors determined whether intragastric and intravenous infusions of [^{13}C]bicarbonate would achieve the same $^{13}CO_2$ enrichment in expired air during steady state. A second aim of this study was to determine the minimum time required to reach steady state during intragastric infusion. Ten preterm infants received a primed continuous [^{13}C]bicarbonate infusion intragastrically, followed by an intravenous infusion the next day. Breath samples were obtained every 30 min by the direct sampling method. $^{13}CO_2$ isotopic enrichment, expressed as atom percent excess, was measured by isotopic ratio mass spectrometry. Two-tailed *t* tests were used to detect statistically significant differences between the infusion routes. The isotopic enrichment at plateau did not differ between intragastric and intravenous infusion. A steady state of $^{13}CO_2$ enrichment was achieved after 60 min of intravenous infusion and after 120 min of intragastric infusion. In conclusion, intragastric infusion of [^{13}C]bicarbonate may serve to estimate the whole-body CO_2 production rate in preterm infants. To reach $^{13}CO_2$ steady state, a minimum of 120 min of bicarbonate administration is required. **(*Pediatr Res* 58: 861–864, 2005)**

Abbreviations

CI, confidence interval
IG, intragastric
IV, intravenous
AP, atom percent
APE, atom percent excess

The past two decades have seen the increased use of stable isotopes to study amino acid metabolism in humans. These isotopic tracer techniques have greatly enhanced our understanding of nutrient daily requirements and metabolism (1).

For determining the oxidation rates of specifically labeled substrates such as amino acids or glucose, we need to quantify substrate oxidation in each individual by measuring the $^{13}CO_2$ production rate during IV infusion of labeled bicarbonate (2). The production of $^{13}CO_2$ is made up of total CO_2 production rate and $^{13}CO_2$ enrichment in expired breath. Although total CO_2 production rate is traditionally assessed by indirect calorimetry, $^{13}CO_2$ enrichment is measured by isotopic ratio mass spectrometry. A certain amount of CO_2, and thus $^{13}CO_2$ as well, is retained in the body. Because this amount is related to caloric intake, a correction factor is necessary to calculate substrate oxidation rates (3). A method that makes correction factors and indirect calorimetry superfluous is the infusion of $NaH^{13}CO_3$ before the labeled substrate infusion (4).

Kien *et al.* (5) compared IG infusion of [^{13}C]bicarbonate with indirect calorimetry by the use of a correction factor. This study showed the validation of the use of dilution stable tracer technique to estimate CO_2 production. However, those authors did not compare the $^{13}CO_2$ enrichment during IV infusion with IG infusion of [^{13}C]bicarbonate.

The general purpose of this study was to determine whether in preterm infants IG infusion of $NaH^{13}CO_3$ yields the same enrichment as IV infusion at steady state. To this aim, we compared $^{13}CO_2$ enrichment in expired breath during IG and IV infusion of labeled bicarbonate at plateau. In addition, we quantified the minimal tracer infusion time required to establish steady state during IG infusion.

We hypothesized that $^{13}CO_2$ enrichment at steady state would not differ between IG administration and IV administration of [^{13}C]bicarbonate.

METHODS

Subjects. We studied 10 preterm infants (8 male, 2 female) admitted to the Neonatal Intensive Care Unit of the Erasmus MC–Sophia Children's Hospital, Rotterdam, The Netherlands. Their mean gestational age was 27 wks (range

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26–30 wks, SD ± 1.3 wks), and they were free of gastrointestinal diseases and were clinically stable during the 2-day study. Five of them needed artificial ventilation, and five breathed spontaneously with O_2 supplementation by nasal prong ($n = 5$). Eight infants tolerated full enteral feeding, and two infants received partial enteral and partial parenteral feeding. For all neonates, the feeding regimen was the same on both study days. All infants were fed through a nasogastric feeding tube because this is a standard procedure in our unit. The study protocol was approved by the Erasmus MC Institutional Review Board, and written and informed consent was obtained from both parents of all neonates.

Tracer protocol. For the purpose of validating this route of labeled sodium bicarbonate the study was designed as a randomized, crossover study. The 10 infants received a primed (10 μmol/kg/min) continuous (10 μmol/(kg·h) infusion of [^{13}C]bicarbonate (sterile pyrogen free, 99% APE; Cambridge Isotopes, Woburn, MA). The study was set up as a true crossover design: in five infants the IV infusion was started for 6 hours on the first day, followed by the IG infusion on the second day. The other five infants received the IG infusion the first day and the IV infusion the second day. One hour before the start of the study, the usual hourly feeding regimen was changed to continuous drip feeding. Enterally infused tracer was mixed with the milk (either fortified or nonfortified breast milk, or preterm infant formula; Nenatal, Nutricia Nederland B.V., Zoetermeer, The Netherlands) and infused continuously *via* the nasogastric tube.

Breath samples were obtained by use of the direct sampling method described by van der Schoor *et al.* (6). Briefly, in mechanically ventilated neonates, a syringe was connected to the ventilator tubing, and breath was taken slowly during expiration with a total volume of 15 mL. When infants were breathing spontaneously, a 6F gastric tube (6 Ch Argyle; Cherwood Medical, Tullamore, Ireland) was placed 1 to 1.5 cm into the nasopharynx, and end-tidal breath was taken slowly with a syringe connected at the end. Collected air was transferred into-10 mL sterile, non–silicon-coated evacuated glass tubes (Van Loenen Instruments, Zaandam, The Netherlands) and stored at room temperature until analysis.

Baseline samples were obtained 15 and 5 min before tracer infusion was started. During the experiment, duplicate ^{13}C-enriched breath samples were collected every 30 min and every 15 min during the last 45 min of tracer infusion.

Analytic methods. $^{13}CO_2$ isotopic enrichment in expired air was measured by isotope ratio mass spectrometry (ABCA; Europe Scientific, Van Loenen Instruments, Leiden, The Netherlands) and expressed as APE above baseline. The APE was plotted relative to time. Steady state was defined as three or more consecutive points with a slope not different from zero. Estimated body CO_2 production (mmol/kg/h) was calculated for each infant with the following equation (7):

Estimated body CO_2 production = IE infusate * tracer infusion rate * 1000 IE breath bicarbonate

where IE infusate is the ^{13}C enrichment of the tracer (APE), IE breath bicarbonate is the ^{13}C enrichment in the expired air (APE), and tracer infusion rate is the rate of [^{13}C]bicarbonate infusion (μmol/kg/h).

Statistical analysis. Descriptive data are expressed as mean ± SD. To define the slope of the curve of the two different methods, a repeated measurements linear model was used. Steady state was achieved when the linear factor of the slope was found to be not significantly different from zero ($p > 0.05$) (8). Whole-body CO_2 production and baseline enrichments between the two methods were analyzed by paired *t* tests.

Differences in steady state between IG and IV administration were also analyzed by paired *t* tests. Statistical significance was defined as $p < 0.05$. Pitman's test (9) was used to test the null hypothesis if the variance of two-paired measurements (IG and IV infusion) were the same. To detect significant differences between the two-paired measurements, a paired *t* test could be performed. Pearson's correlation coefficient was performed to show correlation between IG and IV. The analysis of Bland and Altman (10) was performed to show accuracy between the two different infusions. All statistical analyses were performed by the use of SPSS version 11.0 (SPSS, Chicago, IL, USA).

RESULTS

The clinical characteristics of the infants are given in Table 1. The mean study weight of the infants was 1.18 ± 0.32 kg. The postnatal age at the start of the study was 28 ± 20 d. Their energy intakes did not differ between both study days ($p = 0.75$). The mean ^{13}C enrichments, expressed as AP, in breath CO_2 from time point t = 60 to t = 360 min are shown in Fig. 1. All neonates achieved isotopic steady state in both administration routes. Baseline enrichments did not differ between IG and IV infusion (1.0875 AP ± 0.0022 *versus* 1.0869 AP ± 0.0338, $p = 0.29$).

The mean APE at plateau ($t_{120-360}$) during IG infusion was 0.0365 ± 0.0055; during IV infusion it was 0.0371 ± 0.0067. IG enrichment was slightly lower, though not significantly, than IV enrichment ($p = 0.59$).

The Pitman's tests (9) showed no significant difference between variance in IV and IG infusion ($p = 0.308$), and the Pearson's correlation coefficient was 0.359. Agreement between the two different routes of administration was determined by the analysis of Bland and Altman (10). Figure 2 shows on the *x* axis the average of the IV plateau and the IG plateau ($n = 10$), whereas the *y* axis shows the difference between the two measurements ($n = 10$). The mean difference is 0.0006 APE. Note that all measurements lie between the range of the mean difference +2 SD (0.0076 APE) and the mean difference −2 SD (−0.0064 APE). The 95% CI of the mean difference is −0.0019 to 0.0031 APE. Therefore, from 120 min onward, there was no statistically significant difference in CO_2 enrichment in expired air between IV or IG infusion, nor did we find a sequence effect (no significant difference in $^{13}CO_2$ between infants who received $NaH^{13}CO_3$

Table 1. *Clinical characteristics of 10 studied infants*

	Sex	Gestational age (wk)	Birth weight (kg)	CRIB score	Respiratory support	Feeding regimen e/p	Energy intake kcal/kg/d IV	Energy intake kcal/kg/day IG
1	M	26	0.97	–	np	e	154	153
2	M	26	0.70	13	np	e	128	118
3	F	27	1.13	2	v	e	102	104
4	M	26	0.79	4	v	e	121	123
5	M	27	1.05	2	v	e	98	98
6	F	29	0.81	4	np	e	131	134
7	M	27	1.18	1	np	e	134	131
8	M	27	0.67	9	v	e	109	109
9	M	30	1.17	1	np	e+p	112	112
10	M	27	0.88	4	v	e+p	95	98
Mean		27	0.93	4			118	118
± SD		1	0.19	4			19	18

Key: e = enteral intake; p = parenteral intake; v = ventilation; np = nasal prong.

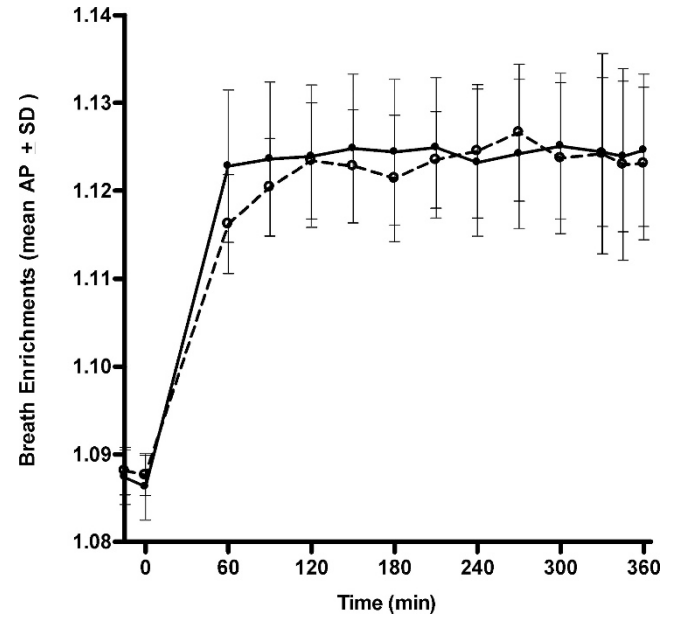

Figure 1. Breath $^{13}CO_2$ enrichments of 10 infants, expressed as mean AP ± SD. IV (●) *vs* IG (°) infusion of [^{13}C]-bicarbonate. At plateau (t_{120}–t_{360}) ^{13}C enrichment IV is not significantly different from IG ($p = 0.59$).

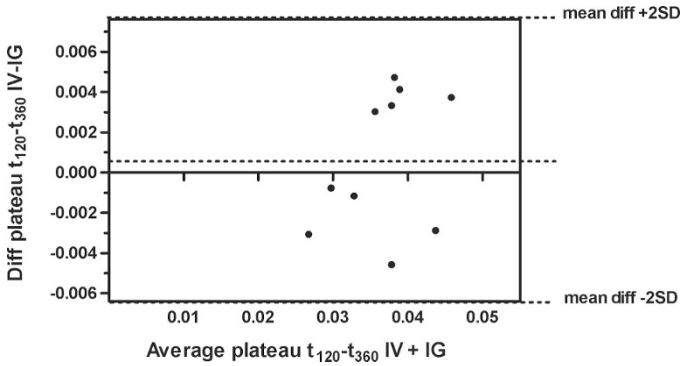

Figure 2. Bland-Altman analysis showing the difference between IG and IV enrichments of $^{13}CO_2$ in breath of 10 infants. The mean difference is 0.0006 APE (dotted line). All measurements are within two standard deviations: + 2 SD (0.0076 APE) and −2 SD (−0.0064 APE). The 95% CI of the mean difference is −0.0019 to 0.0031 APE.

IV the first day or those who received $NaH^{13}CO_3$ IG the first day).

The estimated CO_2 production did not differ between the IG (27.68 ± 5.38 mmol/kg/h) and IV (27.67 ± 5.64 mmol/kg/h) infusions ($p = 0.99$).

Steady state was achieved from 60 min onward when the tracer was infused IV and from 120 min onward when it was infused IG.

DISCUSSION

The main purpose of this study was to validate the use of IG administration of [^{13}C]bicarbonate compared with IV administration for metabolic oxidation studies in preterm infants. Clinical studies in addition to experimental research are of great value in elucidating metabolism and nutrition in preterm infants. Information about amino acid metabolism and protein synthesis and oxidation is needed to provide these infants with optimal nutrition and consequently improved growth and survival.

A principal goal of many tracer kinetic experiments is to determine the oxidation rate of the tracer substance by the appearance in breath of labeled C originating from the tracer (11). The gold standard for determining whole-body CO_2 production is indirect calorimetry (3). An alternative method is a primed continuous IV infusion of $NaH^{13}CO_3$. We found the estimated body CO_2 production (27.67 + 5.64 mmol/kg/h) to be similar to that previously described (0.725 ± 0.021 mol/kg/day) (12). Also, others have shown that $NaH^{13}CO_3$ can be adequately used as a method of determining CO_2 production rate (4,5,13). The infusion of labeled bicarbonate before a ^{13}C-labeled substrate carries the advantage that no correction factor is needed to calculate substrate oxidation. In addition, IG infusion of the tracer reduces the invasiveness of metabolic studies. Finally, in studying the metabolic fate of an enteral substrate, it is preferable to administer the tracer enterally as well.

Hoerr *et al.* (11) studied in adults the effects of IG and IV infusion of labeled bicarbonate on recovery of ^{13}C in breath and concluded that administration route did not affect recovery. When it is considered that placing an IV catheter in preterm infants is highly invasive, it is very important to search for methods minimizing discomfort.

To achieve steady state during IG administration, tracer infusion should last at least 120 min. Sample collection is accomplished during steady state. Consequently, breath samples should be obtained from 120 min onward. To prevent intrasubject variation, at least four breath samples should be obtained at 10-min intervals, thus between 120 and 160 min of infusion.

We need to emphasize the small sample size of this study. However, we presented a 95% CI (−0.0019 to 0.0031 APE) of the mean difference to obtain an impression of a type II error. We considered a difference of <10% between IG plateau and IV plateau to be acceptable. We calculated the difference of the minimal (−5%) and maximal (8%) of the 95% CI limit of the average plateau of IG and IV (0.0368 APE). As we assumed, the plateau of IV and IG infusion can vary from −5% to 8% in the general population.

Additionally, we wish to stress that in metabolic studies in parenterally fed infants, [^{13}C]bicarbonate should preferably be administrated IV.

In conclusion, our findings are consistent with the absence of significant differences in $^{13}CO_2$ enrichment between IG and IV infusion after 120 min of infusion, and therefore it would be valid to infuse [^{13}C]bicarbonate IG for the determination of whole-body CO_2 production rate in preterm infants.

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