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Novel intravenous ^{13}C -methionine breath test as a measure of liver function in children with short bowel syndrome

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Abstract

Monitoring hepatic function in children with short bowel syndrome (SBS) and parenteral nutrition-associated liver disease (PNALD) is currently limited to conventional blood testing or liver biopsy. Metabolism of the stable isotope L[1- ^{13}C]methionine occurs exclusively in liver mitochondria and can be noninvasively quantified in expired breath samples. We hypothesized that the ^{13}C -methionine breath test (^{13}C -MBT) could be a safe, noninvasive, and valid measure of hepatic mitochondrial function in children with SBS and PNALD.

Methods—Baseline breath samples were collected in 8 children with SBS before intravenous administration of 2 mg/kg of L[1- ^{13}C]methionine. Six paired breath samples were obtained at 20-minute intervals. The $^{13}\text{CO}_2$ enrichment was analyzed using isotope ratio mass spectrometry.

Results—All 8 patients (5 males; mean age, 8.9 months) tolerated the ^{13}C -MBT without adverse events. Two patients underwent serial testing. One patient, tested before and after resolution of cholestasis, demonstrated increased cumulative percentage dose (4.7% to 6.6%) and area under the curve (AUC) (270–303). A second patient with progressive PNALD demonstrated decreased cumulative percentage dose (from 7.8% to 5.9%) and AUC (from 335 to 288).

Conclusion—The ^{13}C -MBT is a feasible, safe, and potentially clinically relevant measure of hepatic mitochondrial function in children with SBS and PNALD.

Keywords

^{13}C -methionine; Stable isotope; Breath test; Short bowel syndrome; Parenteral nutrition-associated liver disease; Liver function

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Discussion Dr. David Sigalet, MD (Calgary, Alberta): You said that this relates to mitochondrial function. Do you have any background laboratory data to link mitochondrial dysfunction with synthetic function in liver because that is what we are mostly interested in?

Dr. Duro (response): There are data published in rats by Katayama et al linking hepatic mitochondrial dysfunction to PN. More importantly, there are additional data that the deterioration of hepatic mitochondrial function leads to the end point of hepatic failure.

Short bowel syndrome (SBS) is a malabsorptive state occurring as a result of surgical resection or congenital disease of a significant portion of the small intestine [1]. The mortality rate of the condition is quite high [2], with reported survival rates in pediatric SBS ranging from 73% to 89%, making pediatric SBS one of the most lethal conditions in infancy and childhood [3–6]. Parenteral nutrition (PN) has become widely accepted as the primary supportive therapy in infants with SBS, and mortality because of dehydration and malnutrition has been essentially eliminated [7]. This lifesaving therapy, however, has been plagued by the recognition that infants with prolonged dependence on PN commonly develop progressive and severe hepatic dysfunction. Our own data confirmed a 90% mortality rate in infants with SBS and cholestatic liver disease who were unable to wean from PN [8].

The diagnosis of PN-associated liver disease (PNALD) has historically been performed with routine biochemical tests of hepatic function, including hepatic transaminases, conjugated bilirubin, albumin, and prothrombin time. The “gold standard” test remains liver histopathologic examination, but the young age, small size, and precarious medical status of many infants with SBS and suspected PNALD make routine liver biopsy difficult to perform. Because PNALD has been associated with a poor prognosis among SBS patients, early identification of infants with progressive disease is needed and should allow earlier treatment and potentially avoid the need for liver or liver-intestine transplantation.

Hepatic mitochondrial dysfunction is a consequence of a wide range of liver pathologic condition and is a useful marker of overall liver function. In cases of liver cirrhosis, fatty liver, or injury from xenobiotics, oxidative metabolism of various substrates is impaired because of the dysfunction of the electron transport chain in hepatic mitochondria [9,10]. A study designed to evaluate mitochondrial function in immature rats suggested that PN use induced marked metabolic changes to the mitochondria leading to deficiencies in mitochondrial phosphorylation and respiratory chain function compared to a control group [11].

Because the amino acid methionine is oxidized by the liver mitochondria, $^{13}\text{CO}_2$ production measured after the intravenous injection of a known quantity of L-[1- ^{13}C]methionine may be used to assess liver function. The purpose of this study was to establish a reliable and valid stable isotope breath test to serially monitor the progression or resolution of PNALD in young SBS patients.

1. Material and methods

After institutional review board approval and written informed consent was obtained, 8 patients observed by the Center for Advanced Intestinal Rehabilitation at the Children's Hospital Boston (Mass) were enrolled. The inclusion criteria were (1) a diagnosis of SBS (defined as dependence on PN for ≥ 90 days), (2) corrected gestational age at 36 weeks or more, and (3) the provision of PN to fulfill part or all of their energy and protein requirements. The diagnosis of PNALD was made by (1) serum conjugated bilirubin at 2mg/dL or more or (2) liver histopathologic examination showing moderate to marked fibrosis in the setting of exposure to PN for at least more than 30 days. Patients were excluded if they met any of the following criteria: (1) receipt of parenteral antimicrobials and/or vasopressors, (2) inadequate nutrition support (< 80 kcal/kg per day), (3) receipt of general anesthesia within 48 hours, (4) mechanical ventilation, or (5) any inborn errors of metabolism that affected the methionine metabolic pathways. Direct and total bilirubin tests, pertinent clinical data, and liver biopsy results were obtained from the medical chart.

2. ^{13}C -methionine breath test

Pyrogen-free and sterilized L-[1- ^{13}C]methionine powder was purchased from Cambridge Isotope Laboratories Inc (Andover, Mass). The compound was made into a sterilized solution by the research pharmacist in the Department of Pharmacy, Children's Hospital, Boston. The sterility and pyrogenicity were tested again after purchase.

The procedures were conducted on patients after 2 hours of complete fasting. Patients received intravenous infusion of 5% dextrose with normal saline throughout the study period. An expired air sample was collected for the measurement of baseline $^{13}\text{CO}_2$ enrichment. This procedure was accomplished by slowly withdrawing the expired air into a 30-mL syringe with its tip placed close to the patient's nostril [12]. The samples were collected in duplicate every 20 minutes for accuracy. This was followed by the intravenous injection of L-[1- ^{13}C]-methionine (2 mg.kg $^{-1}$). Six additional breath samples were obtained at 20-minute intervals up to 120 minutes after the ^{13}C -methionine injection. During the study, indirect calorimetry (V_{max} legacy, Viasys Healthcare Inc, Conshohocken, Pa) measurements were conducted during the last 0.5 hour to assess total CO_2 production rate. Breath samples were kept at room temperature and analyzed for $^{13}\text{CO}_2$ enrichment within 72 hours of collection using gas isotope ratio mass spectrometry (Finnigan TraceMat, Thermo Electron Corporation, Waltham, Mass). The $^{13}\text{CO}_2$ enrichment is reported as atom percentage excess above the baseline.

3. Analytical procedures

The ratio of the accumulated total $^{13}\text{CO}_2$ production during 120 minutes vs the amount of L-[1- ^{13}C]methionine injected was used as an indicator of hepatic function during the study. The accumulated total $^{13}\text{CO}_2$ production [$V^{13}\text{CO}_2$ (time)], calculated based on the rate of $^{13}\text{CO}_2$ production at each time-point of air sample collection, was calculated as follows: $V^{13}\text{CO}_2 = V_{\text{CO}_2} \times \text{VE}^{13}\text{CO}_2$ (time), where V_{CO_2} is the total CO_2 production rate (in micromole per kilogram per minute) measured by indirect calorimetry, and $\text{VE}^{13}\text{CO}_2$ is the isotopic enrichment, above baseline, of $^{13}\text{CO}_2$ obtained from air samples collected at each time-point = {0, 20, 40, 60, 80, 100, 120} minutes. The accumulated percentage $^{13}\text{CO}_2$ recovery from the injected dose of L-[1- ^{13}C]methionine at each time-point was calculated as

$$\sum_{\text{time}=0}^T \frac{V^{13}\text{CO}_2(\text{time})}{L - [1-^{13}\text{C}]}, T = \{20, 40, 60, 80, 100, 120\},$$

where L-[1- ^{13}C]MET is the injected dose of L-[1- ^{13}C]methionine in micromole per kilogram. Both accumulated percentage $^{13}\text{CO}_2$ recovery and $^{13}\text{CO}_2$ enrichment at different time-point were plotted as a function of time after the injection of L-[1- ^{13}C]methionine up to 120 minutes.

The mean, SD, and 95% confidence intervals based on standard errors were estimated for all patients at each 20 minutes from 0 to 120 minutes. As a summary of each patient's entire $^{13}\text{CO}_2$ cumulative percentage recovery, we used the final $^{13}\text{CO}_2$ cumulative percentage recovery obtained at 120 minutes. As a summary of the patient's $^{13}\text{CO}_2$ percentage difference from baseline curve, we estimated the area under the curve (AUC). The AUC of each patient was obtained using the Lagrange method [13]. Reliability of these summary values was assessed based on the coefficient of variation

$$\left(100 \times \frac{\text{Standard Deviation}}{\text{Mean}} \right).$$

All analyses were performed using S-plus 8.0 software (Insightful, Seattle, Wash).

4. Results

A total of 8 infants, 5 males, with median age of 5.0 months (interquartile range = 4.3, 3.8 months), with SBS were studied (Table 1). Seven patients had PNALD at the time of the study, demonstrated by elevated direct bilirubin, abnormal liver biopsy result, or both. The full ^{13}C cumulative percentage recovery was approximately linear for all patients with all time values and, for 6 patients, lay within confidence limits (the curve for patient 7 was above the limits, and the curve for patient 8 was below the limits) (Fig. 1A). Each line in Fig. 1A represents a single subject and the ^{13}C percentage of administered isotope recovered at each 20-minute interval. The final value reflects what percentage of the total administered dose of ^{13}C was recovered at 120 minutes. The mean (SD) final value of the $^{13}\text{CO}_2$ cumulative percentage recovery was 7.2% (4.4%). Another way of expressing the isotopic data is using the difference from baseline enrichment (DOB) demonstrated by (Fig. 1B). The y-axis of Fig. 1B shows the difference between the ^{13}C enrichment in expired CO_2 minus the enrichment at baseline at each 20 minute time-point. The shape of the full curve for $^{13}\text{CO}_2$ difference from baseline varied according to the patient with peak values ranging from 1.4 to 7.1 (Fig. 1B). The peak was achieved between 20 and 120 minutes after administration of ^{13}C -methionine with a mean (SD) of 70 (30) minutes. The mean (SD) of the AUCs of $^{13}\text{CO}_2$ difference from baseline (AUC) was 391 (192), ranging from 108 to 688.

Two patients underwent repeat studies after clinically suspected changes in liver function (Fig. 2). One patient was first tested while cholestatic (direct bilirubin = 3.7 mg/dL) and retested when cholestasis had resolved (direct bilirubin = 0.1 mg/dL). The ^{13}C difference from baseline agreed with the observed clinical improvement of this patient. Final cumulative percentage recovery increased from 4.7% to 6.6% and AUC from 270 to 303. In a second patient with suspected worsening liver function (direct bilirubin > 5 mg/dL at 2 time-points), final cumulative percentage recovery decreased from 7.8% to 5.9% and AUC from 335 to 288.

5. Discussion

Parenteral nutritional-associated liver disease is a common metabolic complication seen in children on long-term nutritional support for SBS. Development of progressive cirrhosis and liver failure in these patients is an indication for transplantation. As such, it is vital that clinicians be able to follow a reliable indicator of hepatic function in patients receiving PN.

Our results demonstrate that the ^{13}C -MBT is well tolerated in this pediatric population. No adverse events were reported in the 10 tests performed on 8 patients. This confirms the existing literature on the safe use of stable isotopes in a clinical setting, specifically in neonatal and pediatric populations [14,15].

The noninvasive nature of this test is a further attribute making it particularly attractive in a pediatric practice. Given that serial testing is often required to follow a patient's hepatic function, the avoidance of repeated liver biopsies, is a distinct advantage. Although intravenous access must be used for the administration of the L-[1- ^{13}C]methionine in SBS patients, the breath test itself is entirely noninvasive.

Finally, preliminary results in the 2 patients undergoing serial testing indicate that the ^{13}C -MBT promises to be a clinically relevant measure of hepatic function. The 2 measured outcomes, cumulative percentage dose and AUC, appeared to correlate with the changing hepatic function as assessed clinically and biochemically in these patients.

Several studies have used the oral administration of ^{13}C -MBT to quantify liver function in adult patients. Spahr et al [16] used the ^{13}C -MBT to monitor mitochondrial function changes in a patient with biopsy-proven, acute, valproate-associated microvesicular steatosis. Abnormal breath test improved together with the recovery of liver function, reaching the lower range of values obtained in controls 6 weeks after the acute injury. In another study, Spahr and colleagues [17] performed ^{13}C -MBT on a series of patients with severe liver steatosis before bariatric surgery for morbid obesity. They demonstrated that the ^{13}C -MBT values were significantly reduced as compared to healthy subjects. Additional oral studies with ^{13}C -MBT have been done in liver transplantation patients. Di Campli et al [18] showed that the percentage of expired $^{13}\text{CO}_2$ progressively increased in patients with successful transplantation, reaching control values in the early postoperative period. In contrast, levels remained low in patients who went on to develop primary organ nonfunction. In aggregate, these studies suggest the ^{13}C -MBT may provide noninvasive monitoring of hepatic mitochondrial function in vivo.

In this study, we demonstrated the safety and feasibility of using a novel intravenous ^{13}C -MBT to quantify hepatic mitochondrial activity in children with SBS and PNALD. Although further testing is needed, the intravenous ^{13}C -MBT is potentially a clinically relevant means of measuring hepatic function in these patients who may have significant hepatic dysfunction and unreliable uptake of oral tracer.

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Fig. 1. $^{13}\text{CO}_2$ cumulative percentage recovery (A) and $^{13}\text{CO}_2$ percentage difference from baseline (B) as a function of time from administration of the ^{13}C -methionine. Solid lines and circles represent the mean and 95% confidence interval of all subjects, respectively, and dashed lines represent the curves of each subject.

Fig. 2. Serial measurements of ^{13}C -MBT in 2 patients. Solid line represents the final $^{13}\text{CO}_2$ cumulative percentage recovery (at 120 minutes), and dashed line represents the AUC of the $^{13}\text{CO}_2$ difference from baseline.

Table 1

Demographic and clinical characteristics at the time of the ^{13}C -MBT^a

Patient	Gestational Age (wk)	Age (mo)	Corrected gestational age (mo)	Diagnosis	Total bilirubin (mg/dL)	Direct bilirubin (mg/dL)	Time on PN (mo)	Liver Bx
1	36	15.2	13.9	AV malformation, bowel ischemia	20.7	12.4	9	Cholestasis
2	29	5.2	2.5	NEC	5.2	3.7	5	Fibrosis
3	36	4.8	3.7	Jejunal atresia	0.3	0.1	4	Fibrosis
4	26	4.1	0.9	NEC	12.2	7.8	4	Early cirrhosis
5	40	34.4	34.4	Intestinal ischemia because of MC	0.2	0.1	3	No Bx
6	25	5.7	2.0	IA	3.2	2.2	6	Fibrosis
7	37	4.3	3.5	Gastroschisis	14.1	9.8	4	No Bx
8	39	1.5	1.3	Jejunal atresia	7.7	5.8	2	No Bx

AV indicates arteriovenous malformation; NEC, necrotizing enterocolitis; MC, mesenteric cyst; IA, intestinal aganglionosis.

^aPatients 1 and 2 underwent 2 tests. Here we present their description at the time of the first test.