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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 $^{13}CO_2$ 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.

Short bowel syndrome (SBS) is a malabsorptive state occuring 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

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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, $^{13}CO_2$ production measured after the intravenous injection of a known quantitiy 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 $^{13}CO_2$ 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 acurracy. 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 $^{13}CO_2$ enrichment within 72 hours of collection using gas isotope ratio mass spectrometry (Finnigan TraceMat, Thermo Electron Corporation, Waltham, Mass). The $^{13}CO_2$ enrichment is reported as atom percentage excess above the baseline.

3. Analytical procedures

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