Stable Isotope Studies of Normal and Abnormal Digestion and Absorption in Infants

Buford L. Nichols, Robert J. Shulman, Carlos H. Lifschitz, and Peter D. Klein

USDA/ARS Children's Nutrition Research Center, Section of Nutrition and Gastroenterology, Department of Pediatrics, Baylor College of Medicine, Texas Children's Hospital, Houston, Texas 77030

This chapter describes the use of stable isotopes for the investigation of normal and abnormal digestion and absorption in infants. Isotopes are atoms with equal numbers of protons but different numbers of neutrons. The word isotope is used because these various atoms appear at the same (iso) place (topos) in the periodic table. As an example, 99% of carbon in nature is $^{12}$C. Only 1% is the stable isotope $^{13}$C, which has a heavier mass because of the addition of one neutron in the nucleus of the atom. Radioactive isotopes such as $^{14}$C are not stable, and their instability is associated with the generation of radioactive particles.

Stable isotopes have several advantages for use in human investigation: they are nonradioactive, they can be measured with extreme precision, and, in many cases, noninvasive sample collection techniques are available. The measurement of the amount or abundance of $^{13}$C in organic materials can be reported in several ways. The classic geochemical notation is given as per mil (‰) difference compared to a standard. A simpler notation sometimes is used, parts per million (ppm) $^{13}$C/$^{12}$C.

A mass spectrometer is used to measure the relative abundance of $^{13}$C to $^{12}$C. Molecules of interest are ionized and enter a magnetic field. As the ions pass through the magnetic field, those that have greater weight will have a larger radius, and this results in the displacement of the heavier mass from the lighter mass at the point of ion detection. This technique is most easily understood by a description of the isotope ratio mass spectrometer. When this instrument is used, the sample and a reference standard are introduced alternately into the ion source. The ions generated are passed through a magnetic sector and are detected in dual Faraday cups. The relative concentration of the two masses of the ion then is processed electronically and appears as analytical information.

Breath samples, which may be collected by noninvasive means from a pediatric patient by a research nurse, are used for the determination of the relative ratios of $^{13}$C and $^{12}$C in expired air. The breath samples are then analyzed by a gas isotope ratio mass spectrometer. A series of automated devices extract the carbon
dioxide from the samples in the collector and introduce the purified carbon as CO₂ gas into the ion source of the spectrometer. The magnetic sector segregates the carbon dioxide molecules of different mass. Computer instrumentation controls the equipment and data reduction.

The stable isotope methodology most often applied for the measurement of digestion and absorption of nutrients utilizes substances highly enriched with stable isotopes. The glucose that has been used for such experiments has 95 atom percent excess ¹³C; that is, 95% of the carbon present is in the form of ¹³C. The lactose utilized has 42.5 atom percent excess of ¹³C. Two methods were used to study the utilization of these substrates. In the first, the percent dose oxidized to breath CO₂ was determined by measuring total CO₂ production and the change in the ¹³C abundance of the CO₂ with time. The second approach involved the measurement of the percent of the dose administered that was excreted in the stool. In this test, the isotope ratio of carbon in the stool was determined and expressed as change in ¹³C abundance after ingestion of enriched glucose. The results of this type of investigation, carried out in normal infants by MacLean et al. (1), revealed that 32.6% of orally administered lactose label was recovered in breath CO₂. Under the same experimental circumstances, 35.5% of an oral dose of ¹³C glucose was collected as breath ¹³CO₂. In normal infants, approximately 1.9% of the lactose and less than 1% of the glucose carbon appeared in the stool. These results suggest comparable digestion and utilization of these two forms of carbohydrate.

The use of highly enriched pure substrates has allowed the development of experiments for the study of absorption and oxidation of specific nutrients. These experiments, however, do not give information about the digestion and utilization of more complex or polymeric dietary components. In order to meet this objective, a methodology was chosen that utilizes naturally occurring differences in ¹³C enrichment of nutrients from various food sources (2). The difference in ¹³C content results from differential discrimination of certain enzyme pathways in plant and animal tissues against the ¹³C normally present in the atmosphere. For instance, corn syrup solids, commonly used as a carbohydrate in infant formulas, have a ¹³C content of 10,983 ppm. Beet sucrose, which is from another branch of the plant kingdom, has a ¹³C abundance of 10,849 ppm. This means that there is a difference of 134 ppm between chemically identical carbohydrates from the two sources. Similar differences can be observed in lipids obtained from corn or soy; i.e., an average difference of 159 ppm can be observed when the ¹³C content of soy oil is compared to that of corn oil.

Exploiting this naturally occurring difference in the ¹³C abundance of foods, our group developed an experiment to demonstrate directly the utilization of dietary carbohydrates including glucose, glucose polymers (corn syrup solids), and corn cereal when they were introduced into the infant's diet. In addition, the role of colonic fermentation in the utilization of complex dietary carbohydrates in young infants was examined. In the past, the ability of the young infant to utilize cereal has been demonstrated only by the absence of ingested starch in the stool (3,4).
In the protocol for this investigation, 16 1-month-old infants were studied who had produced hydrogen after a test load of lactulose, a nonabsorbable carbohydrate. The infants were placed on a formula that contained soy protein, soy oil, and beet sucrose, substances naturally low in $^{13}$C. On the test days, the sucrose was replaced by corn glucose, corn glucose polymers, or corn starch cereal, substances naturally enriched in $^{13}$C. All substances were administered as a single feeding in formula. The test dose was 1 g/kg body weight. Breath samples were collected for measurement of $^{13}$C abundance in CO$_2$ by gas isotope ratio mass spectrometry and for H$_2$ and CO$_2$ concentration by gas chromatography. Although the sequence of test doses for the 16 infants was randomized, the generalized protocol was: day 1, lactulose administration; day 4, glucose administration; day 7, corn starch; and day 10, glucose polymers. The results of these studies are shown in Figs. 1 and 2.

When the soy formula that contained beet sucrose was fed, the level of $^{13}$CO$_2$ in breath remained constant. There was no rise following individual feedings. When carbohydrates from corn sources were substituted for the beet sucrose, there was a prompt rise in the level of breath $^{13}$CO$_2$ abundance following ingestion of glucose, Polycose®, or corn cereal. In this case, the rise of $^{13}$C abundance in the breath CO$_2$ indicated digestion, absorption, and oxidation of the $^{13}$C in the diet. These results were reproducible in each of the infants investigated. The total quantity of the dietary carbohydrate that was oxidized to breath CO$_2$ over

---

**FIG. 1.** Response of breath CO$_2$ isotope ratios in infant #1 to the feeding of depleted beet sugar and Polycose® (corn syrup solids) and corn cereal. The results are expressed as the change relative to PDB limestone standard concentration of $^{13}$C/$^{12}$C. Following the feeding of $^{13}$C-enriched glucose, Polycose®, or corn cereal, the ratio of $^{13}$C/$^{12}$C increased, indicating substrate oxidation.
FIG. 2. Response of breath CO₂ isotope ratios in infant #2. Feeding regimen is described in Fig. 1.

6 hr was 34.9% for glucose, 30.4% for glucose polymers, and 34.2% for the corn cereal. These results were not significantly different from each other and prove that the energy derived from the part of the corn cereals that was digested was utilized as efficiently as that from glucose polymers or glucose alone.

It is well known that pancreatic amylase activity is low in infants at 1 month of age (5,6). To determine whether colonic flora plays a role in the utilization of complex carbohydrates, breath hydrogen was measured as illustrated in Fig. 3. Lactulose, a carbohydrate completely unabsorbed by the small bowel, was administered and generated the expected high breath hydrogen concentration from its fermentation by colonic bacteria (2). Glucose, which requires no digestion by the small bowel before absorption, had the lowest breath hydrogen production after the test feeding. Polycose®, corn syrup solids, and the corn cereal had intermediate but increased breath hydrogen production when compared with an oral glucose load. This indicates that increased complexity of carbohydrates is associated with increased small bowel malabsorption of starches and increased utilization of colonic mechanisms for carbohydrate digestion and absorption.

Four of the subjects studied were also monitored for the appearance of increased ¹³C abundance in fecal carbon following the feeding of 3 g/kg per day of the corn cereal. Within detection limits, no significant amounts of ¹³C enrichment were present in the stools of three of these infants. In one subject, 13.0% of the total corn cereal ¹³C passed into the stool.
FIG. 3. Mean breath hydrogen production expressed as parts per million (ppm) following a test load of lactulose and the feeding of a formula containing glucose, Polycose®, or corn. Note that with increasing complexity, from monosaccharide (glucose), to small-molecular-weight starch (Polycose®), to large-molecular-weight starch (corn cereal), there was an increase in mean breath hydrogen production. Note also that the production of breath hydrogen was less on these dietary carbohydrates than on the malabsorbed lactulose.

It was concluded from this investigation that absorption and utilization of glucose polymers and corn starch by 1-month-old infants can be demonstrated by their ability to oxidize these nutrients to CO$_2$. Equal quantities of breath $^{13}$CO$_2$ were produced by feeding equal quantities of glucose, glucose polymers, or corn starch. Hydrogen production accompanied carbohydrate absorption and increased when more complex carbohydrates were fed. This suggests that the colon may play an important role in complex carbohydrate utilization by young infants (1,2).

An ongoing investigation of carbohydrate intolerance in children is under way in our clinical service. The infants have symptoms that are typical of the clinical syndrome called intestinal decomposition described by Finkelstein in 1924 (7). Figure 4 illustrates the clinical course of one such infant. During the first days of rehydration following admission with combined *Salmonella* and rotaviral diarrhea, this child tolerated a 5% glucose solution. The infant was begun on a soy formula containing corn syrup solids. Following an increase in caloric intake necessary to meet energy needs, the child had increased frequency of stools associated with the presence of glucose. Despite adequate caloric intake, the child’s weight faltered. Following intravenous rehydration and fasting, the number of stools fell to zero. The child again was offered an oral hydrating
FIG. 4. The hospital course of a typical infant with acquired carbohydrate intolerance. Prosobee® was first fed at half-strength and then full-strength concentration, as indicated in the middle panel. The child was placed on nothing by mouth, and a central line was inserted on day 51 of age. Peripheral intravenous nutrition had been given from day 47 to day 51. In the lowest panel, glucose in the stools as determined by Testape® is indicated in the cross-hatched area.
solution that contained 5% glucose, which was well tolerated, and then was begun on a soy-based formula with three different levels of glucose concentration. When the child reached 5% glucose concentration in the formula, diarrhea increased, and glucose again was found in the stool. On the following day, a casein-based formula was substituted with a lesser amount of glucose; nevertheless, the diarrhea persisted, as did the presence of glucose in the stool. On the following 2 days, the child received an oral hydrating solution containing 5% glucose and continued to have increased numbers of stools with glucose present. Subsequently, the child was subjected to more intensive intravenous therapy with total parenteral nutrition, and good weight gain was achieved.

A mucosal biopsy was obtained that indicated the presence of complete villus atrophy with a reduction in goblet cells and mitotic figures. There was scant infiltration of the mucosal layer by round cells. This patient, however, recovered complete nutritional status and digestive abilities. At the time of follow-up, the patient was 5 months of age and was receiving cow's milk formula. Clearly, this child suffered a progressive degree of carbohydrate intolerance which was partially caused by mucosal atrophy.

We have undertaken a series of investigations on these patients with severe carbohydrate intolerance to determine the relative rates of absorption and oxidation of glucose and acetate given orally and rectally. Acetate is the product

![Graphs showing breath 13CO2 response to oral and rectal administration of glucose and acetate.](image-url)

**FIG. 5.** Breath $^{13}$CO$_2$ response to the oral or rectal administration of glucose or acetate highly enriched with $^{13}$C. Note: rectal administration of glucose or acetate was associated with prompt and rapid excretion of $^{13}$CO$_2$ in the breath and indicates oxidation of the substrate.
of carbohydrate fermentation of glucose. The $^{13}$CO$_2$ abundance in breath is used to determine the percent of the dose oxidized per minute. These studies are in a preliminary stage, and no conclusions can be made at present concerning the statistical significance of observations. Some illustrative data are shown in Fig. 5. In children with abnormal carbohydrate digestion and absorption associated with the presence of glucose in the stool, different patterns of CO$_2$ oxidation have been observed following rectal administration of a test dose of 1 g glucose/kg per day. We hypothesize that these children with intestinal decomposition have a combination of defects in glucose absorption. The first occurs at the level of the upper small bowel and is associated with marked villus atrophy of the mucosa (7). The second is the failure of colonic scavenging of the malabsorbed carbohydrate that presents at the level of the colon (9). It is hoped that the rates of appearance of $^{13}$C in breath after oral, intravenous, and rectal administration of $^{13}$C-labeled substrates will permit the exploration of mechanisms that result in small bowel and colonic carbohydrate intolerance in young infants.

Based on the results of these investigations, we propose that the use of safe, noninvasive stable isotope methodologies provides valuable quantitative information concerning intestinal function in normal and ill children.

ACKNOWLEDGMENTS

We thank Dr. Milton Finegold, who interpreted the mucosal biopsies reported in the case discussion, and Dr. William Wong, who prepared the gas isotope ratio analyses of the breath and fecal samples. This is a publication of the USDA/ARS, Children's Nutrition Research Center in the Department of Pediatrics, Baylor College of Medicine and Texas Children's Hospital.

REFERENCES