Water, fat, nitrogen, and sugar content in feces: reference intervals in children

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Malabsorption-maldigestion syndromes are commonly found in several gastrointestinal diseases. Quantitative measures of fecal nutrients are important tools for the detection and diagnosis of these syndromes. Adequate food intake is important in the nutrition of children, especially during the first year of life. We have analyzed 180 stools of healthy children, divided into four age groups, to obtain the reference intervals of the major nutrients such as water, fat, nitrogen, sugar, and starch. Quantification of the nutrients was done by means of a near-infrared analyzer (Fenir 8820). Results show that this instrument exhibits a low coefficient of variation for all the nutrients except for starch. Fecal water, fat, nitrogen, and sugar concentrations ranged from 68.7 to 96.1 g/100 g, 0 to 14.5 g/100 g, 1.3 to 2.3 g/100 g, and 0.7 to 3.8 g/100 g, respectively. The results for the starch analyses were not acceptable because of instrument limitations. Near-infrared reflectance spectroscopy appears to be an alternative to standard chemical methods.

Pancreatic insufficiency, cystic fibrosis, celiac disease, Crohn’s disease, and hepatic disorders are among several diseases that can produce malabsorption-maldigestion. In these syndromes, diarrhea, steatorrhea, and important nutrient stool losses can be present. Protein and carbohydrate losses can also be considerable in these diseases. The quantification in feces of principal nutrients such as water, fat, nitrogen, sugar, and starch remains one of the most important diagnostic tools for these syndromes (1, 2).

Fat malabsorption is not an early finding in these syndromes, and other methods often identify the disorder before steatorrhea develops. Nevertheless, measurement of daily stool fat excretion is the most direct and accurate method of demonstrating fat malabsorption (3). Water content is also indispensable for assessing digestive functions and certain syndromes such as constipation and diarrhea (4). Protein malabsorption (measured as nitrogen excretion) can be found in many gastrointestinal diseases, and the evaluation of nitrogen balance is essential in the management of patients with nutritional problems (5) and in subjects with suspected protein malabsorption (6). Fecal carbohydrate loss measurement is also important in the diagnosis of malabsorption syndromes (7, 8), although certain amounts of starch are malabsorbed even under physiologic conditions (9).

Established and usual methods for the measurement of fecal water, fat, nitrogen, sugar, and starch are very unpleasant, labor-intensive, and time-consuming (10–13). In recent years, a new method based on near-infrared reflectance spectroscopy (NIRRS) has presented an alternative for the development of the “fecalogram” that avoids cumbersome chemical techniques (5, 14–15). Fecal analysis by NIRRS presents some advantages: it is very fast (1 min); it does not require reagents; and very little sample manipulation is necessary.

Determinations of the major nutrients in feces are of special interest for the diagnosis in infants of some of the pathologies mentioned above and for the evaluation of their diets. Adequate food intake and absorption is of great importance for the development of children, especially during the first year of life. Fat malabsorption, for example, is considered one of the major causes of poor growth in infancy.

The aim of this study was to obtain the reference intervals for nutrients such as water, fat, nitrogen, sugar, and starch in the feces of children.

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Materials and Methods

INSTRUMENT: PRINCIPLE
Analyses were performed with an infrared analyzer (Fenir 8820, Alerbio S.A.) connected to a computer and printer, using the manufacturer’s software. In short, the method is based on the relationship between the reflectance intensity spectrum of the fecal sample at specific wavelengths and the composition of the sample. Each component to be measured has specific absorption bands in the near-infrared (1400–2600 nm) range, so the reflectance from a fecal sample is related to the concentration of the compound as follows (16):

\[ x = z - f_1 \log R_1 - f_2 \log R_2 - \ldots - f_n \log R_n \]

Where \( x \) is the concentration of the analyte, \( R_n \) is the reflectance for the filter \( n \), \( f_n \) is the scaling factor for each filter, and \( z \) is a constant of bias correction. The selected filters rotate automatically in turn into the light pathway, and the reflected energy from the sample is recorded and analyzed by a microprocessor. The instrument must be calibrated to select the optimal wavelengths and calculate the coefficient of correction to be applied to obtain the best approximation to the results of the chemical reference methods.

SUBJECTS
A total of 180 apparently healthy children from 20 days to 14 years of age were enrolled for the study after informed parental consent was obtained. These children were both outpatients and inpatients. The latter were included in the study after excluding patients with any gastrointestinal disease. The children were divided into four age groups established according to the different diets of children:

- group 1: ≤6 months (n = 42);
- group 2: 7–18 months (n = 46);
- group 3: 19 months–4 years (n = 45); and
- group 4: >4–14 years (n = 47).

Group 1 consisted of 15 females and 27 males; group 2 consisted of 18 females and 28 males; group 3 consisted of 18 females and 27 males; and group 4 consisted of 21 females and 26 males. Children ate their usual diets, which for group 1 was composed of human milk or formula milk, supplemented with cereal, fruits, and vegetables in some cases. There were five breast-fed infants in this group.

Stools were collected in preweighed plastic containers for 24 h and carefully mixed manually immediately before measurement. Three small fecal samples were pressed in a disposable cup to give a smooth surface. The cup was then inserted into the instrument.

IMPrecision
The within-run CV of the analytical method was obtained from the analysis of three or four samples 15 times each in one analytical run, whereas the between-run CV was obtained from the analysis of a different set of three or four samples one time in 15 different analytical runs.

Statistical analysis
Statistical analysis was performed according to the Expert Panel on the Theory of Reference Values of the International Federation of Clinical Chemistry (17).

To know if the studied populations had a gaussian distribution, we applied the Anderson-Darling test and the skewness and kurtosis coefficients. The fractiles and the estimated \( \beta \) confidence intervals were also calculated. When the populations did not have a gaussian distribution, nonparametric statistics were used to obtain the reference interval. Outliers were detected using the Dixon test (18). Differences between the four groups were tested by using the Kruskal–Wallis test (H statistic), with \( \alpha = 0.05 \) as the threshold for statistical significance.

Results

Table 1 summarizes the results of the within- and between-run imprecision studies. As shown in Table 1, the CVs were satisfactory, except for those obtained for starch at low concentration (g/100 g). Likewise, the between-run imprecision for starch and sugar was somewhat high at 6.0 and 1.4 g/100 g, respectively. The lowest imprecision was obtained for water concentration.

Reference Intervals
When results were expressed as concentrations (g/100 g of feces), all the substances except for starch followed a gaussian distribution. The central 0.95 reference intervals in g/100 g for water, fat, nitrogen, and sugar content, the 0.025 and 0.975 fractiles, and their 0.90 confidence intervals in the analyzed stools of the children are given in Table 2. The highest reference interval endpoint for fat was found in group 1, for water in group 2, for nitrogen in group 3, and for sugar in group 1.

We would highlight the results for starch content. In the four groups, the medians (which were 10 g/100 g, 7.8 g/100 g, 10 g/100 g, and 10 g/100 g for groups 1, 2, 3, and 4, respectively) were practically the same as the 0.975 fractile. This phenomenon was due to the inability of the analyzer to read more than 10 g of starch/100 g of feces.

On the other hand, when results were expressed in output excretion (g/day), all the substances had a non-gaussian distribution; these results were analyzed using nonparametric statistics. Fecal weight was highly variable: weights ranged from 5.00 to 67.74 g/day, 11.04 to 80.80 g/day, 5.6 to 175.69 g/day, and 12.5 to 147 g/day in groups 1, 2, 3, and 4, respectively. Reference intervals in g/day for each nutrient are summarized in Table 3, where 0.025 and 0.975 fractiles and the median are shown. Significant differences were found between the groups for each substance (\( P <0.005 \)), using the Kruskal–Wallis test.

Discussion
NIRRS is a method based on the measurement of the scattered radiation in the near-infrared range over the surface of a sample. Absorption is most often associated
with the overtone and combination bands of the fundamental molecular vibrations of \(-\text{OH}, -\text{NH}, \text{and} -\text{CH}\) functional groups. Most substances exhibit a characteristic near-infrared spectrum, which enables their quantification. As mentioned previously, NIRRS presents some major advantages: it is fast (1 min), needs no reagents, and the analysis is performed without additional processing of the stools (16). The only disadvantage is that the analyzer has first to be calibrated with the results of the standard chemical methods (10–13); therefore, the instrument is not more accurate than chemical methods. Unfortunately, neither a gold standard nor calibrators exist for stool analysis.

The Fenir 8820 Analyzer allows the determination of five major fecal components in a single sample. As shown in Table 1, the within-and between-run CVs are <10%, except for starch, which shows very high imprecision at low concentrations. These results are generally similar to those reported in the literature (3, 5, 15, 19, 20). In our opinion, the imprecision for starch is unsatisfactory for clinical use.

Homogenization can be avoided if repeated analyses on different portions of the same sample are performed. Picarelli et al. (20) found very similar CVs for water, fat, and nitrogen in homogenized and nonhomogenized stools, and Stein et al. (15) found similar CVs for carbohydrate. Nevertheless, we homogenized the feces by manual mixing. If a stool sample must be conserved, some authors (15, 21) recommend maintaining it at \(-20^\circ\text{C}\) until analysis. However, we analyzed specimens at the moment that we received them at the laboratory.

Benini et al. (14) reported that variation in stool matrix (e.g., liquid stools) could influence the results of fecal analysis. In our study, we did not focus on this possibility because the children were apparently healthy and did not have diarrhea. Because several intestinal, pancreatic, or biliary disorders can cause malabsorption syndromes, the importance of quantitative measurement of fecal nutrient output in the diagnosis of malabsorption syndromes has been recognized (1, 2). Some of these diseases are prevalent in children. Moreover, evaluation of adequate food intake and its absorption is of great importance for assessing the development of infants, especially during the first year of life. For this reason, the determination of reference intervals for these nutrients in feces is important.

The children were divided into four age groups, mainly according to their different diets. Children from

### Table 1. Imprecision of nutrient assays by the NIRRS procedure.

<table>
<thead>
<tr>
<th></th>
<th>Within-run (n = 15)</th>
<th></th>
<th>Between-run (n = 15)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>(\bar{x})</td>
<td>SD</td>
<td>CV, %</td>
</tr>
<tr>
<td>Water (g/100 g)</td>
<td>67.6</td>
<td>0.07</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>84.5</td>
<td>0.19</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>93.2</td>
<td>0.15</td>
<td>0.1</td>
</tr>
<tr>
<td>Fat (g/100 g)</td>
<td>1.9</td>
<td>0.08</td>
<td>4.5</td>
</tr>
<tr>
<td></td>
<td>6.5</td>
<td>0.07</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td>10.3</td>
<td>0.12</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td>14.9</td>
<td>0.07</td>
<td>0.5</td>
</tr>
<tr>
<td>Nitrogen (g/100 g)</td>
<td>1.4</td>
<td>0.02</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>1.6</td>
<td>0.01</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td>1.7</td>
<td>0.01</td>
<td>0.8</td>
</tr>
<tr>
<td>Sugar (g/100 g)</td>
<td>0.9</td>
<td>0.07</td>
<td>8.3</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>0.06</td>
<td>3.9</td>
</tr>
<tr>
<td></td>
<td>3.1</td>
<td>0.06</td>
<td>2.1</td>
</tr>
<tr>
<td>Starch (g/100 g)</td>
<td>0.9</td>
<td>0.29</td>
<td>31.8</td>
</tr>
<tr>
<td></td>
<td>7.1</td>
<td>0.13</td>
<td>1.9</td>
</tr>
<tr>
<td></td>
<td>7.9</td>
<td>0.14</td>
<td>1.8</td>
</tr>
</tbody>
</table>

* Mean.

### Table 2. Reference intervals for nutrients in stools according to age.*

<table>
<thead>
<tr>
<th></th>
<th>Water, g/100 g</th>
<th>Fat, g/100 g</th>
<th>Nitrogen, g/100 g</th>
<th>Sugar, g/100 g</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(\bar{x})</td>
<td>RI ((\beta = 0.90))</td>
<td>(\bar{x})</td>
<td>RI ((\beta = 0.90))</td>
</tr>
<tr>
<td>Group 1 (n = 42)</td>
<td>80.7</td>
<td>68.7-92.7 (±2.7)</td>
<td>7.3</td>
<td>0–14.5 (±1.6)</td>
</tr>
<tr>
<td>Group 2 (n = 46)</td>
<td>85.4</td>
<td>74.8–96.1 (±2.2)</td>
<td>4.4</td>
<td>0.4–8.5 (±0.9)</td>
</tr>
<tr>
<td>Group 3 (n = 45)</td>
<td>81.5</td>
<td>69.8–93.1 (±2.5)</td>
<td>4.7</td>
<td>0.2–9.2 (±1.0)</td>
</tr>
<tr>
<td>Group 4 (n = 47)</td>
<td>78.6</td>
<td>68.8–88.5 (±2.1)</td>
<td>4.6</td>
<td>1.6–7.6 (±0.6)</td>
</tr>
</tbody>
</table>

* Mean (\(\bar{x}\)), reference intervals (RI; 0.95), and confidence interval (\(\beta = 0.90\)) are shown.
weights varied considerably in every group. Interestingly, introduction of fecal weight into the calculations. Fecal nutrient excretion (g/day), possibly explained by the

table some specific aspects of our own study. The plausible studies on this subject. Nevertheless, it is interesting to point out that the excretion of all nutrients (g/day) rises according to the age of the children, possibly because each child ingests more nutrients as he or she grows older.

In our review of the literature, we found very few comparable studies on this subject. Nevertheless, it is interesting to point out some specific aspects of our own study. The population was not gaussian if data were expressed as nutrient excretion (g/day), possibly explained by the introduction of fecal weight into the calculations. Fecal weights varied considerably in every group. Interestingly, Thorsgaard Pedersen et al. (22) found that the 3-day fat concentration (g/100 g) is as effective as fecal fat excretion (g/day), that fat concentration is more constant than fecal weight, and that 1-day fat concentration measurement is as effective as the 3-day measurement.

The lowest amount (g/day) of water was found in group 1 (Table 3). Constipation and hard stools are a major complication in formula-fed children (23). Of the children in group 1, 88% were formula fed. Nevertheless, we found a larger concentration of water than had been reported by others (4, 24).

On the other hand, fat malabsorption in the first months of life is a well-known phenomenon, which has been attributed to possible factors such as reduced lipase activity (25) or the composition of the feeding fat (26). We did not find steatorrhea (described as fat >5 g/day) in group 1, although in this group, this nutrient is the major one in percentage terms (Table 2). On the contrary, a high excretion output of fat was found in groups 3 and 4 (Table 3). We cannot compare our results with those in the literature, either because others have studied an adult population or because the techniques used are different from ours. Only a single publication (27) was similar to ours. These authors found that formula-fed children had a fecal fat lipid of 10.3 ± 3 g/100 g.

Protein intake is kept low in the first months of age to preserve the kidney from future disorders. Therefore, the lowest quantity of protein measured as nitrogen was found in group 1 (Table 3). Likewise, children have lower nitrogen values than adults because they metabolize proteins better than adults.

Cereal is usually the first solid food given to young children. Children in group 1 were fed with cereal as a source of starch. Unfortunately, our results for starch were not satisfactory. The lower reference limits for starch were close to 0 g/100 g of feces, and the higher reference limit was close to the median in the four groups. We believe that the reference intervals obtained for the starch were wrong because of the instrument limitations. The Fenir 8820 cannot read >10 g of starch/100 g of feces. For this reason, we have not reported the results for starch determination in this paper.

The highest concentration of sugar was found in the stools of group 1 (Table 2), but this group had the lowest total output (g/day; Table 3). Human and formula milk contain ~7% and 5.4–8.6% sugar, respectively, and formula milk contains substantial quantities of unabsorbable carbohydrate (28).

In summary, this new technology for measuring the major nutrients in stools is a major advance in the nutritional-digestive area. The method is simple to perform. Every laboratory should obtain its own reference intervals, especially when, as in this case, diet plays an important role.

### References


6. DiMagno EP, Go VLW, Summerskill HJ. Relationship between pancreatic enzyme outputs and malabsorption in severe pancre-

### Table 3. Reference intervals in g/day for each substance in stools of children.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Water, g/day</th>
<th>Fat, g/day</th>
<th>Nitrogen, g/day</th>
<th>Sugar, g/day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>f₀.025</td>
<td>f₀.975</td>
<td>Median</td>
<td>f₀.025</td>
</tr>
<tr>
<td>Group 1 (n = 42)</td>
<td>4.08</td>
<td>61.03</td>
<td>14.03</td>
<td>0.34</td>
</tr>
<tr>
<td>Group 2 (n = 46)</td>
<td>8.98</td>
<td>97.47</td>
<td>26.17</td>
<td>0.39</td>
</tr>
<tr>
<td>Group 3 (n = 45)</td>
<td>4.40</td>
<td>153.13</td>
<td>33.68</td>
<td>0.53</td>
</tr>
<tr>
<td>Group 4 (n = 47)</td>
<td>9.98</td>
<td>123.09</td>
<td>50.99</td>
<td>0.61</td>
</tr>
</tbody>
</table>

*a Fractiles 0.025 and 0.975 (f₀.025, f₀.975) and median are shown.*