Intestinal Blood Loss During Cow Milk Feeding in Older Infants

Quantitative Measurements

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Objective: To determine the response, in terms of fecal hemoglobin excretion and clinical symptoms, of normal 9½-month-old infants to being fed cow milk.

Design: Longitudinal (before-after) trial in which each infant was fed formula for 1 month (baseline) followed by 3 months during which cow milk was fed.

Setting: Healthy infants living in Iowa City, Iowa, a town with a population of about 60000.

Main Outcome Measures: Hemoglobin concentration in spot stools, 96-hour quantitative fecal hemoglobin excretion, stool characteristics, feeding-related behaviors, and iron nutritional status.

Results: Fecal hemoglobin concentration during formula feeding (baseline) was higher than previously observed in younger infants. Nine of 31 infants responded to cow milk feeding with increased fecal hemoglobin concentration. Fecal hemoglobin concentration (mean±SD) of the 9 responders rose from 1395±856 µg/g of dry stool (baseline) to 2711±1732 µg/g of dry stool (P=.01). The response rate (29%) was similar to that in younger infants, but the intensity of the response was much less. Quantitative hemoglobin excretion was in general agreement with estimates based on spot stool hemoglobin concentrations. Cow milk feeding was not associated with recognizable changes in stool characteristics, nor were there clinical signs related to fecal blood loss. Iron status was similar, except that after 3 months of cow milk feeding responders showed lower (P=.047) ferritin concentrations than nonresponders.

Conclusions: Cow milk–induced blood loss is present in 9½-month-old infants but is of such low intensity that its clinical significance seems questionable. Nevertheless, infants without cow milk–induced blood loss were in better iron nutritional status than infants who showed blood loss.


When young infants are fed pasteurized cow milk, nutritionally significant amounts of blood are lost in the feces.1,2 Although this occult blood loss occurs only in a minority of infants, in some infants the amount of blood lost can be very large.2 Recent changes in feeding practices have included later introduction of cow milk,3 so that in recent years only a small percentage of infants are fed cow milk by 6 months of age (J. Boettcher, MEd, RD, written communication, Mead Johnson Nutritionals, Evansville, Ind, 1998). However, cow milk is gradually introduced during the second 6 months of life. By 12 months of age, 39% of infants were receiving cow milk in 1997 (J. Boettcher, MEd, RD, written communication, Mead Johnson Nutritionals, 1998).

Little is known about cow milk–provoked fecal blood loss in late infancy. In the study by Anyon and Clarkson,4 44% of stools of infants fed cow milk at 4 months of age showed a positive orthotolidine reaction (similar to the guaiac reaction), whereas by 8 months the proportion of infants with positive reactions had dropped to 27%. Fuchs et al5 studied 6- to 12-month-old infants who had been fed formula from birth and found that fecal hemoglobin concentrations were the same in infants fed cow milk as in those fed formula. These findings suggest that older infants might react differently to cow milk than young infants. One of the objectives of the present study was to obtain information on how infants handle cow milk during the last 3 months of the first year of life.

Although some of the previous studies2,3 used quantitative methods (as opposed to nonquantitative color reactions) for the determination of fecal hemoglobin concentrations, only semiquantitative estimates of total fecal hemoglobin loss were possible with the use of
SUBJECTS AND METHODS

STUDY DESIGN

The study used a longitudinal (before-after) design in which each infant served as his or her own control. The study plan is schematically presented in Figure 1. Beginning within 4 days of 252 days of age, infants were fed a milk-based formula for a 28-day baseline period. After this period, cow milk was fed for 12 weeks, ie, from 280 through 364 days of age. Spot stools were collected on 3 occasions during the baseline period, on 6 occasions during the first 2 weeks of cow milk feeding, and once every 2 weeks during the remainder of the cow milk period. Ninety-six–hour stool collections were obtained once during the baseline period and twice during the cow milk period, but a substantial number of infants had 1 or 2 additional collections. Feeding-related behaviors and stool characteristics were recorded every other week throughout the study. Blood was obtained at intervals for the monitoring of iron nutritional status. All stated ages are nominal ages, with actual ages being within 4 days of nominal ages.

SAMPLE SIZE

Quantitative fecal hemoglobin excretion was the main outcome measure. We wished to detect an increase from baseline equivalent to a difference in fecal hemoglobin iron excretion of 0.1 mg/d. On the basis of earlier results with spot stools from our unit, this corresponded to approximately a difference of 1.0 SD. To detect such a difference at α = .05 and power of 0.8, we needed 16 subjects. Assuming that 60% of infants would not respond to cow milk feeding with hemoglobin excretion at all, we estimated that we needed 40 infants. However, when interim data analysis with 32 infants completed and/or enrolled showed that there was only a trend toward higher fecal hemoglobin concentrations, enrollment was stopped.

SUBJECTS

Thirty-two healthy full-term infants (18 males and 14 females) with birth weight greater than 2500 g and gestational age greater than 37 weeks entered the study at 252 days of age. One infant left the study because the parents believed that he was not tolerating cow milk. The parents assumed stool weights. Therefore, a second objective of the present study was to determine fecal blood loss quantitatively for the first time with the use of 96-hour stool collections.

None of the previous studies of cow milk–provoked fecal blood loss noted overt clinical signs that accompanied blood loss. However, more subtle signs could have escaped casual observation. Recognition of clinical signs would be useful in that it might trigger appropriate laboratory tests and thus lead to the identification of infants with clinically important fecal blood loss. Therefore, in the present study, a systematic effort was made to record signs and symptoms that might accompany blood loss.

The present study was thus undertaken with 3 objectives. We determined whether older infants respond to cow milk feeding in the same way as younger infants; we assessed quantitatively fecal hemoglobin excretion during cow milk feeding; and we observed for any possible clinical signs that might accompany occult fecal blood loss in infants.

RESULTS

STOOL HEMOGLOBIN CONCENTRATION

Data are summarized in Table 1 and are graphically displayed in Figure 2. During the baseline period, hemoglobin concentration differed somewhat (not significantly) between infants subsequently classified as responders and nonresponders. The average baseline he-
294 days of age and subsequently every 2 weeks. For the collection of spot stool specimens, parents were provided clean wide-mouthed plastic containers and wooden spatulas. The parents were asked to place 5 to 10 g of stool in the labeled container, place the container in a sealable plastic bag, and store it in the freezer compartment of the home refrigerator until transport to the laboratory. Specimens were transported frozen and were stored in the laboratory at −20°C until analyzed.

Quantitative stool collections were obtained during the baseline period in most infants at 267 days of age and in all 30 infants during cow milk feeding at 309 and at 337 days of age. In 19 and 16 infants, respectively, additional collections were obtained at 295 and at 365 days of age. The quantitative stool collections were performed with the use of disposable diapers (Huggies; Kimberly-Clark, Neenah, Wis). The collections were performed entirely in the homes of the infants. Parents were asked to place diapers in labeled sealable plastic bags and to store them in the freezer compartment of the home refrigerator until transported to the laboratory. When there was solid stool, parents scraped the stool off the diaper and placed it into labeled containers, which were stored in the sealable bags along with the diapers.

STOOL CHARACTERISTICS AND FEEDING-RELATED BEHAVIORS

Observations of stool characteristics and feeding-related behaviors were obtained every other week during the entire week. The parents were provided recording sheets and were asked to record each stool and describe its characteristics such as color, consistency, and odor. The occurrence of fussiness (and crying) and events such as regurgitation (spitting up), vomiting, constipation, and passing of gas were to be recorded throughout the week.

LABORATORY METHODS

Solid stools were pooled and weighed. Diapers soiled with stool were cut into small pieces and placed into a blender. After addition of 100 to 150 mL of detergent solution (1% Tween 20), the contents were blended vigorously. The extraction liquid was manually squeezed from the diapers and added to the solid stool pool, followed by homogenization with a Teflon blender. Recovery experiments (n=12) with random stool specimens applied to diapers showed that by this method 98.4% (SD, 10.9%) of fecal hemoglobin was extracted.

Hemoglobin in spot stools and pool homogenates was determined quantitatively by the HemoQuant assay, which is based on fluorometric measurement of preformed porphyrin and porphyrin derived in vitro from nonfluorescing heme; results are expressed as hemoglobin equivalents. Dry weight of spot stools was determined by drying to constant weight so that results for spot stools could be expressed as micrograms of hemoglobin equivalents per gram of dry stool. Results for stool pools (fecal hemoglobin excretion) were expressed as milligrams of hemoglobin equivalent per day.

Blood hemoglobin was measured with a hematology counter ( Coulter Counter, model M430; Coulter Electronics, Hialeah, Fla), and plasma ferritin by radioimmunoassay (Quantimune, kit 190-2001; Bio-Rad Laboratories, Hercules, Calif).

DATA ANALYSIS

As in the previous study from this unit, an infant was classified as a “responder” if he or she had 2 or more spot stools with hemoglobin concentration greater than the mean + 2 SD value during the baseline period. This value was 2416 µg/g of dry stool in the present study. Data for spot stools were treated by calculating arithmetic mean values for fecal hemoglobin concentration for individual subjects for the baseline and for each consecutive 1-month period during cow milk feeding. These mean values were not distributed normally and were therefore transformed logarithmically for statistical treatment. However, the results are presented as arithmetic means and SDs. Data for stool pools were directly subjected to statistical analysis.

Repeated-measures analysis of variance was performed on the transformed spot stool data and the stool pool data to evaluate changes from baseline to cow milk feeding as well as differences between responders and nonresponders. Other analyses used paired t tests and the non-parametric Mann-Whitney test. The relation between spot stool hemoglobin concentrations and hemoglobin excretion was examined by Pearson correlation coefficient. Data analyses were performed with SYSTAT statistical software (SYSTAT for the Macintosh, version 5.2, SYSTAT, Evanston, Ill). A P value <.05 indicated a significant difference between the groups. Unless otherwise indicated, data are given as mean ± SD.

Fecal hemoglobin concentration was 1192 ± 612 µg/g of dry stool. During cow milk feeding, 9 (29%) of the 31 infants had 2 or more spot stools with hemoglobin concentration greater than 2416 µg/g of dry stool and were classified as responders. Three of the responders were initially breast-fed. During the first 28 days of cow milk feeding, hemoglobin concentration of the responders rose significantly (P=.01) to a mean of 2711 ± 1732 µg/g of dry stool. The difference between responders and nonresponders was also statistically significant (P=.001) during that period. The average (responders plus nonresponders) hemoglobin concentration during the first 28 days was 1640 ± 1181 µg/g of dry stool. During the succeeding 2 months of cow milk feeding, fecal hemoglobin concentration of the responders decreased somewhat but remained significantly greater than in nonresponders. If the entire cohort of 31 infants was considered, fecal hemoglobin concentration during cow milk feeding was not significantly (P=.09) different from baseline.

In 1 of the responders, fecal hemoglobin concentration increased nearly 7-fold (from 1661-11021 µg/g) within 2 days of starting cow milk. In this infant, the mean fecal hemoglobin concentration during the first 28 days of cow milk feeding was 5981 µg/g of dry stool.

FECAL HEMOGLOBIN EXCRETION

The results of the 96-hour stool collections are summarized in Table 2. During baseline, hemoglobin excretion averaged 11.6 ± 4.6 mg/d in responders and 10.4 ± 5.2
mg/d in nonresponders. The difference was not statistically significant. During cow milk feeding, hemoglobin excretion increased in responders and also, to a lesser degree, in nonresponders. In neither group was the increase statistically significant. However, for responders and nonresponders combined at 295 days of age, the increase above baseline (to 14.9 mg/d) was statistically significant ($P = .03$). Also, combining data for 295 and 309 days of age, hemoglobin excretion was significantly higher during cow milk feeding ($r = 0.38$, $P < .001$). The infant with the highest spot stool concentrations had a marked trend (not statistically significant) toward less regurgitation among responders than among nonresponders. All the behaviors or events showed a marked decrease with increasing age.

IRON NUTRITIONAL STATUS

Data concerning iron nutritional status are summarized in Table 4. There were no differences in hemoglobin concentration either between baseline and the cow milk period or between responders and nonresponders. Plasma ferritin concentrations, as expected, decreased significantly during the study period. Although there was no difference in the rate of decrease between responders and nonresponders, at the conclusion of the study plasma ferritin concentrations were significantly ($P = .047$) lower in responders (20.3 ± 13.5 µg/L) than in nonresponders (29.6 ± 15.6 µg/L). No infant developed iron deficiency (plasma ferritin level, <12 µg/L) or anemia (hemoglobin...
bin level, <110 g/L). There was no relationship between plasma ferritin concentration and fecal hemoglobin excretion or spot stool hemoglobin concentration throughout the course of the study.

We found that 29% of healthy 9 1/2-month-old infants responded to cow milk with an increase in spot stool hemoglobin concentration. The proportion of infants who responded was somewhat lower than the 40% of 5 1/2-month-old infants reported previously. However, fecal hemoglobin concentration was decidedly less in the older infants of the present study. Whereas in 5 1/2-month-old infants hemoglobin concentration averaged 3598±1047 µg/g of dry stool during the first month of cow milk feeding, in the present study the average value was only 1640±1181 µg/g of dry stool. Thus, at 9 1/2 months of age the response to cow milk occurred with somewhat lower frequency than at 5 1/2 months of age and, especially, with less intensity. The finding is in general agreement with other data from this unit concerning the effect of cow milk feeding on spot stool hemoglobin concentration of 7 1/2-month-old and 12-month-old infants. At 7 1/2 months of age, the response to cow milk was diminished and at 12 months it was no longer demonstrable.

A single infant in the present study showed exceedingly high fecal hemoglobin concentrations, which was somewhat reminiscent of the 1 infant in a previous study from our unit who became anemic after 4 weeks of receiving cow milk. Although we cannot estimate the prevalence of such strong responses in the healthy infant population, we cannot assume that strong responses are very rare. Contrary to findings in 5 1/2-month-old and 7 1/2-month-old infants, at 9 1/2 months of age formerly breast-fed infants were not more likely to respond to cow milk with increased fecal blood loss and actually responded less frequently to cow milk than infants who had not been breast-fed. Among the formerly breast-fed infants, 20% responded to cow milk with blood loss, compared with 37% of those who were never breast-fed.

In the present study, baseline hemoglobin concentrations were higher than observed in 5 1/2-month-old infants or 7 1/2-month-old infants and were similar to those of 12-month-old infants. This age-related rise in baseline hemoglobin concentration probably reflects increas-

### Table 2. Fecal Hemoglobin Excretion in 96-Hour Stool Collections*

<table>
<thead>
<tr>
<th></th>
<th>Baseline (267 d)</th>
<th>295 d</th>
<th>309 d</th>
<th>337 d</th>
<th>365 d</th>
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</thead>
<tbody>
<tr>
<td><strong>Nonresponders</strong></td>
<td>10.4 ± 5.2 (19)</td>
<td>13.7 ± 5.8 (16)</td>
<td>10.9 ± 4.5 (21)</td>
<td>11.1 ± 4.2 (21)</td>
<td>15.2 ± 8.9 (9)</td>
</tr>
<tr>
<td><strong>Responders</strong></td>
<td>11.6 ± 4.6 (6)</td>
<td>21.5 ± 15.5 (3)</td>
<td>13.8 ± 6.3 (9)</td>
<td>13.8 ± 5.3 (9)</td>
<td>21.3 ± 9.0 (7)</td>
</tr>
</tbody>
</table>

*Values are given as milligrams per day (mean ± SD). Numbers in parentheses are numbers of stool collections tested.

### Table 3. Feeding-Related Behaviors of 31 Subjects

<table>
<thead>
<tr>
<th></th>
<th>Baseline (252-280 d)</th>
<th>Cow Milk Feeding</th>
<th>281-308 d</th>
<th>309-336 d</th>
<th>337-364 d</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Infants with fussiness, No. (%)</strong></td>
<td>25 (81)</td>
<td>20 (65)</td>
<td>12 (39)</td>
<td>15 (48)</td>
<td></td>
</tr>
<tr>
<td><strong>Days with fussiness†</strong></td>
<td>1.2</td>
<td>1.0</td>
<td>0.9</td>
<td>0.9</td>
<td></td>
</tr>
<tr>
<td><strong>Infants with spitting up, No. (%)</strong></td>
<td>20 (65)</td>
<td>21 (68)</td>
<td>13 (42)</td>
<td>14 (45)</td>
<td></td>
</tr>
<tr>
<td><strong>Days with spitting up†</strong></td>
<td>0.7</td>
<td>0.7</td>
<td>0.6</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td><strong>Infants with gas, No. (%)</strong></td>
<td>19 (61)</td>
<td>17 (55)</td>
<td>12 (39)</td>
<td>9 (29)</td>
<td></td>
</tr>
<tr>
<td><strong>Days with gas†</strong></td>
<td>0.7</td>
<td>0.6</td>
<td>0.6</td>
<td>0.5</td>
<td></td>
</tr>
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</table>

*One or more episodes per week.
†Number of days per infant per week.

### Table 4. Blood Hemoglobin and Plasma Ferritin Concentrations

<table>
<thead>
<tr>
<th></th>
<th>Age, d</th>
<th>280 (Baseline)</th>
<th>308</th>
<th>336</th>
<th>364</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hemoglobin, mean ± SD, g/L</strong></td>
<td></td>
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</tr>
<tr>
<td>Nonresponders (n = 22)</td>
<td>121 ± 10</td>
<td>122 ± 8</td>
<td>121 ± 8</td>
<td>121 ± 9</td>
<td></td>
</tr>
<tr>
<td>Responders (n = 9)</td>
<td>126 ± 7</td>
<td>128 ± 7</td>
<td>124 ± 9</td>
<td>127 ± 10</td>
<td></td>
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<tr>
<td><strong>Ferritin, mean ± SD, µg/L</strong></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Nonresponders (n = 22)</td>
<td>39.0 ± 16.7</td>
<td>33.4 ± 17.6</td>
<td>31.3 ± 16.3</td>
<td>29.6 ± 15.6*</td>
<td></td>
</tr>
<tr>
<td>Responders (n = 9)</td>
<td>36.9 ± 35.8</td>
<td>29.8 ± 19.8</td>
<td>35.1 ± 21.8</td>
<td>20.3 ± 13.5</td>
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*P<.05 compared with baseline; P<.05 compared with responders at same age.
ing consumption by the infants of heme-containing foods such as meats. The assay we used picks up heme or porphyrin, regardless of whether it is of dietary or endogenous origin. This assay therefore overestimates somewhat the true fecal hemoglobin concentration.

The novel feature of the present study was the 96-hour stool collections. The results from this labor-intensive undertaking generally confirm and validate the results obtained with spot stools. Since the rate of hemoglobin excretion is likely to be highly variable within each infant, timed stool collections give a much more reliable estimate of fecal hemoglobin excretion than can be obtained from spot stools. We are not aware of previous reports of quantitative measurements of fecal hemoglobin loss in nonanemic healthy infants.

Fecal loss of hemoglobin iron (3.47 µg of iron per milligram of hemoglobin) has previously been estimated from spot stool hemoglobin concentrations assuming a daily stool weight (wet) of 60 g. In this way, hemoglobin iron loss was estimated at 0.15 mg/d for 5½-month-old responders during the first month of cow milk feeding and amounts to 0.168 mg/d in 7½-month-old responders. In the present study, using the same assumption, hemoglobin iron loss would have been estimated at 0.112 mg/d in responders during the first month of cow milk feeding. However, the 96-hour stool collections showed that the 9 responders lost, on average, 21.5 mg of hemoglobin each day during the first month of cow milk feeding, equivalent to iron loss of 0.075 mg. Although the discrepancy is not implausible given the variability of hemoglobin excretion, the fact that the estimate based on spot stools was higher may simply reflect a somewhat excessive assumed stool weight. In nonresponders, the 2 methods yielded almost identical values in the present study. Hemoglobin iron loss during cow milk feeding, estimated on the basis of spot stools, averaged 0.050 mg/d, whereas it averaged 0.048 mg/d on the basis of 96-hour stool collections (hemoglobin loss of 13.7 mg/d).

Infants who responded to cow milk with increased fecal blood loss showed evidence of lower iron stores (lower plasma ferritin concentration) after 3 months of cow milk feeding than infants who did not respond. The other studies from our unit did not detect effects on iron nutritional status, presumably because the infants were younger (and had fuller iron stores) and/or because cow milk was fed for shorter periods.

The undesirability of cow milk for infants was addressed by the Committee on Nutrition of the American Academy of Pediatrics in 1992. The use of cow milk in infant feeding has diminished greatly over the years. Whereas 61% of 5- to 6-month-old infants were fed cow milk in 1971, the percentage had dropped to a mere 4% by 1991. Nevertheless, older infants continue to be fed cow milk. In the United States, about 80% of 12-month-old infants were fed cow milk in 1991, but by 1997 the percentage of 12-month-old infants receiving cow milk dropped to 39% (J. Boettcher, MEd, RD, written communication, Mead Johnson Nutritional, 1998).

In conclusion, the present study shows that fecal blood loss in response to cow milk feeding continues to occur in 9½-month-old infants, albeit with diminishing intensity as compared with younger infants. The amount of iron that is lost as stool hemoglobin seems insignificant from a nutritional point of view. This may be pointing to inhibition of nonheme iron absorption by cow milk as the primary mechanism for iron deficiency in cow milk-fed infants. Cow milk is high in casein and calcium, both known inhibitors of nonheme iron absorption. Nevertheless, infants who responded to cow milk with blood loss showed evidence of an adverse effect on iron nutritional status. There was, again, the occasional infant with exaggerated blood loss who may be at considerable risk of iron deficiency. We further conclude, as we have before, that cow milk–induced blood loss is clinically silent. There are no laboratory tests that would allow the clinician to identify the occasional infant with substantial blood loss. The fact that feeding infants cow milk leads to poor iron nutritional status is well documented in the literature, although it is not clear whether the mechanism mainly responsible is intestinal blood loss or, perhaps more likely, the inhibition of absorption of iron from other dietary sources. Therefore, even if the average fecal blood loss that we observed between 9½ and 12 months of age was relatively mild, we believe that cow milk is undesirable before 12 months of age.

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