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 60,000.

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.

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HEN YOUNG infants are fed pasteurized cow milk, nutritionally significant amounts of blood are lost in the feces.²,³ 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,⁴ 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 Clarkson⁵ 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 al³ 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 studies⁵,³ used quantitative methods (as opposed to nonquantitative color reactions) for the determination of fecal hemoglobin concentrations, only semi-quantitative 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, i.e., 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 of another infant provided only spot stools. Thus, 30 infants provided data on 96-hour stool collections and 31 infants provided spot stool data. All but 5 of the infants had previously participated in feeding studies conducted by our unit. A total of 31 infants were initially breastfed. Of these, 3 were breastfed for less than 2 months, 3 for 2 to 4 months, and 7 for 6 to 7 months. At the time of recruitment into the present study, all initially breastfed infants had been weaned to formula. Seventeen infants were fed formula from birth. The nature of the study was reviewed in detail with 1 or both parents and written consent was obtained. The study protocol was reviewed and approved by The University of Iowa Committee on Research Involving Human Subjects.

FEEDINGS

From 252 to 280 days of age, all infants were fed a milk-based, casein-predominant formula (Similac With Iron; Ross Products Division, Abbott Laboratories, Columbus, Ohio) with an iron concentration of 12 mg/L. From 281 through 364 days of age, all infants were fed pasteurized, homogenized full-fat cow milk produced by 1 of 3 local dairies. Cow milk was provided in half-gallon (1.89-L) paper cartons and was delivered to the family twice weekly. Parents were requested to keep this milk separate from milk used by other family members. In addition to cow milk, infants were permitted to receive, at the parents' discretion, commercially prepared and home-prepared food with the exception of cereals of any kind. Iron supplements were neither recommended nor provided.

PROCEDURES

The infants visited the Fomon Infant Nutrition Unit, The University of Iowa, Iowa City, within 4 days of 252, 280, 308, 336, and 364 days of age. At each visit, an interval medical history was obtained and completed questionnaires regarding feeding-related behaviors and stool characteristics were received and reviewed for completeness. Weight and length were measured by methods described by Fomon and Nelson and a capillary blood sample was obtained by heel stick with a disposable spring-loaded device (Tenderfoot; International Technidyne Corp, Edison, NJ).

Spot stools were collected from 31 infants during the baseline period at 252, 266, and 280 days of age, and during the cow milk period at 282, 284, 286, 288, 290, and

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 seizable 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 (Quantimmune, 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.

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 in responders (mean, 16.1±6.5 mg/d) was significantly ($P = .02$) higher than during baseline and significantly ($P = .049$) higher than in nonresponders (12.3±3.6 mg/d). The highest fecal hemoglobin excretion was observed in the infant with the highest spot stool concentrations. At 295 days of age, hemoglobin excretion was 37.5 mg/d.

The relationship between fecal hemoglobin excretion and hemoglobin concentration in spot stools was close during the baseline period (to 14.9 mg/d) was statistically significant ($P = .03$). Also, combining data for 295 and 309 days of age, hemoglobin excretion in responders (mean, 16.1±6.5 mg/d) was significantly ($P = .02$) higher than during baseline and significantly ($P = .049$) higher than in nonresponders (12.3±3.6 mg/d). The highest fecal hemoglobin excretion was observed in the infant with the highest spot stool concentrations. At 295 days of age, hemoglobin excretion was 37.5 mg/d.

The relationship between fecal hemoglobin excretion and hemoglobin concentration in spot stools was close during the baseline period ($r=0.53$, $P = .006$) and during cow milk feeding ($r=0.38$, $P < .001$). The infant with the very high hemoglobin concentrations in spot stools had three 96-hour collections during cow milk feeding with an average hemoglobin excretion of 19.2 mg/d.

### STOOL CHARACTERISTICS

For the entire cohort of infants, the number of stools increased from an average of 2.1 per day during baseline to 2.3 per day ($P = .04$) during the first month of cow milk feeding, but decreased somewhat subsequently and ended at 1.9 per day. During the baseline period there was no significant difference between responders and nonresponders, but during the first month of cow milk feeding, the number of stools per day was significantly ($P = .009$) lower in responders (1.9 per day) than in nonresponders (2.5 per day). With regard to stool consistency, color, and odor, there were no differences between baseline and cow milk feeding or between responders and nonresponders (data not shown).

### FEEDING-RELATED BEHAVIORS

Data on feeding-related behaviors are summarized in Table 3. With the initiation of cow milk feeding, there were no noteworthy changes in the incidence of fussiness, spit-up, and intestinal gas. Similarly, there were no changes in vomiting, constipation, or other feeding-related behaviors (data not shown). No differences were seen between responders and nonresponders with regard to any of these behaviors. The exception was 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 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 concentration either between baseline and the cow milk period or between responders and nonresponders).
There was no relationship between plasma ferritin concentration and fecal hemoglobin excretion or spot stool hemoglobin concentration throughout the course of the study.

**Comment**

We found that 29% of healthy 9½-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 5½-month-old infants reported previously. However, fecal hemoglobin concentration was decidedly less in the older infants of the present study. Whereas in 5½-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 9½ months of age the response to cow milk occurred with somewhat lower frequency than at 5½ 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 7½-month-old and 12-month-old infants.

At 7½ 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 5½-month-old and 7½-month-old infants, at 9½ 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 5½-month-old infants or 7½-month-old infants and were similar to those of 12-month-old infants. This age-related rise in baseline hemoglobin concentration probably reflects increas-
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 Nutritionals, 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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