Palm olein in infant formula: absorption of fat and minerals by normal infants$^{1-3}$

Steven E Nelson, Ronald R Rogers, Joan A Frantz, and Ekhard E Ziegler

**ABSTRACT** Palm olein, a low-melting fraction of palm oil, and soy oil can be combined to obtain fat blends with proportions of palmitic and oleic acids similar to those of human milk. We compared the absorption of fat and calcium by infants fed a formula containing a blend of palm olein (53%) and soy oil (47%) (Formula PO/S) with that by infants fed a formula containing a blend of soy oil (60%) and coconut oil (40%) (Formula S/C). In a randomized crossover design, one study was performed with each formula in each of 11 normal infants ranging in age from 27 to 161 d. Six of the infants were admitted for 72-h metabolic balance studies. In the other five infants, feces (with some admixture of urine) were collected at home for 96 h by using acid-washed cloth diapers. Mean (± SD) absorption of fat was 90.6 ± 1.6% of intake when Formula PO/S was fed and 95.2 ± 1.1% of intake when Formula S/C was fed; the difference was significant ($P < 0.001$). The difference in excretion of fat by infants fed the two formulas was explained by the difference in excretion of palmitic acid. Absorption of calcium averaged 39.0 ± 8.3% of intake with Formula PO/S and 48.4 ± 10.3% with Formula S/C; the difference was significant ($P < 0.01$). We conclude that fat is less well absorbed from a mixture of 53% palm olein and 47% soy oil than from a mixture of 60% soy oil and 40% coconut oil, and that absorption of calcium is less from a formula containing palm olein, presumably because of the formation of insoluble calcium soaps of unabsorbed palmitic acid.

**KEY WORDS** Fat absorption, palm olein, infants, calcium absorption

**INTRODUCTION** Various mixtures of vegetable oils used in infant formulas are absorbed by infants to about the same extent as is the fat of human milk (1). Yet, such blends may differ markedly from human milk in fatty acid composition. For example, in a blend of soy oil (60%) and coconut oil (40%), palmitic acid (16:0) comprises only 9% of fatty acids and oleic acid (18:1) comprises 17%, whereas in human milk palmitic acid typically provides ≈23% of fatty acids and oleic acid ≈34% (2). Higher proportions of palmitic and oleic acids in infant formulas can be obtained with the use of palm olein, a low-melting fractionation product of palm oil, which contains less palmitic acid (40% of fatty acids) and more oleic acid (43% of fatty acids) than palm oil itself (44% and 39% of fatty acids, respectively) (3). Combinations of palm olein and soy oil yield proportions of palmitic and oleic acids similar to those in human milk. Thus, in a blend of 53% palm olein and 47% soy oil, palmitic acid provides 25% of fatty acids and oleic acid provides 34% (Table 1), proportions that are similar to those in human milk. The efficiency with which this fat blend is absorbed by infants is not known.

The extent of absorption of palmitic acid is related to the position of the fatty acid on the triacylglycerol molecule (4–7). Pancreatic lipase preferentially releases fatty acids in the sn-1 and sn-3 positions and thus produces fatty acids and 2-mono-glycerides. The 2-mono-glyceride of palmitic acid is well absorbed, whereas free palmitic acid is poorly absorbed (5). In vegetable oils, including palm oil, palmitic acid is predominantly esterified in the sn-1 and sn-3 positions (8, 9), whereas in human milk fat palmitic acid is primarily present in the sn-2 position (6, 10). Thus, there was reason to suspect that absorption of fat would be somewhat limited from a fat mixture containing 53% palm olein.

The present study was undertaken to determine fat absorption by normal infants fed a formula containing this blend of vegetable oils. In a crossover study we compared the absorption of fat and of individual fatty acids as well as the absorption and retention of minerals from two formulas that were similar in composition, except that one contained palm olein and soy oil, whereas the other (control formula) contained soy oil and coconut oil.

**SUBJECTS AND METHODS**

**Study design**

In a randomized crossover design, balance studies were performed in 11 infants with each of the two study formulas. The interval between the two studies was 14 d (17 d in one infant) and the order in which formulas were studied was predetermined and random. The formulas were fed for ≈7 d before the start of the balance study.

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TABLE 1
Composition of study formulas1

<table>
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<tr>
<th></th>
<th>Formula S/C</th>
<th>Formula PO/S</th>
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<tr>
<td>Protein (g/L)2</td>
<td>15.0</td>
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<tr>
<td>Lactose (g/L)</td>
<td>71.9</td>
<td>71.3</td>
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<td>Calcium (mg/L)2</td>
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<td>Phosphorus (mg/L)2</td>
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<td>Magnesium (mg/L)2</td>
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<td>Fat (g/L)</td>
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Fatty acids (% by wt)

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<td>0.4</td>
<td>3.2</td>
<td>2.3</td>
<td>18.0</td>
<td>7.3</td>
<td>—</td>
<td>9.5</td>
<td>0.1</td>
<td>4.5</td>
<td>16.7</td>
<td>32.9</td>
<td>4.0</td>
<td>0.4</td>
<td>0.2</td>
<td>0.1</td>
</tr>
</tbody>
</table>

1 Based on manufacturer’s analyses except where indicated otherwise. Formula S/C, 60% soy oil and 40% coconut oil; Formula PO/S, 53% palm olein and 47% soy oil.
2 Based on investigators’ analysis: protein = nitrogen × 6.25.
3 Fat blend of 60% soy oil and 40% coconut oil.
4 Fat blend of 53% palm olein and 47% soy oil.

Subjects

Eleven normal infants (eight females, three males) participated in the study. An additional infant became ill during the second balance study; the study could not be rescheduled and data for this 12th infant are not presented. Eight infants (six females, two males) were full term, with birth weights > 2500 g. Twin infants born after 36 wk gestation had a birth weight of 2295 g (female) and 2410 g (male); one (female) infant born after 31 wk of gestation weighed 1760 g. At the time of the balance studies, the full-term infants ranged in age from 27 to 137 d and weighed between 4000 and 7260 g. The three preterm infants were between 140 and 161 d of age and weighed between 5010 and 7680 g. All infants lived at home between studies. Six infants were admitted to the Lora N. Thomas Metabolism Ward for 72-h metabolic balance studies; the other five infants had 96-h stool collections performed at home. The study protocol was approved by the University of Iowa Committee on Research Involving Human Subjects. The study was explained to one or both parents and written consent was obtained.

Feedings

The two study formulas were similar in composition as was possible, except for the sources of fat. The formula containing 60% soy oil and 40% coconut oil (Formula S/C) was Similac with Iron (Ross Products Division, Abbott Laboratories, Columbus, OH) as marketed at the time of the study (1992–1993). The other formula (Formula PO/S) provided fat from palm olein oil (53%) and soy oil (47%). The fatty acid composition of both formulas is listed in Table 1. In both formulas protein was derived from nonfat cow milk (casein-predominant) and the carbohydrate was lactose. The formulas were provided in ready-to-feed form in 946-mL (32-oz) cans. Study formulas were fed for 7–13 d before stool collections began.

Procedures

Studies in which infants were admitted to the metabolism ward and in which separate fecal and urinary collections were performed are referred to as metabolic balance studies. Studies in which stools were collected at home with the use of cloth diapers are referred to as home stool collections.

Metabolic balance studies (72 h)

Six infants were admitted to the metabolism ward for 4 d and 3 nights. Stools (between carmine markers) and urine were collected separately by using methods described by Fomon (11). Formula intake was determined by weighing the full containers and weighing back empty or partially empty cans. Seventy-two-hour stool pools were weighed and homogenized by vigorous stirring, and aliquots were saved for mineral analyses. A separate aliquot for fat determination was made into a slurry by adding deionized water and was acidified with hydrochloric acid (final concentration 1 mol/L). Urine aliquots were saved for analysis.

Home stool collections (96 h)

Five infants were brought to the metabolism ward for application of the first diaper and administration of carmine with the first feeding. The infants then returned home, where feces were collected between carmine markers with the aid of acid-washed cloth diapers. Briefly, the method involved collection of stool and urine with cloth diapers that were prepared by laundering, followed by rinsing in ample volumes of 1% nitric acid followed by deionized water. All soiled diapers were returned to the laboratory daily, where diapers containing fecal matter were stored at −20°C. Diapers containing only urine were laundered and acid-washed for reuse. After thawing, solid fecal matter was scraped from the diapers and treated in the same manner as the feces from the 72-h balance studies. Diapers were then soaked in and eluted four times with generous volumes of 1% nitric acid, the pooled eluate filtered, and an aliquot of the filtrate saved for calcium analysis. The particular matter retained by the filter paper and the diapers, after drying at room temperature, was extracted with hot 95% ethanol in a Soxhlet (Ace Glass Inc, Vineland, NJ) apparatus for 24 h. Aliquots of the ethanol extract were saved for fat determination and also for determination of calcium, of which the ethanol extract contained small quantities. Intake of formula was determined as described for the metabolic balance studies.

Stool collections obtained by this method inevitably include some urine. For nutrients that are not excreted in the urine (ie, fat), this admixture is inconsequential and for nutrients whose urinary excretion is low in infancy (ie, calcium), a urinary admixture introduces a trivial error. However, for nutrients that are substantially excreted in the urine (eg, magnesium, nitrogen, phosphorus), an admixture of urine would create large errors in fecal excretion and absorption. Absorption of these
nutrients can therefore not be determined by our home stool-collection method.

**Methods**

Excreta were analyzed essentially as described previously (12). Aliquots of formulas and of stool homogenates were dried and ashed overnight at 525 °C in a muffle furnace and ashes were dissolved in dilute hydrochloric acid. Determinations of calcium and magnesium in ashes, urine (directly), and ethanol extracts of diapers (after evaporation of the ethanol) were performed by atomic-absorption spectrophotometry (model 560; Perkin-Elmer, Norwalk, CT). Fat was determined in stool slurries and in ethanol extracts by using a modification of the method of van de Kamer et al (13). Nitrogen in formula, stool, and urine was determined by the micro-Kjeldahl method and microdiffusion (14). Phosphorus in ashes and urine was determined by the phosphomolybdate method by using a Gilford SBA 300 (CIBA-Corning, Oberlin, OH) automated laboratory analyzer.

Determination of stool fatty acids was performed on chloroform:methanol (2:1, by vol) extracts of stool homogenates from metabolic balance studies, or aliquots of the ethanol extracts for home stool collections. Extracts were dried under nitrogen, lipids were saponified with 0.5 mol KOH/L in methanol at 100 °C for 10 min and derivatized in 12–15% BF₃ in methanol as described by Metcalfe and Schmitz (15), and fatty acids were separated by capillary gas chromatography (model 5890A; Hewlett-Packard, Palo Alto, CA) equipped with a flame-ionization detector and using an Omegawax 320 column (Supelco Inc, Bellefonte, PA). Because of a laboratory error, fatty acid analysis was incomplete in one subject and data are reported for only 10 subjects.

**Data analysis**

Absorption of nutrients was calculated as intake minus fecal excretion, and retention as intake minus fecal plus urinary excretion. In home stool collections, fecal fat was calculated as the sum of fat in the solid fecal matter plus that in the ethanol extract; fecal calcium was calculated as the sum of calcium in the solid fecal matter plus calcium in the eluate plus calcium in the ethanol extract. Absorption of individual fatty acids was calculated as intake (total formula fat × fractional fatty acid composition) minus fecal excretion (total fecal fat × fractional fatty acid composition).

Absorption and retention (% of intake) data were analyzed by randomized block, mixed-effects analysis of variance, equivalent to a paired t test analysis. Data on excretion, absorption, and retention of minerals (mg·kg⁻¹·d⁻¹) and fat (g·kg⁻¹·d⁻¹) were analyzed by randomized block analysis of covariance with adjustment for intake. Carryover and feeding-order effects were not detected and were therefore eliminated from the statistical models. Data from metabolic balance studies and from home stool collections showed homogeneity (similar means and variances) and were therefore combined for analysis. Partial correlations between fat and calcium excretions were determined by general linear models with adjustment for formula. SAS version 6.08 (SAS Institute Inc, Cary, NC) was used for the statistical analysis.

**RESULTS**

Data regarding intake, excretion, and absorption of total fat and selected fatty acids are summarized in Table 2. Intake of total fat was similar with the two formulas. Fecal excretion of...
total fat averaged 0.47 g·kg⁻¹·d⁻¹ with Formula PO/S and 0.24 g·kg⁻¹·d⁻¹ with Formula S/C (P < 0.001). Mean percentage absorption of fat was 90.6% of intake with Formula PO/S and 95.2% of intake with Formula S/C (P < 0.001). It is evident from Figure 1 that absorption of fat was less from Formula PO/S than from Formula S/C in every infant.

Fatty acids with a chain length of 10:0 or shorter were not detected in fecal extracts (detection limit: 0.1% of fatty acids), and only very small amounts of lauric acid were excreted in feces. Fecal excretion of myristic acid, on the other hand, accounted for 7.4% of intake from Formula S/C and 12.5% of intake from Formula PO/S. Intake of palmitic acid was substantially higher with Formula PO/S (1.253 g·kg⁻¹·d⁻¹) than with Formula S/C (0.473 g·kg⁻¹·d⁻¹). Fecal excretion of palmitic acid was 0.313 g·kg⁻¹·d⁻¹ when Formula PO/S was fed and 0.090 g·kg⁻¹·d⁻¹ when Formula S/C was fed. The difference in palmitic acid excretion (0.223 g·kg⁻¹·d⁻¹) thus accounted quantitatively for the difference in total fat excretion (0.227 g·kg⁻¹·d⁻¹). Absorption of palmitic acid was lower (75.1% of intake) from Formula PO/S than from Formula S/C (81.1% of intake; P < 0.001). Excretion of stearic acid was slightly greater from Formula PO/S than from Formula S/C and the percentage absorption was slightly less from Formula PO/S than from Formula S/C (P < 0.05). Intake of oleic acid was greater from Formula PO/S than from Formula S/C, but the percentage absorption of oleic acid was equally high (97.1% and 97.3%, respectively) from both formulas. Intake, excretion, and absorption of linoleic acid were similar with the two formulas.

Table 3 presents balance data for calcium, phosphorus, magnesium, and nitrogen for the six infants who underwent metabolic balance studies. Intake of calcium was similar with the two formulas, but fecal excretion of calcium was significantly greater (P < 0.05) and the percentage absorption of calcium was significantly lower (P < 0.01) when Formula PO/S was fed than when Formula S/C was fed. Retention of calcium was also significantly (P < 0.01) lower with Formula PO/S than with Formula S/C. For all 11 infants, including the 5 who had home stool collections, the percentage calcium absorption was 39.0% when Formula PO/S was fed and 48.4% when Formula S/C was fed; the difference was significant (P < 0.01). Paired values for individual infants (Figure 2) show that the percentage calcium absorption was lower in the majority of subjects when Formula PO/S was fed. The correlation between percentage absorption of calcium and fat was significant (partial correlation coefficient r = 0.589, P < 0.05). The relation between the fecal excretion of fat and calcium is illustrated in Figure 3. The correlation was significant (r = 0.734, P < 0.001). The correlations between fecal excretion of palmitic acid and calcium (r = 0.785, P < 0.001) and stearic acid and calcium (r = 0.704, P < 0.001) were also significant.

The percentage absorption of phosphorus was greater in infants fed Formula PO/S than in those fed Formula S/C (Table 3; P < 0.05); however, urinary excretion of phosphorus was also greater (P < 0.05) and retention of phosphorus was lower (P < 0.05) by infants fed Formula PO/S. There were no significant differences in excretion, absorption, or retention of magnesium or nitrogen.

Results obtained with the home stool collection method were similar to those obtained with the metabolic balance method. Absorption of fat from Formula PO/S averaged 90.2% with metabolic balances (n = 6) and 91.0% with home stool collections (n = 5). Absorption of fat from Formula S/C averaged 95.2% with metabolic balances (n = 6) and 95.2% with home stool collections (n = 5). Similarly, calcium absorption from Formula PO/S averaged 38.2% with metabolic balances and 39.9% with home stool collections. Absorption of calcium from Formula S/C averaged 53.3% with metabolic balances and 42.4% with home stool collections.

Note that the three preterm infants, all of whom underwent metabolic balance studies, differed somewhat from the full-term infants with regard to calcium absorption. While fed Formula S/C, the premature infants had an average calcium absorption of 57.5% whereas the three full-term infants who underwent metabolic balance studies had an average calcium absorption of 49.1%. Premature infants generally tend to absorb calcium more efficiently (16) than full-term infants (17). Differences of similar magnitude were noted for calcium absorption in studies with Formula PO/S. There were no noteworthy differences in fat absorption between premature and full-term infants.

**DISCUSSION**

Our results showed that a mixture of palm olein and soy oil, although it provides proportions of palmitic and oleic acids similar to those of human milk fat, was less well absorbed by infants than was a blend of soy and coconut oils. A comparison of these results with published data (1, 18) and unpublished data from our laboratory also showed that the palm olein and soy oil blend was less well absorbed than the soy oil and coconut oil blend used in the present study, was less well absorbed than other vegetable fat blends used in infant formulas, and was less well absorbed than human milk fat.

We used the method of van de Kamer et al (13) to determine total fecal fat. It has been recognized for some time that the method does not yield quantitative extraction of medium-chain fatty acids with a carbon chain length ≤ 12:0 (19–22). The method is therefore not suitable when feces contain significant amounts of medium-chain fatty acids. In the present study, 23.9% of the fatty acids provided by Formula S/C had 12 or fewer carbons. However, fatty acid analysis of hot ethanolic or
TABLE 3
Intake, excretion, absorption, and retention of minerals and nitrogen in metabolic balance studies from Formula S/C and PO/S.

<table>
<thead>
<tr>
<th></th>
<th>Intake (mg · kg⁻¹ · d⁻¹)</th>
<th>Excretion (mg · kg⁻¹ · d⁻¹)</th>
<th>Absorption (mg · kg⁻¹ · d⁻¹)</th>
<th>Retention (mg · kg⁻¹ · d⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Urine</td>
<td>Feces</td>
<td>% of intake</td>
<td>% of intake</td>
</tr>
<tr>
<td>Calcium</td>
<td>S/C</td>
<td>82.2 ± 13.9</td>
<td>5.4 ± 4.5</td>
<td>38.5 ± 9.3</td>
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<tr>
<td></td>
<td>PO/S</td>
<td>77.5 ± 12.3</td>
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<td>Phosphorus</td>
<td>S/C</td>
<td>71.3 ± 12.1</td>
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<tr>
<td></td>
<td>PO/S</td>
<td>68.4 ± 10.8</td>
<td>31.3 ± 7.7²</td>
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<tr>
<td>Magnesium</td>
<td>S/C</td>
<td>8.6 ± 1.5</td>
<td>3.0 ± 0.5</td>
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<tr>
<td></td>
<td>PO/S</td>
<td>8.3 ± 1.3</td>
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<tr>
<td>Nitrogen</td>
<td>S/C</td>
<td>329 ± 56</td>
<td>155 ± 35</td>
<td>42 ± 9</td>
</tr>
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<td></td>
<td>PO/S</td>
<td>326 ± 52</td>
<td>153 ± 38</td>
<td>46 ± 4</td>
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¹ ± SD; n = 6. Formula S/C, 60% soy oil and 40% coconut oil; Formula PO/S, 53% palm olein and 47% soy oil.
²,³ Significantly different from Formula S/C. ²P < 0.05, ³P < 0.01.

chloroform:methanol extracts prepared from acidified feces showed only very small amounts of myristic acid (Table 2) and essentially no fatty acids of shorter chain length. Thus, because there were only minuscule amounts of medium-chain fatty acids in the feces of our study subjects, it appears highly unlikely that the use of the method of van de Kamer et al (13) could have resulted in significant underestimation of total fat in stools collected while infants were fed Formula S/C.

Human milk fat is very well absorbed by infants (1) despite its high content in saturated fatty acids (45% of fatty acids, including 23% of palmitic acid). This good absorption is attributed to the fact that ~70% of the palmitic acid is in the sn-2 position (6, 10). In contrast, in fats of vegetable origin < 15% of palmitic acid is located in the sn-2 position (8, 9). In palm oil, which contains 44–48% palmitic acid, only ~9% of the palmitic acid is in the sn-2 position (8, 9).

Free palmitic acid liberated by pancreatic lipase from the sn-1 and sn-3 positions of triacylglycerol is poorly absorbed, whereas palmitic acid is well absorbed as the 2-monopalmitin (5, 23–25). The superior absorption of palmitic acid esterified in the sn-2 position is supported by results of absorption studies in infants (7). In the present study, overall absorption of palmitic acid was 81.1% and 75.1%, respectively, from Formulas S/C and PO/S. Although the absorption of stearic acid was equally low, it contributed only ~5% of fatty acids in the two study formulas. It's relatively poor absorption therefore had little effect on overall fat absorption.

Our study also showed greater fecal excretion of calcium and, hence, a lower percentage absorption of calcium when a blend of palm olein and soy oil was the source of fat. Fecal excretion of calcium was closely related to the fecal excretion of fat (Figure 3). Palmitic acid that is not absorbed is prone to form insoluble calcium soaps in the intestinal tract. Therefore, a plausible explanation for the lower calcium absorption is that unabsorbed palmitic acid led to the formation of insoluble calcium soaps, rendering a portion of dietary calcium unavailable for absorption. The observation that the presence of unabsorbed dietary fatty acids leads to increased fecal calcium loss is, of course, not new. Widdowson et al (26, 27) and others (28) showed clearly that in normal infants poor fat absorption...
is associated with poor absorption of calcium. In the present study, urinary phosphorus excretion increased and phosphorus retention decreased when infants were fed Formula POS, presumably reflecting lower availability of calcium for deposition in bones.

Similarly, the fact that we used two different methods for stool collection, the established metabolic balance technique and our newly developed technique for home stool collections, could not have affected the results of the paired comparisons. Furthermore, in direct comparisons of infants, the two methods have yielded similar results for fat and calcium absorption (unpublished observations, 1996). In the present study, absorption of fat and calcium was similar with the two methods.

Whether the reduced absorption of fat and calcium caused by the inclusion of palm olein in infant formula is clinically relevant is a matter of perspective. Fecal loss of an additional 0.231 g fat/kg represents a loss of ≈10.45 kJ/kg (≈2.5 kcal/kg) each day. Normal infants are certainly capable of increasing energy intake proportionately to make up for an energy loss of this magnitude. Similarly, decreased retentions of calcium and phosphorus suggest decreased deposition of bone mineral. Yet, retentions of calcium and phosphorus (mg/kg) by infants fed Formula POS were only slightly lower than those observed in breast-fed infants (17). On the other hand, there is no evidence of an advantage of feeding infants formulas with percentages of palmitic and oleic acids similar to those in human milk. Unless a source of fat is used with a high percentage of palmitic acid in the sn-2 position, a formula with a palmitic acid content similar to that of breast milk seems to offer no advantages.

We conclude that increasing the proportions of palmitic and oleic acids in infant formula by replacing coconut oil with palm olein leads to decreased absorption of fat and calcium compared with a blend of soy oil and coconut oil. The difference in overall fat absorption was quantitatively explained by the behavior of palmitic acid, which was poorly absorbed. Commensurate with decreased fat absorption, the absorption and retention of calcium and the retention of phosphorus decreased, suggesting diminished bone mineral deposition. The clinical relevance of these effects is uncertain.

REFERENCES