Original Paper

Absorption of Fat and Calcium by Infants Fed a Milk-Based Formula Containing Palm Olein

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Key words: Palm olein, infant formula, fat absorption, calcium absorption

Objective: The study tested the hypothesis that inclusion of palm olein (45% of fat) in the fat blend of a milk-based infant formula decreases the absorption of fat and calcium.

Methods: Formula PO contained palm olein (45%) in addition to soy, coconut and high-oleic sunflower oils (20%, 20%, and 15%, respectively); Formula HOS contained high-oleic safflower oil (42%) in addition to coconut and soy oils (30% and 28%, respectively) and no palm olein. Fat and calcium levels in the two formulas were similar. In a balanced crossover design, fat and calcium absorption were determined in 10 normal infants ranging in age from 22 to 192 days. In three infants metabolic balance studies with complete separation of urine and feces were performed, whereas in seven infants excreta were in part collected at home, resulting in incomplete separation of urine and feces.

Results: Mean (±SD) fecal excretion of fat was higher when Formula PO was fed than when Formula HOS was fed (0.55±0.29 vs. 0.09±0.04 g/kg/day; p<0.001). Hence % fat absorption was lower with PO than with HOS (90.0±6.4 vs. 98.5±0.6% of intake; p<0.01). The difference in percent fat absorption was explained by significantly (p<0.05) lower % absorption of palmitic (16:0) and stearic (18:0) acids when Formula PO was fed than when Formula HOS was fed. Fecal excretion of calcium was higher with Formula PO than with Formula HOS (53.4±12.0 vs. 37.4±14.9 mg/kg/day; p<0.01), and hence % calcium absorption was lower with Formula PO than with Formula HOS (37.5±11.5 vs. 57.4±14.9%; p<0.001).

Conclusion: Absorption of fat and calcium by normal infants is lower when palm olein provides a substantial proportion of formula fat than when formula does not contain palm olein.

INTRODUCTION

The blends of vegetable oils used in infant formulas are selected to match the excellent absorption by the infant of human milk fat, but they differ considerably from human milk fat in their fatty acid profile. Aside from the virtual absence of long-chain polyunsaturated fatty acids, most formula fat blends are lower than human milk fat in palmitic acid (C16:0) and oleic acid (C18:1) content. Palm oil is one of the few plant oils that are rich in palmitic acid. However, contrary to human milk fat, in which palmitic acid is esterified predominantly in the sn-2 position [1,2], in palm oil palmitic acid is esterified predominantly in the sn-1 and sn-3 positions [3]. When present predominantly in the sn-1 and sn-3 positions, palmitic acid is known to be absorbed to a lesser degree by rats [4,5] and human infants [6,7] than when it is present predominantly in the sn-2 position.

Palm olein, a lower-melting fraction of palm oil, contains 40% palmitic acid and 43% oleic acid. It is used in some commercially available infant formulas. In a previous study [8] we found that when palm olein provided the major proportion (53%) of the fat blend of a milk-based infant formula, with soy oil providing the remainder (47%), fat and calcium absorption were significantly lower than from a milk-based formula containing coconut oil (40%) and soy oil (60%). The lower overall fat absorption was nearly quantitatively explained by poor absorption of palmitic acid from the palm olein-containing formula. Lower calcium absorption was presumed to be secondary to formation of insoluble calcium soaps.

In our previous study [8] the two fat blends differed greatly...
in fatty acid composition. For example, the formula with coconut and soy oils provided 18% of fatty acids from lauric acid (C12:0) and an additional 5.9% from shorter chain fatty acids (C6:0-C10:0), whereas the palm olein-containing formula provided only 0.2% of fatty acids from lauric acid and no shorter-chain fatty acids. It remained unclear whether the inclusion of palm olein would also cause a reduction of overall fat absorption when compared to a fat blend with similar proportions of medium chain-length fatty acids. The present study was undertaken to answer this question. We tested the hypothesis that inclusion of palm olein (45% of fat) in the fat blend leads to a decrease of fat and calcium absorption compared to a blend without palm olein but of otherwise similar fatty acid composition. The study showed that inclusion of palm olein caused a decrease in absorption of fat and calcium of a magnitude similar to that observed in the previous study [8].

**MATERIALS AND METHODS**

**Study Design**

In a randomized crossover study design, metabolic balance studies were conducted with each of the two study formulas in ten normal infants. The interval between studies was 7 or 14 days in four infants each, and was 21 and 28 days in one infant each. The study formula was fed for at least 3 days before the start of a balance study. The order in which formulas were studied was predetermined and random. The formulas were code-labeled and the investigators were thus blinded.

**Subjects**

Ten infants (four females and six males) participated in the study. All were singletons. Eight infants were born at term (gestation 39 to 40 weeks) with birth weights between 3545 and 4850 g. One infant was born at 37 weeks of gestation weighing 2665 g and one at 34 weeks weighing 2975 g. At the start of the first study, nine infants were between 22 and 91 days old and one infant was 192 days old. Infants lived at home between studies. The study protocol was approved by the University of Iowa Committee on Research Involving Human Subjects and one or both parents provided written informed consent.

**Feedings**

The two study formulas were similar in composition except for the source of fat (Table 1). One formula (Formula PO) provided fat from a blend of palm olein (45%), soy oil (20%), coconut oil (20%) and high-oleic sunflower oil (15%). The other formula (Formula HOS) contained a blend of high-oleic safflower oil (42%), coconut oil (30%) and soy oil (28%).

<table>
<thead>
<tr>
<th>Table 1. Composition of Study Formulas*</th>
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<tr>
<td>Protein (g/L)**</td>
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<tr>
<td>Lactose (g/L)</td>
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<td>Calcium (mg/L)**</td>
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<td>Phosphorus (mg/L)**</td>
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<td>Magnesium (mg/L)**</td>
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<td>Fat (g/L)</td>
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<td>Palm olein (%)</td>
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<tr>
<td>Soy oil (%)</td>
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<td>Coconut oil (%)</td>
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<tr>
<td>High-oleic sunflower oil (%)</td>
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<tr>
<td>High-oleic safflower oil (%)</td>
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<tr>
<td>Fatty acids (% by wt)</td>
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<td>8:0</td>
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<tr>
<td>10:0</td>
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<td>12:0</td>
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<tr>
<td>20:0</td>
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* Manufacturer’s analyses except where indicated otherwise.

**Authors’ analysis (protein = nitrogen × 6.25).**

† Reference values from Lammi-Keefe & Jensen [9].

fatty acid profiles are included in Table 1, along with the typical profile of breast milk. Both formulas contained oleic acid in a proportion similar to human milk; palmitic acid was lower in Formula HOS than in human milk. The protein of both formulas was derived from cow milk with added bovine whey proteins, and the carbohydrate was lactose. Both formulas contained the customary amounts of vitamins and trace minerals believed to satisfy the needs of normal infants. Formula HOS contained added nucleotides. The formulas were supplied in ready-to-feed form in 32-oz. cans labeled only by code. Formula PO was Enfamil® (Mead Johnson Nutritional Group, Evansville, IN) as marketed at the time of purchase (1993), and Formula HOS was similar to the formula subsequently marketed as Improved Similac® with Iron (Ross Products Division, Columbus, OH).

**Procedures**

Three infants underwent metabolic balance studies, for which they were admitted to the Lora N. Thomas Metabolic Unit for 3 nights and 4 days. Stool and urine were collected separately for 72 hours between carmine markers using the procedures described by Fomon [10]. Seven infants had 96-hour stool collections performed using a combination of the metabolic balance technique and the home stool collection method. As described previously [8], with this combined method infants were admitted during the daytime hours to the
Metabolic Unit where separate collections of stool and urine were performed. Infants spent the evening and night hours at home, where collections of feces and urine together were performed with the use of cloth diapers. Intake of formula was determined by weighing the full containers and by re-weighing empty or partially empty containers. Excreta were handled as described previously [8].

Methods

Stools collected during the 72 hour metabolic balance studies were pooled for each subject. They were homogenized by vigorous stirring and aliquots saved for analyses of fat and calcium. In home collections, fecal matter was scraped from the diapers, added to the fecal pool from the daytime collection, and homogenized. Diapers were then eluted with nitric acid directly before studies. Nevertheless, data from the study with Formula PO of Subject #6451 revealed unusually low absorption of fat and calcium. Stool volume and consistency were normal. Results from this study were therefore included, but statistical analyses were also performed after excluding all data from this subject.

Intake, fecal excretion and absorption of total fat and of selected fatty acids are summarized in Table 2. Total fat intake was not different, but, as expected, intake of palmitic acid from Formula PO was about three times that from Formula HOS. Intakes of other fatty acids were similar. Fecal excretion of total fat averaged 0.55 g/kg/day when Formula PO was fed, but averaged only 0.09 g/kg/day when Formula HOS was fed. The difference was statistically significant (p<0.001) and remained so (p<0.001) after exclusion of data from Subject #6451. Mean fecal fat excretions after excluding Subject #6451 were 0.46 and 0.09 g/kg/day, respectively, with Formula PO and Formula HOS. Fecal excretion of palmitic acid averaged 0.34 g/kg/day with Formula PO and 0.04 g/kg/day with Formula HOS. The difference was statistically significant (p<0.01). More than half the excess fecal fat with Formula PO was accounted for by palmitic acid. Fecal excretion of stearic acid was also significantly (p<0.01) higher with Formula PO, but, because stearic acid contributed only 4.7% of the fatty acid intake from Formula PO, the contribution of stearic acid to total fecal fat was much smaller than that of palmitic acid.

Absorption of fat averaged 5.09 g/kg/day from Formula PO and 5.66 g/kg/day from Formula HOS. The difference was statistically significant (p<0.05). If data for subject #6451 were excluded, mean absorption of fat was 5.29 g/kg/day with Formula PO and 5.66 g/kg/day with Formula HOS; the difference was of borderline significance (p=0.07). Expressed as a percentage of intake, fat absorption was on average 90.0% with Formula PO, and 98.5% with Formula HOS. The difference was statistically significant (p=0.003). After exclusion of data for Subject #6451, mean values for % absorption were 92.0% and 98.4%, respectively. The difference remained statistically significant at p<0.001. Fig. 1 indicates that in every infant percentage fat absorption was less than Formula PO was fed than when Formula HOS was fed.

Absorption of palmitic acid from Formula PO averaged only 70.5% of intake, significantly (p<0.05) lower than from Formula HOS, from which average absorption was 91.7%. Similarly, absorption of stearic acid was significantly (p<0.04) lower from Formula PO than from Formula HOS. Fecal excretion of these two saturated fatty acids was significantly (p<0.001) correlated with total fat excretion (r=0.899 for

RESULTS

Intake of formula averaged 919 (SD 228) g/day with Formula PO and 945 (SD 244) g/day with Formula HOS. The difference was not statistically significant. No infant showed temperature elevation or other signs of illness during or immediately before studies. Nevertheless, data from the study with Formula PO of Subject #6451 revealed unusually low absorption of fat and calcium. Stool volume and consistency were normal. Results from this study were therefore included, but statistical analyses were also performed after excluding all data from this subject.

Data Analysis

Absorption (net) of nutrients was calculated as intake minus fecal excretion. Intakes of fat and calcium were calculated from the weight of formula consumed, multiplied by the respective fat and calcium concentrations. In studies with home collections, fecal fat was calculated as the sum of fat in the solid fecal matter plus fat in the ethanol extract, and fecal calcium was calculated as the sum of calcium in the solid fecal matter plus fat in the ethanol extract. The absorption of individual fatty acids was calculated as fatty acid intake [formula fat intake×0.95×formula fatty acid wt%/100] minus fecal fatty acid excretion [fecal fat×fecal fatty acid wt%/100]. The 0.95 reflects the fatty acid proportion in a typical triacylglycerol.

Data on absorption and excretion were analyzed by repeated measures analysis of variance. Data obtained by the 72-hour metabolic balance studies were combined with those obtained by 96-hour home collections after preliminary analysis demonstrated the absence of effects of the collection method on absorption data. Data were also tested for carryover effects (at p<0.10) and for order effects (at p<0.05) using a crossover analysis with two-sample t-tests. No carryover or order effects were detected. Partial correlations between fecal calcium and fecal fat, palmitic (16:0) or stearic (18:0) acid were determined by general linear models procedures with adjustment for the effect of formula.
palmitic acid and \( r = 0.635 \) for stearic acid. The major unsaturated fatty acids, oleic (C18:1n9) and linoleic (C18:2n6) were also significantly less well absorbed from Formula HOS than from Formula PO, but the contribution of these fatty acids to total fecal fat excretion was negligible.

**Intake, Excretion, and Absorption of Calcium**

Table 3. Intake, Excretion and Absorption of Calcium

<table>
<thead>
<tr>
<th>Intake (mg/kg/day)</th>
<th>Fecal excretion (mg/kg/day)</th>
<th>Absorption** (mg/kg/day) (% of intake)</th>
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<tbody>
<tr>
<td>Formula PO</td>
<td>86.0 (15.9)</td>
<td>53.4 (^1) (12.0)</td>
</tr>
<tr>
<td>Formula HOS</td>
<td>86.8 (14.2)</td>
<td>37.4 (14.9)</td>
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</table>

*Mean (SD).  
**Intake minus fecal excretion.

1 Significantly \((p<0.01)\) different from Formula HOS.

2 Significantly \((p<0.001)\) different from Formula HOS.

**DISCUSSION**

The results of this study confirm our previous finding [8] that palm olein is poorly absorbed by infants from a milk-based formula. In the previous study [8] palm olein provided 53% of fatty acids, with soy oil providing the remainder. In the present study palm olein provided 45% of fatty acids, with the remainder being provided by soy, coconut and high-oleic sunflower
oils. Total fat absorption from the palm olein-containing fat blends was similar in the two studies: 90.6% of intake in the previous study and 90.0% of intake in the present study. The conclusion that fat absorption is somewhat reduced when palm olein is used as part of the fat blend of milk-based infant formulas is thus well supported. The control formula in the previous study contained a substantial proportion of medium-chain fatty acids, whereas the control formula in the present study did not. Total fat absorption from the two control formulas was 95.2% and 98.5% of intake, respectively.

Palmitic acid accounted for a large proportion of unab sorbed (fecal) fat in both the previous and the present study. In palm oil, only about 9% of the palmitic acid is located in the sn-2 position [3,13], and hence almost all the palmitic acid is present in the sn-1 and sn-3 positions. It is generally agreed that pancreatic lipase liberates preferentially fatty acids esterified in the sn-1 and sn-3 positions, sparing fatty acids in the sn-2 position. The resulting 2-mono-acyl-glycerol is well absorbed regardless of the chain length and/or degree of saturation of the fatty acid. There is good evidence that it is absorbed intact [14]. The excellent absorption of human milk fat [15], despite its high content of palmitic acid (22% of fatty acids), is generally attributed to the fact that 70% of the palmitic acid in human milk fat is present in the sn-2 position [1,2]. On the other hand, the efficiency of absorption of free fatty acids liberated from the sn-1 and sn-3 positions depends on the nature of the fatty acids. Long-chain saturated fatty acids, such as palmitic and stearic acids, are poorly absorbed and are prone to the formation of insoluble soaps with calcium and magnesium. In the present study, absorption of palmitic acid from Formula PO averaged 70.5% and that of stearic acid averaged 63.6%. Together, these two fatty acids accounted for about 80% of the excess fecal fat excreted by infants fed Formula PO.

The present study also confirmed that absorption of calcium is lower when palm olein is present than when it is absent from a milk-based formula. The fact that fecal excretion of calcium was correlated with fecal excretion of palmitic acid and total fat reinforces the interpretation that the formation of insoluble soaps occurs when saturated fatty acids are liberated in the presence of calcium.

Because the present study utilized a balanced crossover design in which each infant served as his/her own control, neither the inclusion of two premature infants nor the wide subject age range affected the interpretability of results. Moreover, there was no detectable trend toward an age effect on fat or calcium absorption. With regard to gestational age, only absorption of calcium, not fat, showed a trend toward higher percent absorption in the two premature infants, an observation that had been noted in our previous study [8].

The clinical relevance of the findings of the present study remains uncertain. Quinlan et al [16] demonstrated that stool hardness is a function of the concentration of calcium soaps of saturated fatty acids. Stool consistency was not determined in the present study, but it appears possible that palm olein in an infant formula may lead to harder stools. Somewhat increased fecal loss of dietary fat and hence energy was associated with feeding of palm olein-containing formula. As we have stated before [8], it is quite likely that infants are able to compensate for the modest loss of energy by consuming more formula. Similarly, the clinical importance of decreased absorption of calcium cannot be assessed from our short-term metabolic balance studies. Long-term effects, e.g., on bone mineral content, although theoretically possible, are not likely to occur since several other factors, besides percentage calcium absorption, interact to determine bone mineral content over the long term.

ACKNOWLEDGMENT

Supported in part by a grant from Ross Products Division of Abbott Laboratories.

REFERENCES

Palm Olein and Fat and Calcium Absorption


Received October 1997; revision accepted December 1997.