The role of fatty acid composition and positional distribution in fat absorption in infants

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Fat digestion and absorption in the infant is a multistep process. An initial gastric phase of lipolysis generates modest amounts of diglycerides, monoglycerides, and free fatty acids. These initial digestion products, as well as bile salts, are required for optimal activity of the intestinal phase of lipolysis. Colipase-dependent pancreatic lipase catalyzes the intraduodenal phase of triglyceride digestion in formula-fed infants; in breast-fed infants this process is also mediated by bile salt-stimulated lipase. Triglyceride fatty acid positional distribution may modulate the efficiency of nutrient absorption. Human milk contains palmitic acid (C16:0) primarily in the sn-2 position; infant formula fat blends contain palmitic acid predominantly in the sn-1 and sn-3 positions. Because pancreatic lipase selectively hydrolyzes triglycerides at the sn-1 and sn-3 positions, free fatty acids and 2-monoglycerides are produced. Free palmitic acid, but not 2-monopalmitin (which is efficiently absorbed), may be lost as a calcium-fatty acid soap in the feces. As a result, many infant formulas contain substantial levels of well-absorbed saturated fatty acids of shorter chain lengths (e.g., C4:0) in place of palmitic acid. Means of increasing the proportion of 2-palmitic acid in infant formula may make possible fat blends closer to that of human milk with acceptable absorption characteristics. (J PEDIATR 1994;125:628)

The rapidly growing neonate requires dietary fat to provide approximately 50% of total dietary energy and to supply essential fatty acids. All infant formulas currently in use are similar to human milk in their total fat content but vary substantially in their fatty acid profile. Saturated and monounsaturated fatty acids predominate in human milk, each accounting for approximately 40% of the total fatty acids. The polyunsaturated fatty acid components of human milk are complex, including both the C18 precursors, linoleic acid (18:2ω-6) and α-linolenic acid (18:3ω-3), and the bioactive very long chain polyunsaturated fatty acids of both the ω-6 and the ω-3 families. The fatty acid profiles of some formulas closely resemble that of human milk (with the exception of VLCPUFA); others differ substantially. Both the fatty acid composition and triglyceride structure may influence the digestion and absorption of dietary fat.

MECHANISMS OF FAT DIGESTION

Triglyceride digestion is initiated by a gastric hydrolysis phase. The lipase involved is derived from either the serous glands on the dorsal posterior of the tongue (lingual lipase) or from glands within the gastric mucosa. This gastric phase involves hydrolysis primarily to diglycerides, monoglycerides, and free fatty acids and occurs at all three triglyceride positions, with the sn-1 and sn-3 positions slightly favored. This lipase is active in the mildly acidic environment of the infant stomach (pH 4.5 to 5.5) and is inactivated by pancreatic proteases. In vitro studies have demonstrated that the enzyme is under feedback inhibition...
from released free fatty acids, which limits this phase of fat digestion. The initial gastric lipolysis of triglycerides is crucial to the digestive process, especially in infants with immature pancreatic function and low concentration of bile salts, because the end products serve as emulsifying agents for the intestinal phase of digestion. After passage of the partially digested triglycerides into the duodenum, mixed micelles are formed and colipase-dependent pancreatic lipase proceeds with subsequent lipolysis, resulting in the generation of free fatty acids and 2-monoglycerides, which are absorbed directly (discussion to follow). Another pancreatic enzyme, carboxyl ester lipase, may play a role in the digestion of triglycerides that contain VLCUFAs. Pancreatic lipase is relatively inefficient in digesting marine oils or arachidonic acid-containing triglycerides; the combination of pancreatic lipase and carboxyl ester lipase yields much more efficient hydrolysis of these triglycerides. This dual-enzyme system may play an important role in the digestion of the lipids added to infant formula that contain VLCUFAs. Bile salt-stimulated lipase, which is present in human milk, plays a role in triglyceride digestion in the breast-fed infant; it is thoroughly reviewed elsewhere in these proceedings.

THE EFFECT OF FATTY ACID SATURATION, TRIGLYCERIDE STRUCTURE, AND MINERALS ON ABSORPTION

A landmark study by Holt et al. evaluated many fats for their potential use in infant formula. This study demonstrated that unsaturated fatty acids and medium-chain saturated fatty acids, including lauric acid, C12:0, were more efficiently absorbed than long-chain saturated fatty acids such as C14:0, C16:0, and C18:0. These investigators demonstrated that the absorption process matured during the first few months of life and that fat absorption was particularly poor in preterm infants. They also demonstrated that high levels of minerals in the diet could be deleterious to fat absorption and that the predominant loss of fat was as fatty acid soaps.

These findings have been confirmed in numerous studies in animals and humans. Fat blends characterized by high levels of LCSFAs are poorly absorbed in both rats and human infants. This poses a problem in attempting to "humanize" the fat blends in infant formulas. Human milk has a high content of LCSFAs (approximately 25% palmitic acid, C16:0, and 9% stearic acid, C18:0), and it may be desirable to include similar fatty acid profiles in formula. Unfortunately, such formulas have resulted in poor absorption of fats and minerals, particularly when studied in infants during the first few weeks of life. For example, Widdowson studied an infant-formula fat blend with a fatty acid profile similar to that of human milk. Surprisingly, the fat absorption in infants fed this formula was substantially lower than that in infants fed human milk, and the calcium absorption was exceptionally poor. These results suggest that there is an interaction of elevated levels of free LCSFAs and calcium in the intestinal lumen. Calcium-saturated fatty acid soaps are formed in this process; they are relatively insoluble in micelles and are lost in the feces. Thus the formation of insoluble soaps leads to a loss of both fatty acids (energy) and calcium.

One explanation for the greater absorption of fat and calcium in breast-fed infants than in those who are fed a formula with the same amount of LCSFAs relates to the chemical structure of the human milk triglycerides. Human milk contains approximately 70% of its palmitic acid in the sn-2 position, whereas most animal and vegetable fats contain this fatty acid primarily in the sn-1 and sn-3 positions. Colipase-dependent pancreatic lipase plays a major role in fat digestion; it hydrolyzes the fatty acids of the sn-1 and sn-3 positions, yielding two free fatty acids and a 2-monoglyceride. Thus most of the palmitic acid of human milk may be released as 2-monopalmitin. The bile salt-stimulated lipase of human milk may continue the hydrolysis process to yield free palmitic acid, but the relative importance of this lipolytic system remains to be determined. Matson and Volpenhein have demonstrated that monopalmitin is absorbed more efficiently in rodents than is free palmitic acid, owing to the formation of insoluble calcium-palmitin soaps and the subsequent fecal loss of these soaps. Several clinical studies have explored the relationship of positional distribution and fat absorption in infants. Filer et al. conducted a study in infants who were fed formulas that contained either lard or randomized lard as the sole source of fat. Lard, like human milk fat and unlike most animal fats, contains approximately 80% of its LCSFAs in the sn-2 position. The process of randomizing this fat involves redistributing the fatty acids in a random manner: approximately one third of each of the fatty acids will be present in the sn-1, sn-2, or sn-3 position of the triglyceride. The LCSFA absorption was greater in infants fed a lard-based formula than in those fed a randomized lard-based formula, which supports the hypothesis that positional specificity may play a role in fat absorption. In this study no significant differences in calcium absorption were noted. Brooke also reported that feeding infants formula that contained lard resulted in efficient fat absorption despite the high content of saturated fatty acids in the formula.

"HUMANIZING" FAT BLENDS IN INFANT FORMULA

The problem of poor LCSFA and calcium absorption may be addressed in several ways. Because unsaturated and
medium-chain saturated fatty acids, including lauric acid, are well absorbed, formula that contains a predominance of these fatty acids may be developed. Simple mixtures of unsaturated vegetable oils (e.g., corn or soy oil) and fats that contain a predominance of lauric acid or shorter saturated fatty acids (e.g., coconut oil) are common fat blends in some formulas. Complex mixtures of oils that contain modest levels of LCSFAs can be used in developing formulas with fatty acid profiles closer to that of human milk. If this approach is followed, carefully controlled clinical studies should be conducted to ensure that adequate fat and calcium absorption is achieved. For example, Williams et al. compared a series of infant-formula fat blends that varied in their levels of LCSFAs; some blends contained palmitic and stearic acid levels similar to those in human milk. An increased level of coconut oil was used in other blends to achieve proportions of unsaturated and saturated fats comparable with those in human milk, but with reduced palmitic acid levels. Coconut oil provides lauric acid, which is well absorbed, in place of high levels of LCSFAs. As would be expected on the basis of the original study by Holt et al., these investigators found a clear relationship between the level of LCSFAs in the formula and the overall fat absorption (related primarily to palmitic and stearic acid absorption). This study also documented the interaction of the excretion of LCSFAs and calcium loss, confirming the findings of Widdowson. As the experimental fat blends approached the composition in human milk, the calcium losses became more pronounced.

The complex interactions of dietary fatty acid composi-
Fig. 2. Excretion by rats of total fatty acids from mixtures of native and corandomized coconut oil and palm olein plotted against (A) the total amount of dietary myristic (C14), palmitic (C16), and stearic (C18) acids and (B) the amount of these acids in the sn-1 and sn-3 positions of the triglycerides. Values are means; n = 10; A, r = 0.72 for native oils, 0.75 for corandomized oils; B, r = 0.88.

Fig. 3. Saturated fatty acid excretion during a 3-day balance study corrected for endogenous fat excretion in rats fed 15% dietary fat (n = 10/group). Values for each fatty acid with different superscripts (a, b, c, d) were significantly different (p < 0.05).

tion and calcium on nutrient absorption have also been thoroughly documented in preterm infants. These infants have relatively underdeveloped hepatic function, which results in low levels of bile salts in the duodenum and a failure to reach the critical micellar concentration. This is further exacerbated by the presence of high levels of calcium in preterm formulas. Chappell et al. demonstrated that the fecal loss of fatty acids was increased by administering additional calcium in preterm infants who were fed term-infant formulas. Medium-chain triglycerides, predominantly C8:0 and C10:0, have been added to some preterm formulas to maximize the fatty acid absorption in preterm infants. These triglycerides can be hydrolyzed in the stomach and the medium-chain fatty acids can be absorbed directly into the portal circulation, thus avoiding the extensive digestion and lymphatic transport of the
longer-chain fatty acids. Unfortunately, high levels of dietary medium-chain triglycerides may result in an undesirable increase in the level of circulating dicarboxylic acids. In addition, medium-chain fatty acids are present in relatively low concentrations in the milk of the mothers of both term and preterm infants; C10:0 or shorter chain lengths make up less than 5% (wt/wt) of the fatty acids in typical human milk. Therefore use of high levels of medium-chain triglycerides is relatively unphysiologic.

**ALTERED TRIGLYCERIDE POSITIONAL DISTRIBUTION IN FORMULA FAT BLENDS**

The issue of altered positional distribution in infant formula fat blends has been examined. Lard may provide a source of LCSFAs with human milk–like triglyceride positional distribution, but there are numerous cultural and religious prohibitions to using this fat. Other sources of long-chain saturated fatty acid triglycerides have an undesirable level of these fatty acids in the sn-1 and sn-3 positions. For example, the level of palmitic acid in the sn-2 position of palm olein ranges from 5% to 15%. In an effort to increase the level of LCSFAs in the sn-2 position, we randomized palm olein, increasing the level of palmitic acid in the sn-2 position to approximately 35%. Our initial absorption studies in rats indicated that the fecal loss of fat from diets that contained native or randomized oils was similar. Adding cholestyramine and calcium carbonate to decrease the functional bile salt concentration in the intestine and to promote the formation of fatty soaps resulted in a greater fecal fat loss; however, no significant difference was observed between the groups given native and randomized oils. When these oils were mixed with coconut oil, a slight but significant improvement in fat absorption with the randomized preparation was noted. It was not surprising that, in the clinical study conducted by Clandinin, Van Arde, Hervada, and Lien in which randomized palm olein was an ingredient of a complete fat blend that contained unsaturated vegetable oils as well as coconut oil, the slight and variable improvement seen in the rat studies could not be duplicated (unpublished data). In this study, infants were given formulas with fat blends that differed only in their source of palmitic acid: oleo in the control group and randomized palm olein in the experimental group. Two 3-day balance studies were conducted during the second and third weeks of life. The acceptance of the formulas and the growth rates were similar in both groups. The level of total fat absorption was relatively high and was not significantly different between groups. In addition, the levels of fecal palmitic and stearic acids were similar in both groups, and the calcium absorption was also similar (Table).

The failure to demonstrate a significant improvement in fat absorption both preclinically and clinically, despite the increased amount of palmitic acid in the sn-2 position in randomized palm olein, may be explained by other modifications in the triglyceride structure that occur during randomization. Native palm olein contains low levels of triglycerides that consist entirely of LCSFAs (palmitic and stearic acids). However, through the process of randomization, the probability of obtaining triglycerides that contain three LCSFAs increases significantly, as documented by aluminum-clad column gas chromatography. For example, native palm olein contains 0.9% tripalmitin and randomized palm olein contains 9.3% (R. Yuhas, C. Kuhlman, E. Lien, unpublished data, September 1992). Because of the poor availability of tripalmitin for digestion, relatively large amounts of this triglyceride and other triglycerides that contain three LCSFAs are lost directly in the feces.

The amount of completely saturated long-chain fatty acid triglycerides that are formed by randomization depends directly on the concentration of the LCSFAs in the oil being randomized. By diluting palm olein with a second oil of low palmitic acid content, such as coconut oil, the palmitic acid concentration of the mixture is reduced and lower levels of completely saturated triglycerides will be formed. The randomization of oil mixtures is termed corandomization. We have studied four such combinations of coconut oil and palm olein in rats to determine the level of fat excretion. As the concentration of LCSFAs increased with increasing amounts of palm olein, the level of fecal fat also increased. The corandomized preparations were superior to the native oils, and the randomized palm olein-native coconut oils were intermediate (Fig. 1). Examining the excretion of the individual saturated fatty acids provided even stronger evidence of this phenomenon. When the total fatty acid excretion is plotted against the sum of the LCSFAs (myristic, palmitic, and stearic acids) in the native oil mixtures and in the corandomized mixtures, substantially less fat is excreted in the corandomized groups (Fig. 2, A). In contrast, when the total fatty acid excretion is compared with the percentage of the LCSFAs in the sn-1 and sn-3 positions, a direct and highly significant relationship is seen (Fig. 2, B). The monorandomized preparations cannot be used in these comparisons because of the increased levels of triglycerides that contain three LCSFAs.

These fat blends may be used to increase the level of LCSFAs in infant formulas while maintaining optimum calcium and LCSFA absorption. This may have practical applications; the current European Society for Pediatric Gastroenterology and Nutrition guidelines and the proposed European Community regulations would limit the levels of both lauric acid (<15%) and linoleic acid (<20%),
leaving the predominant proportions of the fat blends to be either monounsaturated or LCSFAs. Improved positional distribution may make it possible to safely use blends that contain higher levels of LCSFAs than are currently available.

The fat blends described previously will contain no more than one third of their palmitic acid in the sn-2 position (versus ~70% in human milk) and will also contain levels of lauric acid greater than those in human milk. A vegetable-oil preparation (Betapol) has been developed that has a fatty acid profile and a level of sn-2 palmitic acid that match those of human milk. Betapol is prepared by interesterifying a fat high in tripalmitin with a position sn-1-sn-3-selective lipase in the presence of monounsaturated and polyunsaturated fatty acids. This process replaces the sn-1-sn-3 palmitic acid with oleic and linoleic acids. We have conducted a preclinical evaluation in rats with Betapol, the corresponding native oils (which have the same fatty acid profile, but with palmitate predominantly in the sn-1 and sn-3 positions), and mixtures of Betapol and native oils in proportions of 75:25, 50:50, and 25:75. An analysis of the total fat excretion demonstrates that the positionally altered preparation is superior to the native oils and the intermediate mixtures. The differences in the excretion of saturated fatty acids is striking (Fig. 3). For example, only 2% of the palmitic acid is lost in the Betapol group and almost 20% is excreted in the native-oils group. Although both monounsaturated and polyunsaturated fatty acids are well absorbed in all groups, a significant improvement in the absorption of these fatty acids was observed in the groups that were given the mixtures that contained Betapol. Unfortunately, economic considerations currently preclude the development of Betapol as a universal fat blend for infant formulas.

CONCLUSION

Triglyceride lipolysis is initiated by lingual and gastric lipases. In infants fed human milk, both bile salt–stimulated lipase and colipase-dependent pancreatic lipase participate in triglyceride digestion; in formula-fed infants, pancreatic lipase must play the dominant role in triglyceride digestion. Because of considerations of lipase positional specificity, providing triglycerides with elevated sn-2 LCSFAs to formula-fed infants may improve fat and calcium absorption; however, the clinical benefits of such triglyceride preparations remain to be determined.

REFERENCES