Can we define an infant’s need from the composition of human milk?1–3

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ABSTRACT
Human milk is recommended as the optimal nutrient source for infants and is associated with several short- and long-term benefits for child health. When accepting that human milk is the optimal nutrition for healthy term infants, it should be possible to calculate the nutritional needs of these infants from the intake of human milk. These claims can then be used to design the optimal composition of infant formulas. In this review we show that the composition of human milk is highly variable and is dependent on factors such as beginning or end of feeding, duration of lactation, diet and body composition of the mother, maternal genes, and possibly infant factors such as sex.

In particular, the composition of fatty acids in human milk is quite variable. It therefore seems questionable to estimate the nutritional needs of an infant exclusively from the intake of human milk. The optimal intake for infants must be based, at least in part, on other information—eg, balance or stable-isotope studies. The present recommendation that the composition of infant formulas should be based on the composition of human milk needs revision.

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INTRODUCTION
Human milk is recommended as the ideal nutrient source for infants and has been associated with several short- and long-term benefits for child health, such as a reduction in gastrointestinal infections and otitis media, as well as with a reduced risk of allergies and autoimmune diseases (eg, atopic dermatitis, asthma, celiac disease, inflammatory bowel disease, and type 1 diabetes), leukemia, and metabolic syndrome (1, 2). Human milk is proposed to be ideally adapted to the needs of the infant and contains many bioactive components. The exact mechanisms of the protective effects of human milk remain unclear, but it has been suggested that the specific components play an important role in the health benefits.

Human milk is not a uniform body fluid; its composition changes continuously (3, 4). Foremilk differs from hindmilk, and milk changes with time of day as well as during the course of lactation (5–7). The main components of human milk are as follows: carbohydrates, lipids, proteins, minerals, vitamins, and other trace elements. It is known that the variability of these components in human milk is quite high. A study by Wojcik et al (8) analyzed 415 samples of human breast milk and found a high variability in the distribution of lactose, protein, lipids, and energy between samples (Figure 1). Moreover, human-milk composition is influenced by numerous genetic, maternal, and environmental factors (9, 10). In addition, it is impossible to measure the amount of human milk that breastfed infants consume. Nonetheless, human milk has been used as the gold standard to estimate the needs of an infant. The recommendations of authorities worldwide with regard to the composition of infant formula are primarily based on the chemical analysis of human milk. Because the variability of the macronutrients is high, the question is whether we can define the needs of infants on the basis of the composition and intake of human milk. In this review, we give an overview of the macronutrient variation of human milk as well as the factors that influence the macronutrient composition of human milk.

BREASTFEEDING: NUTRIENT CONTENT COMPARED WITH INTAKE
Because the content of macronutrients and the amount of human milk consumed varies, it is difficult to estimate the nutrient intake of infants. How much human milk a breastfed infant consumes changes during the course of lactation. A recent study published by da Costa et al (11) used a stable-isotope methodology to measure the mean human-milk intake during the first 12 mo of lactation. They analyzed human-milk intake from mother-infant pairs from 12 countries across 5 continents and showed a steady increase of human-milk intake from 1 to 4 mo of life, which then reached a plateau after 6 mo of age and declined steadily thereafter. On average, human-milk intake increased from 0.60 kg/d during the first month of life to 0.82 kg/d at 4 mo of age, with a high variation between individual infants (Figure 2). In addition to the variation of human-milk intake by the individual infant, human-milk intake is influenced by other factors, such as sex and nonmilk water intake. The median human-milk intake was higher in boys than in girls, which is likely related to the fact that boys have greater lean mass than do girls during infancy. Further studies have shown that human-milk intake is higher in developed countries than in developing countries (12).

Human-milk intake may also be dependent on maternal milk production. Milk production can vary over the course of lactation.
and between breasts. It has been shown that milk production increases rapidly in the first weeks of life (6). Furthermore, there is evidence that over the first 12 mo of lactation milk production is constant from the first month of life until 6 mo of age and then steadily declines (13–16). Differences in milk production between breasts appear to be associated with a maternal preference for the breast with the most milk production (15, 16).

VARIATION IN MACRONUTRIENTS AND INFLUENCING FACTORS

Carbohydrates

The major carbohydrate in human milk is lactose. In colostrum and during early lactation lactose concentration is relatively low, but it rapidly increases to an average concentration of 6 g/L. After the first few weeks of life, studies consistently show that the concentration of lactose does not change with the stage of lactation (7, 16–18). Some authors found a significantly lower lactose concentration in preterm milk (19, 20). Maas et al (21) found that gestational age did not affect lactose content, whereas postnatal age was related to an increase in lactose concentration. Others found no differences (7, 22). Saarela et al (7) did observe slightly, not clinically relevant, lower lactose concentrations in preterm hindmilk when compared with term hindmilk. Overall, the variation in lactose content was minimal.

Several oligosaccharides have also been identified as important components in human milk. These oligosaccharides have been an area of research especially in the past decade because they are proposed to have important protective functions for newborns (23). As many as 85 different oligosaccharides have identified so far. Thurl et al (24) identified 4 oligosaccharide patterns in human milk that were correlated to the genetic basis of the 4 blood groups of the Lewis blood group system. This means that certain oligosaccharide patterns in human milk are associated with different genetic backgrounds.
HUMAN-MILK COMPOSITION AND INFANTS’ NEEDS

To our knowledge, 2 studies investigated the variation of human-milk oligosaccharides in relation to stage of lactation (18, 20). Thurl et al (18) showed that, in general, the oligosaccharides in human milk decrease over the first 3 mo of lactation. Gabrielli et al (20) investigated oligosaccharide concentrations in 4 milk groups of preterm delivered newborns, collected at 4, 10, 20, and 30 d postpartum. They showed oligosaccharide concentrations to be higher in preterm milk than in term milk. Furthermore, the total oligosaccharide concentration was significantly lower at 30 d than at 4 d postpartum. On the basis of these 2 studies, oligosaccharide concentration appears to decrease with increasing stage of lactation. However, further studies should be conducted to substantiate these results.

Protein

The principal proteins in human milk are casein and whey (α-lactalbumin, lactoferrin, and secretory IgA). The protein content composition of human milk changes considerably during the course of lactation. In early lactation, there is little casein, which increases during the course lactation. The concentration of whey proteins remains high during the whole lactation period.

Colostrum and human milk during the first month of lactation contains high concentrations of protein (25). During the first month of lactation, the protein content in human milk decreases to concentrations of ~9–11 g/L; thereafter, it decreases more slowly until the sixth month of lactation and then remains steady after 6 mo (7, 16, 26–28). In the study by Saarela et al (7) no difference in protein content was found between preterm and term milk.

Over the past decades, it has become clear that certain human-milk proteins, such as lactoferrin and secretory IgA, are excreted in the feces in relatively large amounts. The mechanism that may explain this phenomenon is that these proteins are glycosylated in the intestine, which makes them nondigestible, and consequently are excreted in the feces. Excretion of these proteins indicates that the total protein content of human milk is an overestimation of the protein nutritionally available to the breastfed infant. For this reason, it is still difficult to determine how much protein infants need on the basis of human-milk composition.

Lipids

Lipids form an important part of human milk. Lipids are a mixture of compounds with different chemical properties that follow diverse pathways in the human body. Infants have a high need for cholesterol, which is an important part of the cell membrane. Fats are not only the most important source of energy, they have many more functions. Long-chain PUFA (LC-PUFAs) are seen as essential fatty acids during infancy. They are an important part of the cell membrane, specifically in the eye and the brain. They are considered to have an effect on the immune system, and more importantly, they are regulators of a number of genes (29).

The lipid composition of human milk is quite variable and dependent on a number of factors as indicated in Table 1. All of the studies that reported on the fat content found a great variation in both total lipid content (Figure 1) as well as in composition. Michaelsen et al (30) found a range in lipid content during the first 2 mo of ~2–9 g/100 mL, Szabó et al (31) found at 6 wk mean concentrations of 1.6 g/100 mL with a range of 0.4–2.8 g/100 mL. At 6 mo, the mean concentration was 3.2 g/100 mL with a range of 1.5–4.9 g/100 mL. Saarela et al (7) found in human donor milk an average value of 3.1 g/100 mL with a range of 1.1–5.1 g/100 mL.

It is known that lipid content is different between foremilk and hindmilk. Saarela et al (7) found an average fat content in foremilk of 1.9 g/100 mL compared with 5.2 g/100 mL in hindmilk. Not only is the concentration different but the DHA content is also higher in hindmilk (32). Whether the lipid content is related to the duration of lactation is not clear. Szabó et al (31) found a marked increase in the lipid content, from 1.6 g/100 mL at 6 wk to 3.2 g/100 mL at 6 mo. Mitoulas et al (16) observed a decrease in concentrations between the first and second month, with an increase starting at 9 mo. Michaelsen et al (30) and Mandel et al (33) observed an increase in lipid content only after ~12 mo. Agostoni et al (34) found a decrease in DHA content during the first months of life. This might be due to a decrease in the phospholipid concentration. The concentration of DHA is much higher in phospholipid than in total lipids.

<p>| TABLE 1 |
| Factors associated with changes in the lipid content of human milk |</p>
<table>
<thead>
<tr>
<th>Factor</th>
<th>Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration of a nursing or feeding</td>
<td>Increases</td>
</tr>
<tr>
<td>Age postpartum or stage of lactation</td>
<td>Constant or small decrease first month; increases after 6–12 mo</td>
</tr>
<tr>
<td>Diurnal rhythm</td>
<td>Variable; related to the time of samples and maternal meals</td>
</tr>
<tr>
<td>Between breasts</td>
<td>Occurs</td>
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<tr>
<td>Gestational age at birth: preterm vs term</td>
<td>Small differences</td>
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<tr>
<td>Diet</td>
<td>Strong effects</td>
</tr>
<tr>
<td>Nutritional status</td>
<td>Decrease in maldnourished women</td>
</tr>
<tr>
<td>High-carbohydrate, low-fat diet</td>
<td>Higher MCT, lower DHA concentrations</td>
</tr>
<tr>
<td>Parity</td>
<td>Constant or small change</td>
</tr>
<tr>
<td>BMI of mother</td>
<td>Adiposity increases</td>
</tr>
<tr>
<td>Fat composition of diet</td>
<td>Strong effects on LC-PUFAs</td>
</tr>
<tr>
<td>Smoking</td>
<td>Decrease in DHA</td>
</tr>
</tbody>
</table>

1 Adapted from reference 9. LC-PUFA, long-chain PUFA; MCT, medium chain triglyceride.

Abbreviations used: AA, arachidonic acid; ALA, α-linolenic acid; IQ, intelligence quotient; LA, linoleic acid; LC-PUFA, long-chain PUFA.
There are indications that the lipid content is influenced by the body mass of the mother. One study found a positive correlation between maternal BMI and fat content of human milk (35), but another study found no correlation (33). The diet of the mother has an important influence on lipid composition. A maternal diet high in carbohydrates and low in fat is related to higher medium-chain triglyceride concentrations in milk (36). A high-carbohydrate feeding also resulted in higher arachidonic acid (AA) concentrations, and a high-fat diet resulted in higher DHA concentrations (37).

Prentice et al (38) showed that the fat composition of breast milk is influenced by the parity of the mother. They found significantly lower concentrations of medium-chain triglyceride after 9 pregnancies. Concentrations of n–6 PUFAs were higher. These effects might be caused by a change in diet (more vegetable fat) and not by parity itself.

The highest variation in the composition of human milk is seen in the composition of LC-PUFAs. These are essential fatty acids derived from the parent compounds linoleic acid (LA; 18:2n–6) and α-linolenic acid (ALA; 18:3n–3). AA is formed after several enzymatic steps from LA, and DHA from ALA. The term infant as well as the preterm infant is capable to produce these products from the parent compounds. However, plasma concentrations decrease when preformed AA and DHA are given in both term and preterm infants. The content of AA and DHA in human milk is quite variable. LC-PUFAs in human milk are mainly derived from maternal stores (39). The intake of DHA is important during lactation, as shown by Makrides et al (40). Studies have shown marked differences in AA and DHA content in human milk between countries (Figure 3, A and B).

content shows less variation, from 0.3 to 0.6% by weight. Concentrations can, however, also be very different within a country. A study in Malaysian women reported clear differences in the composition of breast milk between different ethnicities (41). This study showed that the LA concentrations in breast milk of Chinese women were higher than those in Indian and Malaysian women, whereas concentrations of DHA were lower. These differences are most likely due to different fatty acid profiles of the typical diets consumed by these ethnic groups. It is advised in modern Western diets to replace saturated fats with polyunsaturated fats to reduce cardiovascular diseases. This change might also have negative effects. A study in Australia showed that the LA content in human milk increased from 1981 to 2000, whereas at the same time the DHA concentration showed a significant decrease (Figure 4) (42).

This significant decrease in DHA concentration in human milk may have been caused by an increased intake of LA, which resulted in a reduction in the conversion of ALA into DHA; however, it may also have been related to a concomitant decrease in DHA intake.

Another factor in the diet that is influencing PUFA status is the intake of trans fatty acids. Szabó et al (31) showed that trans-octadecenoic and trans-octadecadienoic acids were inversely correlated with LA, ALA, AA, and DHA (31). The inverse correlation between trans fatty acids and LC-PUFAs may be explained by the impairment of the synthesis of LC-PUFAs from the parent compounds by these acids.

Finally, LC-PUFA concentrations are not only influenced by the fat and carbohydrate intake of the mother. Agostoni et al (34, 43) showed that LC-PUFA concentrations in breast milk are negatively influenced by smoking due to lower production of LC-PUFAs in the mammary gland.

Taking all of these studies into account it is virtually impossible to define the optimal amount of lipid intake by the infant at different ages or the lipid composition of the milk.

Energy

Carbohydrates, proteins, and lipids are the major components that contribute to the energy content of human milk (44). The mean energy content of human milk ranges from 0.62 to 0.80 kcal/g. On one hand, studies have shown that energy content is positively correlated with infant weight, energy intake, and weight gain (45–47). On the other hand, energy content was not found to be related to gestational or postnatal age (21).

Because the lipid content increases markedly with emptying of the breast, consequently significantly higher energy content is seen in hindmilk than in foremilk (7, 16).

Some conflicting results were found with regard to the energy content and stage of lactation. Mitoulas et al (16) showed that the energy content differed with stage of lactation, decreasing from 1 mo to 2 mo of life, after which the energy content increased again up to 9 mo. In this study the decrease in lipid content at 2 mo probably resulted in the decrease in energy content at that stage of lactation. Whereas the lipid content in human milk decreased until 4 mo, the amount of lipid delivered to the infant increased after 2 mo, which explained the increase in energy content from the second month of lactation. Saarela et al (7), on the other hand, found no significant changes in energy content between 1 wk and 6 mo. This finding may also be explained by the lipid content because they did not find any differences in lipid content during the first 6 mo of lactation. Energy content does, however, differ significantly between women (10, 16). Furthermore, a study by Qian et al (10) showed significant differences in energy content of human milk between regions in China. This study also found a decreased lipid content due to poorer nutrition, which was associated with lower socioeconomic status.

In addition to the amount of nutrients, which is influenced by the diet of the mother, other factors, such as maternal characteristics, breast development, or the demand for energy of infants, might influence the energy content in human milk. For example, it has been suggested that male infants have higher energy requirements than do female infants. Only one study by Powe et al (48), investigated whether energy content was associated with infant sex and breast size. They reported an association between energy content in human milk and infant sex during pregnancy; the mother of male infants produced milk that had a 25% higher energy content than that of mothers of female infants. An increase in breast size during pregnancy was associated with higher energy content in human milk; however, after correcting for sex this

FIGURE 4. Histograms depicting the average LA (A) and DHA (B) content of breast milk in Australian women in 1981 and 2000. Reproduced with permission from reference 42. LA, linoleic acid.
difference disappeared. This was a study conducted in only 25 lactating mothers, and data were collected by self-report. On the basis of this small number, no definitive conclusion can be drawn.

It appears that energy content can differ during the course of lactation and is probably caused by differences in lipid content.

**DISCUSSION**

Human milk is considered to be the gold standard for infant formulas, and there have been many attempts to produce infant formulas that resemble human milk as closely as possible. The composition of human milk, however, is quite variable, as is its intake. It is therefore impossible to estimate the optimal intake of nutrients of healthy term infants from the intake of breast milk. It does not seem reasonable to take the average value of all values obtained by many different studies. Whether the composition of human milk is actually tailored to the individual infant remains unknown, but it does not seem likely given the clear effects of the maternal diet. The composition most likely is dependent on 3 unrelated factors: maternal factors, diet of the mother, and characteristics of the infant. Consequently, there is no real understanding of how to exactly mimic human milk, which means that an outcome measure other than the composition of human milk must be used to assess the optimal nutrient intake for infants.

The lactose content of human milk does not seem to show a high variability. There are clear indications that protein content decreases with the duration of lactation. The highest variability in the composition of human milk is found with regard to fat content. The fat content in human milk is related to the fat mass of the mother, with higher concentrations found in obese mothers and lower concentrations in malnourished mothers. The diet of the mother has a marked effect on the fat content and fat composition of human milk. In particular, the amount of essential LC-PUFAs is highly influenced by the diet of the mother. That the infant him- or herself influences the composition and amount of human milk can be concluded from studies that show that human milk intake varies between individual infants, with male infants consuming more milk than female infants (11).

Because it is virtually impossible to define the needs of a growing healthy term infant from the composition of human milk, other ways need to be found to ensure optimal nutrition. The most frequent measure used is growth. Weight gain, however, can be an increase in water, protein, or fat. A gain in protein is considered to be healthy, but an abundant increase in fat is not advised because a high fat increase in early life might predispose the infant to obesity later in life. Weight gain alone, therefore, is not the best indicator in determining the optimal intake. Measuring waist circumference and skinfold thickness can provide a more reliable indication of fat increase (49, 50). Measuring length gain is important because this is a reflection of protein availability. More recently, measurements of intraabdominal fat mass have been introduced as a method to assess adequate postnatal growth. Rapid postnatal growth was associated with greater abdominal fat mass (51). Further studies should be conducted to assess whether abdominal fat mass is a good indicator for the risk of developing obesity and whether it could be used as a method to assess optimal nutrient intake of infants.

Psychomotor development is, next to physical growth, an important indicator of optimal nutritional intake. Studies in preterm infants have shown less optimal psychomotor development with lower protein intake (52–54). Deficient iron intake also was related to suboptimal development (55). Evaluating the psychomotor development therefore might be an important indicator for optimal nutrition.

The nutritional needs of infants may also be reflected by functional outcome. When the literature is reviewed, there are indications that breastfed infants have a significantly higher intelligence quotient (IQ) than their formula-fed counterparts; this difference in IQ was even higher for preterm infants (56). This finding may indicate that human milk contains some special components that lead to better functional outcomes. A recent study by Holme et al (57) evaluated IQ and neurocognitive abilities and found similar results in 1200 children aged 9 y before adjustment of confounders (57). After correcting for several maternal and economic confounders, however, no significant relation of human milk with functional outcome was found. Functional outcome is clearly not only determined by adequate nutrition.

The composition of formula is presently based on mean values found in human milk. It might be better to base the composition on actual requirements of the newborn infant. Energy needs can be estimated by using stable-isotope methodology. Requirements of individual amino acids can be calculated by using the oxidative indicator technique (58). The intake of the essential fatty acids, LC-PUFAs, can be estimated from the functional outcome of infants receiving supplemental LC-PUFAs. This approach might provide a better indication of the optimal composition of formulas than an average value taken from studies in human milk.

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