Human milk provides all the dietary essential fatty acids, linoleic acid (LA; 18:2n-6) and α-linolenic acid (18:3n-3), as well as their longer-chain more-unsaturated metabolites, including arachidonic acid (20:4n-6) and DHA (22:6n-3) to support the growth and development of the breast-fed infant. Human milk levels of LA have increased in Westernized nations from mean levels (g/100 g total fatty acids) of 6 to 12–16 over the last century, paralleling the increase in dietary intake of LA-rich vegetable oils. DHA levels (g/100 g total milk fatty acids) vary from <0.1 to >1% and are lowest in countries in which the intake of DHA from fish and other animal tissue lipids is low. The role of DHA in infant nutrition is of particular importance because DHA is accumulated specifically in the membrane lipids of the brain and retina, where it is important to visual and neural function. An important question is the extent to which many human diets that contain low amounts of n-3 fatty acids may compromise human development. The present paper reviews current knowledge on maternal diet and human milk fatty acids, the implications of maternal diet as the only source of essential fatty acids for infant development both before and after birth, and recent studies addressing the maternal intakes and milk DHA levels associated with risk of low infant neural system maturation.

Human milk: Essential fatty acids: n-3 Fatty acids: Infant development

Milk fat synthesis and composition

Milk fat consists predominantly of PUFA, which represent about 98 g/100 g milk total fat and are synthesized from glycerol and NEFA in the alveolar cells of the mammary gland (Jensen, 1999; Neville, 2001). Fatty acids of C≥16 are taken up from plasma chylomicrons and VLDL-PUFA through the action of the mammary lipoprotein lipase. As such, the fatty acids in human milk may originate either from the recent dietary fatty acid intake, from fatty acids released from maternal adipose tissue or from de novo synthesis.

Abbreviations: ALA, α-linolenic acid; ARA, arachidonic acid; LA, linoleic acid; PE, phosphatidylethanolamine; RBC, erythrocyte.
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The long-chain SFA, cis-MUFA, trans-fatty acids and n-6 and n-3 PUFA are taken up by the mammary gland and then acylated into PUFA for secretion in milk. Mammary gland-specific acyl transferases preferentially esterify 16:0 and 18:0 total fatty acids and has negligible amounts of EPA and DHA. In man glucose-derived acetyl-CoA is the major source of C2 units for fatty acid synthesis, and the maternal dietary intake of PUFA has a marked effect on the amounts of n-6 and n-3 fatty acids secreted in milk (Innis, 1992, 2004; Jensen, 1999).

The long-chain SFA, cis-MUFA, trans-fatty acids and n-6 and n-3 PUFA are taken up by the mammary gland and then acylated into PUFA for secretion in milk. Mammary gland-specific acyl transferases preferentially esterify 16:0 and 18:0 at the centre sn-2 position of the human-milk PUFA, rather than at the sn-1 or sn-3 positions, as in other tissues (Jensen et al. 1990; Innis, 2004). The products of hydrolysis of human-milk PUFA hydrolysis by gastric and pancreatic lipases are NEFA released from the PUFA sn-1 and sn-3 positions and sn-2 monoaoylglycerols enriched in 16:0 and 18:0 (Innis et al. 1994). This unusual structure of human-milk PUFA is believed to be important because non-esterified 16:0 and 18:0 have melting points (about 63°C and 69°C respectively) that are considerably above body temperatures and readily form insoluble soaps with Ca and other divalent cations at the pH of the intestine, resulting in faecal loss of both fat and important minerals (Kennedy et al. 1999). Whether in breast-fed infants the distribution of fatty acids in the plasma chylomicron PUFA with 16:0 and 18:0 in the centre position also influences tissue n-6 and n-3 fatty acid delivery is not known. After absorption sn-2 monoaoylglycerols are re-esterified mainly via the 2-monoacylglycerol pathway, thus preserving the unusual position of fatty acids in human milk fat (Innis et al. 1994).

LA and ALA cannot be formed by mammalian cells, and as a result all the n-6 and n-3 fatty acids secreted in milk must be derived from the maternal diet, either directly or after storage or further metabolism in maternal tissues. LA and ALA are present in the diet in greatest amounts in unsaturated plant, seed and nut oils, although different oils vary considerably in their LA and ALA content (Innis, 2004). Once obtained from the diet LA and ALA can be further metabolized by Δ6-desaturation, elongation and Δ5-desaturation to form ARA and EPA respectively. The generally-accepted pathway for further metabolism of EPA to DHA is that proposed by Sprecher (1999). It involves two successive elongations of EPA to form 24:5n-3, then desaturation at position 6 to yield 24:6n-3, which is then translocated to the peroxisomes where a single cycle of β-oxidation generates 22:6n-3 (Innis, 2003). Further metabolism of n-6 fatty acids to form docosapentaenoic acid (22:5n-6) is believed to occur through an analogous pathway. A characteristic feature of animals fed an n-3 fatty acid-deficient n-6 fatty acid-sufficient diet is a decrease in tissue levels of DHA and an increase in n-6 docosapentaenoic acid (Innis, 1991, 2003). Essential fatty acid deficiency involving an inadequate intake of both n-6 and n-3 fatty acids, on the other hand, results in a decrease in ARA and DHA and increased desaturation and elongation of the n-9 fatty acid oleic acid (18:1n-9) to give increased levels of 20:3n-9 and 22:5n-9 (Innis, 1991).

As the synthesis of ARA, EPA, DHA and their intermediates is limited to animal cells, they are found in the diet only as part of animal tissue lipids (Innis, 2003). While the richest dietary source of EPA and DHA is fish, most dietary ARA is derived from poultry and other meats (Kris-Etherton et al. 2000; RW Freisen and SM Innis, unpublished results). ARA, EPA and DHA secreted into milk may originate either from the maternal diet or as a result of synthesis from their LA and ALA precursors respectively in the mammary tissues. In 

Maternal dietary lipids and their effect on the composition of milk fat

The presence in human milk of a large number of fatty acids of different chain length and unsaturation, including
many geometric and positional isomers, and the wide variability in milk fatty acid compositions among women have been known for many years. However, only recently has interest been directed towards the possibility that maternal lipid nutrition may adversely affect, as well as promote, the growth and development of the breast-fed infant. Observational and intervention studies have shown that levels of trans-fatty acids, oleic acid, LA, ALA and DHA in human milk show considerable variability, explained largely by the amount of the respective fatty acid in the maternal diet. For example, levels of trans-fatty acids derived from industrial hydrogenation of cis-unsaturated vegetable oils are higher in human milk in North America than in Europe, with levels as high as 18 g trans-fatty acids/100 g total fatty acids in the milk fat of some women (Innis, 2006). High amounts of trans-fatty acids in human milk have raised concerns because of the possible adverse effects on essential fatty acid and lipid metabolism, as well as incorporation of unusual fatty acid isomers into the infant’s tissues (Innis, 2006). The levels of trans-fatty acids in human milk in Canada have now decreased with the introduction of food labelling and regulations to restrict the amounts of trans-fatty acids in retail foods (Friesen & Innis, 2006).

The effects of maternal dietary fat composition on the n-6 and n-3 fatty acid composition of human milk fatty acids are clearly shown by: a consideration of the changes in human-milk fatty acids over the last 50 years; a comparison of human milk fatty acids among women following vegan or mixed diets of different fat composition; the results of controlled interventions to alter maternal dietary fat intake. Early studies by Insull et al. (1959) have shown an LA content (g/100 g total fatty acids) of 7.7 in milk from women following their usual diet, which increases 4-fold to 41.4 when the diet is changed to 40% energy from maize oil (usual LA content of about 52) and to 10.4 when the diet has 40% energy from lard (LA content of about 10). Later studies in the 1960s have noted mean concentrations of 9.5–10 g LA/100 g total fatty acids in human milk (Tomarelli et al. 1968). The mean levels of LA in human milk among women in the USA, Canada and Europe in most studies conducted from 1980 onwards are 12–16 g/100 g total fatty acids (Innis, 1992; Jensen, 1999). However, the amount of LA in human milk varies widely among individual women, from about 6 g/100 g total fatty acids to >30 g/100 g total fatty acids in the milk fat of vegetarians (Innis & King, 1999; Jensen, 1999; Sanders & Reddy, 1992). In the population studied in Vancouver, Canada, LA in human milk (g/100 g total fatty acids) ranges from 6 to 22 (Innis & King, 1999). Assuming an average of 3.7 g fat and 315 kJ (75 kcal)/100 ml milk and an intake of 780 ml/d by the infant, then the average and range of LA intake (g/d) among breast-fed infants are 3.46 and 1.73–6.20 respectively, equivalent to 5.3 and 2.7–9.5% total energy intake respectively. There is a high positive correlation between the LA content of human milk and the LA levels in the plasma PUFA and phospholipids in the recipient breast-fed infant (Innis & King, 1999). Several conclusions can be drawn. First, all human milk meets and exceeds the dietary requirements for n-6 fatty acids, which is met by an intake of only 1–2% energy from LA (Innis, 1991). Second, the intake of LA by the breast-fed infant is similar to that of adults in the same population. The high level of LA in Western diets has led to concern, particularly in relation to the concurrent low intake of n-3 fatty acids and the increasing prevalence of many inflammatory and immune disorders (Simopoulos, 1999; Calder, 2006). Consequently, this area of infant nutrition is now becoming the subject of increasing interest, which should be extended to human milk lipids and the impact on the breast-fed infant (Calder et al. 2000; Innis et al. 2006).

Similar to LA, the levels of DHA in human milk also vary widely within and among populations (Innis, 1992, 2004), which again results in differences in the plasma and cell membrane levels of DHA among breast-fed infants (Gibson et al. 1997; Innis & King, 1999; Jensen et al. 2000; Innis et al. 2001). Milk fat from women following vegan diets, which lack DHA, contains <0.1 g DHA/100 g total fatty acids (Sanders & Reddy, 1992), whereas much higher amounts, often >1 g DHA/100 g total fatty acids, are found in the milk of women consuming diets high in fish and other marine animals (Innis, 1992, 2004). The mean levels of DHA (g/100 g total fatty acids) in milk from women in Canada, the USA, Europe and Australia is 0.2–0.3 (Jensen, 1999; Innis, 1992, 2004), while population averages range from 0.07 in milk fat from women in some areas of Africa to 1.0 in milk fat of women in Japan (Innis, 2004; Yukas et al. 2006). In the study centre in Vancouver lactating women who exclusively breast-feed their healthy term infants and consume about 81 mg DHA/d have 0.17 g DHA/100 g total fatty acids in their milk fat at 30 d postnatally, while women who consume 131 mg DHA/d have 0.32 g DHA/100 g total fatty acids (Fig. 1). Studies in Norway, on the other hand, have shown mean levels of 0.5 g DHA/100 g total fatty acids in milk fat at 1 month post partum among women with a dietary intake of 200 mg DHA/d (Helland et al. 2001).

Similarly to LA, the consideration of mean levels of DHA in milk among groups of women hides the wide variability among individuals, which in the Vancouver study population extends from <0.1 g DHA/100 g total fatty acids to 1.0 g DHA/100 g total fatty acids in the milk fat of women who are not taking DHA supplements (Innis & King, 1999; Friesen & Innis, 2006). This range in mean values is similar across different countries (Innis, 1992, 2004; Jensen, 1999; Yukas et al. 2006). Assuming an average of 3.7 g fat and 315 kJ (75 kcal)/100 ml milk and an intake of 780 ml/d by the infant, then the average intake of DHA among breast-fed infants consuming milk with 0.2 g DHA/100 g total fatty acids is 0.056 g/d, representing 0.086% total energy. A range of 0.1–1.0 g DHA/100 g total fatty acids in human milk fat is equivalent to a potential DHA intake ranging from 0.028 g/d to 0.280 g/d, or from 0.04% total energy intake to 0.4% total energy intake from DHA among breast-fed infants. Although theoretical, these estimations illustrate a very wide variation in n-3 fatty acid intakes, as well as n-6 fatty acid intakes, among breast-fed infants.

An increase in DHA intake among lactating women through supplementation with fish, fish oils, single-cell PUFA, DHA-enriched eggs or other sources of DHA increases the secretion of DHA in milk, and increases the...
PUFA in human milk and the breast-fed infant

Human milk provides the only source of n-6 and n-3 fatty acids to support the growth and development of the breast-fed infant during breast-feeding and for much of early infancy, when foods such as Fe-fortified infant cereals, vegetables, fruits and dairy products, all of which are low in n-6 and n-3 fatty acids unless fortified, are added to the infant diet.

Particularly high concentrations of DHA are present in phosphatidylserine and the ethanolamine phosphoacyl-glycerols (ethanolamine plasmalogens and phosphatidylethanolamine (PE)) of brain grey matter and the outer segments of rod and cone photoreceptors in the retina (Innis, 2003). DHA comprises $\leq 80\%$ of the PUFA in the retina outer segment disks, and phospholipids are present in which both fatty acids are DHA. In *in vitro* studies DHA has provided evidence that DHA plays a role in photoreceptor signal transduction by influencing the ability of photons to transform rhodopsin to the activated meta-rhodopsin II state and the efficiency of G-protein-coupled receptor signalling (Niu et al. 2004). More recently, it has been shown (Soubias et al. 2006) that there are specific binding sites for DHA in the rhodopsin molecules. In the brain DHA is enriched in synaptic terminal membranes and has diverse roles in brain growth and function and in protecting against oxidative stress (Innis, 2007). Although much remains to be understood, recent experimental studies have shown (Bazan, 2006; Innis, 2007) that reduced levels of DHA in the brain impair neurogenesis and neurite outgrowth and alter the metabolism of several neurotransmitters, including dopamine, serotonin and acetylcholine. DHA is also a ligand for nuclear hormone transcription factors, targets for which include genes involved in synaptic plasticity, endocytosis, signal transduction and synaptic vesicle recycling, and the precursor to resolvins and neuroprotectors that protect against oxidative damage.

Thus, DHA is important in the central nervous system, is present in human milk and early deficiencies in key nutrients have the potential to result in long-term effects on cognitive and behavioural functions. These factors have stimulated intense interest in the role of DHA in infant nutrition, particularly in relation to the fatty acid composition of infant formulas (Lauritzen et al. 2001; Heird & Lapillonne, 2005; Fewtrel, 2006; Uauy & Dangour, 2006). Growth of membrane structures, involving cell division, myelination and such processes as neurite extension and dendritic arborization, requires the synthesis of large amounts of new membrane material, which results in a high demand for DHA. The rate of brain growth is at peak velocity during the last 3 months of gestation and the first few months after birth (Innis, 1991), leading to a general concept that DHA requirements in the human infant may be particularly high during the later stage of gestation and in early infancy.

It is now well known that the inclusion of DHA in infant formulas increases DHA in a dose-dependent manner in the blood lipids of infants fed formula (Makrides et al. 1995; Carlson et al. 1996; Innis et al. 1996; Birch et al. 1998). In addition, some studies (Birch et al. 1998, 2000; Hoffman et al. 2003) have reported that the addition of DHA to infant formulas increases visual and neural system maturation in term-gestation infants who are fed formula from birth or after initial breast-feeding. Similarly, although less well appreciated, the circulating blood levels of DHA in breast-fed infants depend on the concentration of DHA in the mother’s milk, which in turn depends on the amount of DHA in the mother’s diet (Gibson et al. 1997; Innis & King, 1999; Fidler et al. 2000; Jensen et al. 2000; Innis et al. 2001). Early studies by Sanders & Reddy (1992) have shown levels of 1·9 (SE 0·32) g DHA/100 g RBC total fatty acids in breast-fed infants whose mothers followed a vegan diet (and thus devoid of DHA) compared with 6·2 (SE 0·41) g DHA/100 g RBC total fatty acids in breast-fed infants whose mothers followed mixed diets. In
Bayley Scales of Mental or Motor Skill Development at 18 months of age or with tests of novelty preference at 9 months of age (Innis et al. 2001; Innis, 2003). In agreement with this work, studies in other countries (Jorgensen et al. 2001; Lauritzen et al. 2004) have also shown a positive relationship between human milk levels of DHA and visual function maturation in breast-fed infants. Some studies (Helland et al. 2003; Jensen et al. 2005) have reported an association between infant DHA status at birth or during breast-feeding and scores for tests of cognitive function when measured later in early childhood. In a follow-up of fifty-five infants from the Vancouver studies (Innis et al. 2001; Innis, 2003), however, correction could not be made for the concurrent child diet at 5 years of age, and it was found that mothers with low milk levels of DHA whose children had a low DHA during breast-feeding also fed their child diets low in DHA. It is recognized that, although consistent with an important role of DHA in retinal and neural function, other dietary or lifestyle variables could explain the statistical associations between DHA and infant development (Innis et al. 2001; Innis, 2003). Also evident is the strong probability that women with low intakes of DHA during lactation also have low intakes of DHA in gestation, which could have long-term effects secondary to the role of DHA in neurogenesis (Innis, 2007).

The current studies in Vancouver are investigating whether women with low levels of DHA in their milk also have a low DHA status in gestation. Using dietary intervention with DHA, these studies are also investigating whether n-3 fatty acid deficiency occurs among pregnant and lactating women and whether it poses a risk of reduced visual and neural system maturation among infants in the study population. Women are asked to take a supplement of 400 mg DHA/d from single-cell PUFA or a maize–soybean oil placebo from 16 weeks of gestation until delivery. Maternal dietary intake and blood lipid DHA are determined at 16 and 36 weeks of gestation, and functions dependent on that factor is not linear but plateaus at intakes equal to and above need, only the individuals who are deficient can benefit from intervention. However, since blood levels of DHA continue to increase with increasing DHA intake, a linear relationship between DHA intake or blood levels of DHA and measures of functions dependent on DHA can occur only when all or almost all individuals in the group are deficient. Careful studies in animals have shown that dietary n-3 fatty acid deficiency results in reduced retinal and visual acuity (Innis, 1991), and clinical studies concur that infant visual acuity at 60 d of age is the most robust reproducible measure of inadequate DHA (SanGiovanni et al. 2000). Different from drug studies, in the Vancouver dietary study the placebo group is not a non-exposure group, and overlap will occur between individuals in the placebo group in this study.

Fig. 2. Visual acuity at 60 and 365 d of age among term-gestation infants grouped by tertile of erythrocyte phosphatidylethanolamine (RBC PE)-DHA status at 60 d of age, at which time all infants were breast-fed. The mean DHA in the mothers’ milk of infants in the lower, middle and upper tertile was 0.17, 0.22 and 0.31 g/100 g total milk fatty acids respectively. Visual acuity at 60 and 365 d of age was significantly higher among infants in the highest tertile compared with lowest tertile: *P<0.05. (Adapted from Innis et al. 2001.)

the same study the RBC fatty acids of infants fed formula with no DHA were found to contain 3.7 (SE 0.21) g DHA/100 g. As in adults, increasing the dietary intake of ALA has no measurable effect in increasing blood levels of DHA in young infants (Ponder et al. 1992). These findings suggest that DHA intake, either from the mother’s breast milk or infant formula, is the most important determinant of the infant’s blood levels of DHA. Research by the author’s group in Canada has focused on the variability in maternal dietary DHA intake, and the implications for infant development of low maternal DHA status during both gestation and lactation.

A prospective approach has first addressed whether the variability in human milk DHA is likely to be related to visual and neural development among breast-fed infants (Innis et al. 2001; Innis, 2003). Healthy term-gestation infants (n 88) were assessed using measures of visual acuity, language, mental and motor skill development from birth to 18 months of age, and the Stanford-Binet intelligence test was undertaken at 5 years of age. Milk samples and infant blood samples were collected at 60 d after birth, at which time all the infants were exclusively breast-fed. The results show that infants in the lowest tertile of RBC PE-DHA at 60 d of age have significantly lower visual acuity at 60 and 365 d of age than infants in the highest tertile of RBC PE-DHA (Innis et al. 2001; Fig. 2). The analysis of the mother’s milk fat indicates that infants in lowest, middle and highest tertile of DHA status were fed milk containing mean levels of DHA of 0.17, 0.20 and 0.31 g/100 g total fatty acids respectively. Multivariate analysis controlling for environmental, maternal and infant variables that are known to influence infant development shows that infant DHA status at 60 d of age is related to language development at 9 and 18 months of age, although there are no significant relationships with scores for the

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group with a high habitual DHA intake and deficient individuals in the DHA intervention group with a low DHA intake. Analysis of the RBC-membrane PE is used as a measure of DHA status because it avoids problems in the analysis of fatty acids in plasma total lipids or phospholipids that results from short-term dietary effects and heterogeneity in plasma lipoproteins (VLDL, LDL and HDL) in which lipids (cholesteryl esters, TAG, phospholipids) are carried. Maternal blood is collected at 36 weeks of gestation to avoid possible variables associated with labour and delivery.

Whether women with low levels of DHA in their milk also have a low DHA status in gestation is demonstrated by the milk fat levels of 0·17 and 0·32 g DHA/100 g total fatty acids at 30 d postnatally for women who had an RBC PE-DHA of < 8·5 (n 17) and > 8·5 (n 22) g/100 g total fatty acids at 36 weeks of gestation respectively (P < 0·05; Fig. 1). Dietary analyses show that the intakes of DHA are different (81 (SE 2) and 131 (SE 2) mg/d respectively for placebo and DHA group respectively. As maternal DHA status, supplement group, infant gender, birth weight, length, gestational age at birth, breastfeeding, breast-feeding duration and maternal ethnicity, which together account for 86% of the variability in infant visual acuity at 60 d of age, show that infants in the placebo group have a 2-fold greater risk (OR 2:1) of having a visual acuity score of < 2·32 cycles/degree (the mean score of the study population (n 99); OR 2:1).

Fig. 3. Visual acuity scores at 60 d of age among infants grouped according to their mother’s DHA status at 36 weeks of gestation. The no. of infants whose mothers were assigned to placebo or 400 mg DHA/d from 16 weeks of gestation until delivery were respectively (according to tertile of erythrocyte phosphatidylethanolamine (RBC PE)-DHA; g/100 g total fatty acids): < 8·50, 21, 1; 8·51–13·56, 27, 33; > 13·57, 2, 15. Visual acuity scores were significantly lower among infants in the lowest tertile compared with the highest tertile (P < 0·05). Using multivariate regression DHA supplementation was associated with a 2-fold decrease in the risk of a visual acuity score of < 2·32 cycles/degree (the mean score of the study population (n 99); OR 2:1).
DHA/d and milk levels of <0.2 g DHA/100 g total fatty acids. Data derived from autopsy analysis indicate that the fetus accumulates about 70 mg DHA/d during the last trimester of gestation (Clandinin et al. 1981), and DHA accretion in the human forebrain follows a linear pattern through at least the 12 months after birth (Martinez, 1992). Assuming an average of 37 g fat/l milk, a daily intake of 780 ml human milk with 0.2 g DHA/100 g total fatty acids will provide 56 mg DHA/d to the infant, which is less than projected requirements.

In summary, while the importance of the differences in n-6 and n-3 fatty acids, particularly differences in DHA between human milk and infant formulas, has been the subject of intense clinical research, the n-6 and n-3 fatty acids in human milk also vary widely. As a result, there are marked differences in n-6 and n-3 fatty acid intake among breast-fed infants. Dietary patterns in relation to selection of fats and oils and the choice of whether or not to eat fish appear to be relatively stable. As a result, differences in the n-6 and n-3 fatty acids in human milk affect not only differences in the supply of n-6 and n-3 fatty acids to the breast-fed infant, but also differences in the supply of these fatty acids to the infant before birth. The dependence of human milk LA and DHA on the maternal dietary fat composition shows that for infant feeding human milk is not a gold standard for these fatty acids. Dietary guidelines aimed at reducing risk of chronic disease need to be cognisant that when followed by pregnant and lactating women they also have a marked effect on the amount and balance of n-6 and n-3 fatty acids transferred to the infant before and after birth. The Vancouver studies emphasize the need for dietary fat recommendations for pregnancy and lactation, specific stages of the life cycle involving anabolism and transfer of nutrients to the developing infant. In the absence of dose–response studies to determine safe upper limits for fatty acid supplements during pregnancy, a practical approach, if supplements are to be used, is to use a supplement within the range that would be achieved by currently-recommended intakes of fish (1 g fish oil/d).

References


development in breast-fed term infants? 

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