Review Article

Implications of maternal diet on breast milk docosahexaenoic acid 22:6n-3 (DHA) and arachidonic acid 20:4n-6 (AA) contents: A narrative review

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Background and Objectives: This narrative review aims to provide recent understanding of the implications of maternal diet on fatty acid composition of breast milk, with focus on the docosahexaenoic acid (22:6n-3 (DHA) and arachidonic acid 20:4n-6 (AA) contents, for fetal growth and development. Breast milk n6/n3 polyunsaturated fatty acids (PUFA) ratio will also be highlighted in relations to maternal lipid intake. **Methods and Study Design:** PubMed and Google Scholar were searched for relevant publications in English focusing on but not limited to the use of the key words stated below. **Results:** Studies since the 1950s of different population groups worldwide affirmed the recognition that breastmilk fatty acid compositions are highly sensitive to maternal diet. Colostrum is richer in long-chain PUFA (LC-PUFA) metabolites of both linoleic and linolenic acids than mature milk. Among these LC-PUFA, both DHA and AA are incorporated preferentially and rapidly within the cerebral cortex and the retina during the last trimester of pregnancy and postnatal 18 months. Maternal supply of DHA and AA include maternal fatty acid stores, endogenous synthesis or directly from diet. Decreasing fish intake concomitant with increased intake of meat and vegetable oil leading to decreased intake of DHA and EPA, and an increase in AA intake, have resulted in an imbalanced n-6/n-3 PUFA ratio in breastmilk. **Conclusions:** A balanced intake of PUFAs during pregnancy and lactation is recommended for fetal and childhood growth and development.

Key Words: breast milk fatty acids, maternal diet, long chain polyunsaturated fatty acids, DHA, AA, n6/n3 PUFA ratio

INTRODUCTION

"*The human mammary gland, acting as a remarkable bioreactor to synthesise milk, and the infant, in utilizing breastmilk, reflects 200 million years of symbiotic co-evolution between producer and consumer*".[1]

Breastmilk composition has been a subject of much interest for centuries. "*In clinical studies many organs of prime metabolic interest are not accessible for examination. In this circumstance the study of human milk fat provides unique advantages since it is a metabolic end-product which may be sampled frequently without risk or discomfort to the patient*".[2]

Among earlier reports of purposeful alterations of human milk fat by dietary means were attempts by Thiemrich in1898,[3] who showed "marked changes in the iodine number of the milk fat after feeding mothers with unsaturated fat" cited by Insull et al.[4] In ensuing feeding investigations, Sodarhjelm[5] reported an increase in breastmilk content of "*diene fatty acids following feedings of corn oil, and of higher polyene fatty acids following feedings of fish oil*". Kneebone et al[6] reported studies that intentionally manipulated the level of unsaturation of human breast milk over a range of values. For example, Insull et al[4] succeeded in reducing the level of saturates in breast milk to about 16% of the total by feeding a diet containing 70% corn oil, whilst Potter and Nestel[7] raised the level of milk saturated fatty acids by increasing the P/S ratio from 0.07 to 1.3.

An earlier investigation of Read et al[8] on the influence of dietary carbohydrates and fat on fatty acids of mature milk in different ethnic groups consuming widely differing macronutrient diets, revealed that a high level of carbohydrate intake was the most important dietary factor influencing the fatty acids. Carbohydrate has long been recognised as an important source of both fatty acids and glycerol in the mammary gland, and glycerol itself has been known to stimulate fatty acid synthesis.[9]

Meanwhile feeding experiments conducted by Insull et al[4] demonstrated that during energy equilibrium, milk fat closely resembles dietary fat, but when deficient calories are fed, milk fat approaches the composition of human depot fat. "*These findings suggest that, under either of these nutritional conditions, transfer of dietary or depot fat dictates the fatty acid pattern of human breast milk*".

According to recommendations made by the WHO/FAO, term infants should be exclusively breast-fed

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for the first 6 months of life and regular n-3 LC-PUFA should be provided to lactating women to ensure adequate LC-PUFA intake for infants.[10] Meanwhile, the International Society for the Study of Fatty Acids and Lipids[11] had suggested the AA intake for infants should be above 0.5% of total fat. In the same year, a Joint WHO/FAO Expert Consultation recommended that the minimum level of DHA + EPA intake was 0.3 g/d for pregnant or lactating women to ensure normal growth and development of the fetus, as explained in this background article.[12]

In general, the nutritional composition of mature human milk is known to comprise approximately 3%-5% fat, 0.8%-0.9% protein, 6.9%-7.2% carbohydrate and 0.2% mineral constituents.13 In an extensive review, Brenna et al[14] reported the worldwide means of DHA and AA as 0.32% and 0.47 % respectively of total breastmilk fatty acids. However, it is evident that DHA and AA levels in human breast milk vary by population and their dietary intake. People residing near the sea may have higher breast-milk DHA levels than those living more inland. This could be partly explained by disparate accessibility to fish or marine foods, a major source of DHA.

The present narrative review aims to provide recent understanding of the implications of maternal dietary fat on breastmilk fatty acid composition, with emphasis on the long chain polyunsaturated fatty acids (LC-PUFA), particularly DHA and AA for fetal growth and development. This descriptive article will also highlight the increasing n-6/n-3 PUFA ratio in breastmilk as a reflection of current changes in maternal lipid intake.

COMPOSITION OF BREASTMILK FAT AND FATTY ACIDS

"*Milk can be characterized as an emulsion of milk fat globules in an aqueous liquid*".[15] Garcia & Innis[16] had similarly described breastmilk as a "sophisticated oil-in-water emulsion comprising a core of nonpolar lipids that encompass 98-99% triacylglycerols (TAGs), enclosed by an unusual trimolecular layer of polar lipids, proteins and cholesterol". The milk fat globules are formed in the mammary alveolar cells and served as the single largest component of the infant's energy supply.

In mature human milk, the majority of the TAGs in the milk fat globule core consists of 20-35% oleic acid (18:1n-9), 18-23% palmitic acid (16:0), and 8-18% linoleic acid (18a:2n6). While medium chain fatty acids (MCFA) comprise 12% of total fatty acids, less than 1% are short chain fatty acids (SCFA).[16] Meanwhile, long chain polyunsaturated fatty acids (LC-PUFA) constitute 14-17% of the total fatty acids in human milk.[17,18] The predominant LC-PUFA in human milk and tissues are arachidonic acid (AA, 20:4n-6), eicosapentaenoic acid (EPA, 20:5n3), and docosahexaenoic acid (DHA, 22:6n-3).

LC-PUFA are synthesized from the essential fatty acids, linoleic acid (LA) of the omega-6 (n-6) series and a-linolenic acid (ALA) of the omega 3 (n-3) series, being unable to convert LA and ALA obtained from foods to n-6 (AA) and n-3 LC-PUFA (DHA & EPA), respectively. "*The contribution of endogenous synthesis from ALA to blood and tissue pools of infants does not match that of diets with preformed DHA*".[19]

Despite its relatively small proportion in breastmilk, the LC-PUFA has been well recognized as playing key roles in the structure and function of human tissues, immune function, and brain and retinal development during gestation and infancy.[15,20,21]

IMPORTANCE OF BREASTMILK LC-PUFA FOR FETAL GROWTH AND DEVELOPMENT

The essential functions of LC-PUFA, particularly DHA and AA, for the maturation of the developing brain, retina and other organs in new born infants, as well as to achieve optimal growth and development has been extensively investigated and reviewed over the years.[21-26]

Lipids constitute 50%–60% of the brain's dry weight and are known to play several fundamental roles in critical structural development of the brain, neural function as structural and signalling molecules.[27] and regulation of crucial physiological processes.[25,28] Both DHA and AA are major components of the brain, with DHA and AA comprising 10–20% and 9% respectively of the total fatty acid composition in the brain, both occurring in the highest concentrations in neural membranes.[29] Together, they make up a third of all lipids in the brain's grey matter,[30,31] with DHA in particularly high concentrations in membranes surrounding neural synapses,[32] retinal phospholipids,[33] and photoreceptors.[34]

Quantitative fatty acid analysis of cerebellum, frontal and occipital brain lobes indicated rapid accretion of chain elongation and desaturation products during the last trimester of a human pregnancy and continues at very high rates up to the end of the second year of life.[35,36] Studies demonstrated that "*uncorrected deficits in brain DHA accrual during perinatal development led to transient and enduring structural and functional neurodevelopmental abnormalities*".[37]

Both DHA and AA are particularly enriched in the phospholipids of cell membrane of neural tissues, mainly localised in phosphatidylethanolamine and phosphatidylserine.[38] The brains of several mammalian species have also been shown to be progressively enriched in DHA at the expense of the n-6 fatty acids, with phosphatidylserine and phosphatidylethanolamine being the principal repositories for DHA.[39,40] The essentiality of DHA in the mammalian brain could be due in part to "*brain energy metabolism (facilitation of glucose entry into the brain), which is significantly influenced by neural omega-3 status*".[25]

Among brain components that depend on dietary components, DHA is limiting because its synthesis from terrestrial plant food precursors is low but its utilization when consumed in diet is very efficient.[41] Dietary preformed DHA in the breast milk of modern mothers supports many-fold greater breast milk DHA than is found in the breast milk of vegans, a phenomenon linked to consumption of shore-based foods. Most current evidence suggests that the DHA-rich human brain required an ample and sustained source of dietary DHA to reach its full potential.[41]

LC-PUFA come mainly from maternal intake, being released from the TAGs in the intestines as chylomicrons that are transported to the liver, from which they are subsequently transported to the mammary glands as very-low-density lipoproteins (VLDLs).[42] The second source of LC-PUFA is systemic biosynthesis, which takes place principally in the liver. Thus, not only the woman's current but also her long-term dietary intake is of marked relevance for milk fat composition.[17,18]

As the biosynthesis of DHA and AA is inefficient in a growing fetus and placenta, increased intake of preformed DHA is required for optimal infant brain development. This is supported by observational findings that "*higher maternal DHA status during pregnancy is associated with enduring neurocognitive benefits in infants and children*".[43] The maternal dietary balance of DHA and AA intake and their interactions are recognised as essential for the development and function of the neonatal brain.[26]

INFLUENCE OF MATERNAL DIET ON BREAST-MILK DHA AND AA

A woman's diet during pregnancy and lactation plays a central role in the adequate contribution of macro and micronutrients during the fetal life and lactation. Essential fatty acids and LC-PUFA including DHA and AA, are directly associated with maternal dietary intake and body store. The functionality of the placenta determines the levels of these fatty acids from the mother to her offspring, first through the placenta (intra-uterine life), and then through lactation.[44]

Preformed DHA was suggested to have been an integral dietary constituent during evolution of the genus Homo to facilitate the growth and development of an encephalizing brain. The basis of this belief came about as it was known that (i) DHA formed a primary constituent of membrane phospholipids within the synaptic networks of the brain essential for optimal cognitive functioning, and (ii) biosynthesis of DHA from n-3 dietary precursors (alpha-linolenic acid, LNA) is relatively inefficient. However, there were differing schools of thoughts with regards to the main resources of the preformed DHA. As preformed DHA had been identified to an appreciable extent within aquatic resources (marine and freshwater), this led to speculation that hominin encephalization was linked specifically to access and consumption of aquatic resources. Nonetheless, there were researchers,[45] who did not support this assumption, claiming that "*DHA being available in a wider variety of sources within a number of terrestrial ecosystems is sufficient for normal brain development and maintenance in modern humans and presumably our ancestors*". Other researchers including Cunnane et al[46] who provided evidence of fossil record that shows some of the earliest hominins were regularly consuming fish, reiterated support for the concept that access to shore-based diets (fish, molluscs, crustaceans, frogs, bird's eggs and aquatic plants), provides the rich dietary sources of brain selective nutrients necessary for hominin encephalization. Thus, by providing access to an enriched dietary source of brain selective nutrients and by permitting evolution of body fat, a shore-based habitat masked the neurodevelopmental vulnerability that is still a hallmark of human infants today. "*Together, these two developments in early hominins led eventually to evolution of the modern human brain*".[47]

The composition of human milk fatty acids depends on three factors: endogenous biosynthesis in the mammary gland,[18] the release of fatty acids from tissue deposits laid down during pregnancy,[48] and most importantly – the current diet of the breastfeeding mother.[17,49] Whilst maternal diet is a key factor affecting human milk composition, the fat content and fatty acid composition are known to be influenced by several other aspects, including the lactation period, maternal nutritional status, and genetic background.[20,50] Conventionally, lactation period has been viewed in three stages: colostrum (day 1–5 postpartum), transitional milk (day 6–15 postpartum), and mature milk (after day 15 postpartum).

Human milk fat composition varies considerably among individuals and is dynamic over the course of lactation, but also varies over a single breastfeed. This characteristic of human milk fatty acid composition subject to change during the course of a feeding had gained early recognition in medical writings since the 15th century as quoted by Hytten.[51] These variations reflect maternal factors including duration of pregnancy, parity, diet, environment, genetics, and body composition, as well as the changing needs of the infant over the period of lactation and the stage of lactation.[52] According to Insulls et al[4] "*there is a very marked diurnal variation in both the volume and the fat content of human milk, but the other major constituents show no systematic change*". In general, this circadian variation in total fat concentration with its nadir (lowest value) in the morning and its acrophase (peak) either in the afternoon or evening, was affirmed in a recent systematic review.[53] It has been shown that fatty acids content in human milk changes during the lactation stages, and factors, such as maternal diet may influence breastmilk short chain fatty acids, and LC-PUFA composition. In a study on temporal changes of human breast milk collected over eight months postpartum of 539 urban mothers in China, Giuffrida et al[54] reported that "*total PUFA n-6 increased from 21.7% in colostrum to 24.1% of total fatty acids in mature milk with linoleic acid (18:2n-6) being the most abundant fatty acid and increasing along the lactation time from 18.9% in colostrum to 22.8% of total fatty acids in mature milk. In contrast, AA (20:4n-6) content decreased from 0.9% to 0.5% of total FA from colostrum to mature milk*". DHA contents for the total population ranged from 0.3%, in mature milk, to 0.5% in colostrum and transitional milk, therefore, lower than the DHA content reported for Chinese marine populations in colostrum (1.5%), transitional (0.6%) and mature milk (0.5%–2.8%).[55]

Researchers who evaluated the effects of variation of maternal dietary polyunsaturated and saturated fats on maternal plasma and milk fatty acids reported that providing a polyunsaturate-rich diet to lactating women markedly increases their plasma and milk concentrations of PUFAs, and diminishes saturated fatty acids.[56] Subsequent studies and reviews by Innis[57,58] also clarified that the levels of n-6 and n-3 fatty acids, particularly LA, ALA and DHA, in human milk varied widely within and among different populations, but "*they are readily changed by the maternal dietary intake of the respective fatty acid*".

A review of 14 studies from 9 European countries and 10 studies from 7 African countries on fatty acids in mature human milk, stated that "*considering the marked differences in methods and dietary composition in different parts of Europe and Africa, the average milk fatty acid composition data are surprisingly consistent*".[59] The diet of lactating women apparently influences, to a certain extent, the milk content of saturated and monounsaturated fatty acids and linoleic acid (18:2n-6), but different self-selected diets in different geographic locations seem to have little effect on the milk content of 20 and 22 carbon LC-PUFA.[60]

Kneebone et al[6] also reported that, despite large variations in their diets among Malaysian women of multi-ethnicity, "*the levels of essential fatty acid (18:2w6) in the breast milk of the mothers was within, or above, the range of values reported for well-nourished Swedish mothers, and furthermore the level of long chain PUFA such as DHA and AA were higher than levels reported in Australian mothers*". This finding was attributed to consumption of local fish which are a rich source of both 20:4n6 and 22:6n3.

In a descriptive meta-analysis of 65 studies of human milk from island/coastal and inland populations, Brenna et al[14] reported that overall, the mean DHA concentration was lower than and more variable than that of AA. Also, the highest DHA concentrations were primarily in coastal populations associated with marine food consumption. Concentrations of DHA and AA in breast milk depend on the amount of these preformed fatty acids in the mother's diet and their biosynthesis from precursors. Milk DHA content appears to be closely linked to maternal dietary DHA intake, with dose dependent linear increases in breast-milk concentrations of DHA with increased maternal intake.[14,61]

Nonetheless, conflicting findings on the importance of LC-PUFA have been reported. In a Cochrane Database of Systematic Reviews, Jasani et al[62] compared outcomes of full-term babies, who were given formula milk enriched with LC-PUFA versus outcomes of full-term babies fed formula milk without enrichment with LC-PUFA. "*Most of the included RCTs reported no beneficial effects or harms of LC-PUFA supplementation on neurodevelopmental outcomes of formula-fed full-term infants and no consistent beneficial effects on visual acuity. Routine supplementation of full-term infant milk formula with LC-PUFA was not recommended at this time*".[62]

DHA and EPA are only found in seafoods with its content of DHA and EPA ranging from several hundred mg to more than 1 g per 100 g of fish. In contrast, AA is found only in animal-derived foods including meat, poultry, eggs, fish and dairy foods. However, the contents of AA are moderate, <200 mg per 100 g of these foods, revealing the wide but small distribution of AA in major animal foods.[63]

Applying the WHO recommendation on exclusive breastfeeding for 6 months, it is estimated that at 6 months, "*infants will be receiving approximately 190 mg/d AA and 130 mg/d DHA, based on a breast milk intake of 850 mL/d and the reported mean concentrations of DHA and ARA in human milk*".[64,65]

BREAST MILK N6/N3 PUFA RATIO

It is postulated that human beings evolved on a diet with a ratio of n-6 to n-3 PUFA of approximately 1 whereas subsisting on the modern Western diets has led to the ratio in excess of 20:1.[66] "*Western diets are deficient in n-3 fatty acids, and have excessive amounts of n-6 fatty acids, compared with the diet on which human beings evolved and their genetic patterns were established*". It was recognized that the dietary imbalance of n-6 to n-3 PUFA can lower the synthesis of n-3 LC-PUFA, particularly DHA, because of the competition generated between the respective precursors for the active sites of desaturase enzymes.[67] An excess of n-6 fatty acid (such as LA) may decrease the synthesis of DHA from ALA.[68]

High dietary ratios of n-6 to n-3 PUFA have been implicated in promoting the pathogenesis and controlling markers of several metabolic disorders, including cardiovascular disease, cancer, inflammatory and autoimmune diseases, whereas increased levels of omega-3 PUFA (thus a lower n-6 to n-3 ratio), exert suppressive effects.[69-71] The anti-inflammatory effects of marine n-3 PUFA may contribute to their protective actions towards atherosclerosis and plaque rupture.[72] Low-ratio n-6 to n-3 PUFA supplementation was found to decrease significantly the concentration of serum TNF-α and IL-6, but not the CRP concentration.[73] The optimal ratio may vary with the disease under consideration.[74]

Dietary strategies have been developed world-wide to improve the quality of the diet of women during pregnancy and lactation, including educational programs with emphasis on promoting consumption of foods that provide LC-PUFA especially DHA from marine origin. Worldwide mean levels of DHA and AA in breast milk were estimated as 0.37% and 0.55% of total fatty acids, respectively.[75] The breastmilk DHA levels from women with accessibility to marine foods were significantly higher than those from women without accessibility. Data from different regions in China affirmed that different dietary habits were largely the drivers behind the different fatty acid profiles in samples obtained from colostrum, transitional milk and mature milk of lactating mothers.[76]

Recent studies worldwide generally showed maternal intake of n-6/n-3 PUFA ratio at undesirably high levels. In assessing Chilean women during the last stage of pregnancy and across the lactation period, Barrera et al[77] found maternal intake high in n-6 PUFA and low in n-3 PUFA, especially LA (owing to frequent consumption of soy or sunflower oil), which can produce a reduction of the capacity of the mother for transferring DHA to her offspring during pregnancy and breast feeding. "*Erythrocytes and breast milk DHA concentration was significantly reduced during lactation compared to pregnancy*".

Several investigators had reported high n-6 to n-3 PUFA ratios among European women e.g., a ratio of about 26 among Greek mothers,[78] and about 13 in Spanish women.[77] Despite residing in the coastal Croatia, the breastfeeding mothers showed sub-optimal dietary intake of n-3 LC-PUFA, resulting in a relatively high n6 to n3 PUFA ratio of about 12.[79] An investigation of lactating women in Latvia reported that "*while macronutrient (fat, protein, and lactose) content in human milk is not affected by maternal diet, conversely, the human milk fatty acid* *profile is affected by the immediate diet consumed by the mother - significant positive correlations were found among C18-2n6, C18-3n3, monounsaturated, and PUFA, n-6 to n-3 ratio and DHA intake in human milk*", the latter being noticeably lower than recommended.[71]

During the past decades, as the result of rapid economic development, conspicuous changes in dietary habits, primarily manifested by a significant increase of vegetable oil consumption, has occurred in China. Data from the National Nutrition Survey in China (CHNS) between 1992-2002 showed vegetable oil intake of urban and rural dwellers increased by 24.1% and 74.8%, respectively.[80] This rapid dietary transition has precipitated the n-6 to n-3 ratio imbalance because the vegetable oils consumed are high in the n-6 fatty acid (linoleic acid, C18:2 n6). The estimated n-6 to n-3 fatty acid ratio of American and Chinese diets were 10:1 and 5-7:1 respectively.[80] The CHNS between 1997 -2011 also reported growing trends of adults ingesting more fat mainly from vegetable oils and their dietary EPA and DHA intakes remained very low owing to low consumptions of fish and seafood.[81]

Globally, with increasing consumption of vegetable oils, interest on the effects of dietary fats on the lipid composition of breastmilk has heightened. The general observation was that when maternal diets were high on corn oil, the content of n-6 PUFA in breastmilk significantly increased.

CLOSING REMARKS

"*Breast feeding is an integral part of the reproductive process, the natural and ideal way of feeding the infant and a unique biological and emotional basis for child development*".[82] This, together with its other important effects, including prevention of infections, child spacing and on the health and well-being of the mother makes it a key aspect of primary health care.

Several reviews have affirmed that maternal dietary intake, particularly fatty acids, and some micronutrients, was related to their contents in breast milk composition.[83,84] It is thus not surprising that for the past several decades, there has been extensive interest in the roles of lipids particularly LC-PUFA, not only in infant growth and development, but also in metabolic functions in brain, immune and cardiovascular systems.[85-87] Nonetheless, there remains a lack of consensus on the need for explicit recommendations on dietary intake for both DHA and AA especially during the early years of life.[65]

Recommendations by WHO and FAO have been established for dietary fat intakes to decrease the burden of chronic diseases caused by excessive intakes of specific dietary fatty acids worldwide: for total dietary fat, 15–35 % of total energy (%TE); for SFA, an upper limit of 10 %TE; and 6–11 %TE for PUFA.[88] As such, "*there is no rational for a specific recommendation for n-6 to n-3 ratio, or LA to ALA ratio, if intakes of n-6 and n-3 fatty acids lie within the recommendation established in this report*". The European Food Safety Authority (EFSA) shared the position that, while currently there are insufficient data to set a value for the n-6/n-3 PUFA ratio, the ratio should be kept as low as possible in order to reduce the risk of many chronic diseases.[89]

Further research including human clinical trials are needed for definitive conclusions that dietary deficiency of n-3 fatty acids during fetal development *in utero* and the postnatal state has detrimental effects on cognitive abilities.[90] While the biological mechanisms for the consequences of a diet rich in cholesterol or saturated fatty acids in the expression or suppression of brain markers are not well understood,[91] a lower ratio of n6 to n-3 fatty acids is desirable in reducing the risk of many of the chronic diseases of high prevalence in developed as well as in the developing countries.

AUTHOR DISCLOSURES

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