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GENETICS (GVZ DEDOUSSIS, SECTION EDITOR)

Maternal Nutrition, Genetics, and Human Milk Lipids

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Abstract Human milk provides the sole source of nutrition and immunological protection for neonates during the first weeks to months after birth. Triacylglycerols are one of the largest components of human milk and they provide fatty acids for energy and growth and development of the infant's storage and membrane lipids. Fatty acids are one of the most variable components of human milk, with an undisputable, strong influence of maternal fatty acid nutrition, and smaller but potentially important contribution of maternal genetic variation. This review begins with the question of whether recent changes to the diet of modern women has shifted the composition of human milk essential n-6 and n-3 fatty acids, potentially resulting in failure to meet the needs of the young infant. This is followed by the emerging area of the link between lipid nutrition and genetic variability and its impact on human milk n-6 and n-3 fatty acids.

Keywords Human milk · Lipids · Fatty acids · n-6 fatty acids · n-3 fatty acids · Linoleic acid · Docosahexaenoic acid · Arachidonic acid · Medium chain fatty acids · Lactating mammary gland · Westernized diet · Fish · Maternal nutrition · Genetics · Fatty acid desaturases · Fatty acid elongases · Traditional diet

Introduction

Human milk is the sole source of nutrition for neonates during the first weeks to months after birth and has evolved to provide nutrition, appropriate modulation of feeding, growth and development, and protection in the extra-uterine environment into which the infant is born. Recent advances have seen a growing appreciation of milk secretion as a unifying characteristic of all mammals, with an ancient evolutionary origin dating more than 150 million years [1–3]. Current theories suggest that the mammary gland and "milk" evolved from the fluid secretions of apocrine-like glands associated with abdominal hair follicles. By providing crucial immunological protection and nutrients, these secretions gave pivotal survival advantages for offspring born into pathogen-laden, nutrient-poor environments, and they have continued to evolve into what is now the wide diversity of species-specific mammalian milks. The early roles of modern milk antecedents of conferring immunological protection, nutrients, and growth-modulating factors remain the hallmark of the many benefits of human milk for the young infant [4–6].

Lipids comprise the second largest component of human milk and provide energy, essential lipids, and bioactive components but in complex structures that vary in content and composition. The first part of this review introduces human milk lipids and the question of whether recent changes to the diet of modern women has shifted the composition of human milk lipids in a timeframe too fast for evolutionary adaptation, potentially resulting in maladaptation, with increased disease risk or loss of developmental potential for the infant. The second part of this review addresses the emerging area of the link between maternal lipid nutrition, human milk lipids, and genetic variability shaped by selective advantage to food resources of the prevailing ecological environment to which different ancestral populations migrated and adapted.

Human Milk Lipid Composition

The major components of human milk are proteins, lactose and oligosaccharides, which in mature milk average approximately 8, 70, and 10 g/L, respectively, with lipids representing the second largest component at 40 g/L [7, 8]. Although the fat content of mammalian milks varies tremendously, all species, including humans, secrete milk lipids in similar, highly

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specialized fat globules. These globules are comprised of a central core of mainly triacylglycerols (TAG) surrounded by a monolayer layer of phospholipids, itself enclosed in a classical phospholipid bilayer membrane with a glycosylated surface [3, 9–11]. This multilayer structure, known as the milk fat globule membrane, is found only in milk and functions to stabilize the milk fat globule as an emulsion within the aqueous milk environment. At the same time, it provides the infant with numerous nutritionally and immunologically important components, of which the glycoproteins and glycolipids are becoming increasingly understood [9, 12–14]. The globule membrane lipids, unesterified cholesterol, phosphatidylcholine, phosphatidylethanolamine, including ethanolamine plasmalogens, phosphatidylserine, phosphatidylinositol, and sphingomyelin are typical of cellular membranes but differ from other species and with stage of lactation [7, 15–19]. However, there is as yet no evidence that human milk globule membrane lipids are altered by nutrition or genetic variability in healthy, well-nourished women.

TAG are comprised of a glycerol backbone to which three fatty acids are esterified. TAG represent approximately 98 % of human milk total lipid and are thus synonymous with the milk total fat [7, 8]. Human milk TAG increase from approximately 2.0 g/dL in colostrum to an average of 4.0 g/dL in mature milk produced after the first 30 days of lactation [7, 8, 20–22]. However, the fat content of mature human milk varies widely, from approximately 2.5 to 4.5 g/dL. Because fat is the major source of the milk energy, this results in considerable variability in caloric density. The high TAG biosynthetic activity of the human mammary gland, involving secretion of 20–30 g/day TAG, requires large amounts of fatty acids. These fatty acids may be derived from three sources: fatty acids transferred directly from the maternal diet, fatty acids stored or synthesized in maternal tissues, and fatty acids synthesized in the mammary gland [23, 24]. These three sources must be balanced to maintain a relatively constant supply of milk fat and energy for the young infant.

Human dietary and endogenously synthesized fatty acids are carboxylic acids, which typically have an even number of 8 to 24 carbons in straight chains with zero, one, or more than one double bonds, thus termed saturated, monounsaturated, or polyunsaturated, respectively. Unsaturated fatty acids are further grouped as n-9, n-7, n-6, or n-3 based on the position of the carbon with the first double bond from the methyl end of the fatty acid [25, 26]. More than 150 different fatty acids have been identified in human milk, many of which are present as a result of transfer from the maternal diet, either directly or after storage and modification in maternal tissues [7, 23]. Regardless of the large number of fatty acids, most work on maternal or infant nutrition and human milk fatty acids has been limited to the 10–20 most common saturated and *cis* unsaturated fatty acids. Among these fatty acids, the n-6 and n-3 fatty acids have attracted the most attention because of the absence of the Δ12 and Δ15 desaturase enzymes needed to synthesize n-6 or n-3 fatty acids, respectively, in mammalian cells [26, 27]. This lack of Δ12 and Δ15 desaturases results in an obligate dietary requirement for n-6 and n-3 fatty acids in animals, including humans. In turn, this means that human milk supplies of essential n-6 and n-3 fatty acids are entirely dependent on the nutrition of the mother and that of the breastfed infant on the mother's milk.

The n-6 and n-3 fatty acids are a complex series of noninterchangeable, often mutually competitive, fatty acids that are unevenly distributed in the diet [28, 29]. Although typically depicted in linear pathways that begin with linoleic acid (18:2n-6) and α-linoleic acid (18:3n-3) from which desaturation and elongation leads to arachidonic acid (20:4n-6, ARA) and eicosapentaenoic acid (20:5n-3, EPA) and docosahexaenoic acid (22:6n-3, DHA), respectively (Fig. 1), the competitive interactions within and between the n-6 and n-3 fatty acids are better appreciated as a cycle. The initial desaturation of 18:2n-6 to 18:3n-6 and 18:3n-3 to 18:4n-3 requires Δ-6 desaturase, which also is required later in the pathway for desaturation of 24:4n-6 to 24:5n-6 and 24:5n-3 and 24:6n-3. Intermediate steps for synthesis of ARA and EPA are accomplished by Δ-5 desaturase, which desaturates 20:3n-6 to produce ARA and 20:4n-3 to produce EPA [25, 28]. Key points are that although human milk 18:2n-6 and 18:3n-3 must originate from the maternal diet, their metabolites, including ARA and EPA and DHA, may be derived from the maternal diet or synthesis from 18:2n-6 or 18:3n-3, respectively, in maternal tissues. The extent to which human milk n-6 and n-3 fatty acids, particularly DHA, depends on maternal nutrition and the implications for infant neurological and immunological development has been the focus of much of the research on human milk fatty acids over the past three decades [23, 29–33].

Human milk saturated and monounsaturated fatty acids may originate from the maternal diet or synthesis in the maternal liver or mammary gland [7, 22, 23]. Humans, like other animals, synthesize palmitic acid (16:0), which can be elongated to stearic acid (18:0) and desaturated by stearoyl CoA desaturase (SCD, Δ9 desaturase) to oleic acid (18:1n-9), the major monounsaturated fatty acid in human milk. Mammary gland fatty acid synthesis differs from classical fatty acid synthesis in the liver and other organs due to the mammary gland-specific enzyme thioesterase II, an enzyme that terminates fatty acid synthesis at carbon chain lengths of 10 to 14. This gives rise to the medium chain fatty acids (MCFA), capric acid (10:0), lauric acid (12:0), and myristic acid (14:0), which are esterified in TAG and secreted in milk. The contribution of human mammary gland synthesis to 16:0 and 18:1n-9 secreted in milk is incompletely understood. Early studies suggested that mammary gland SCD is low and

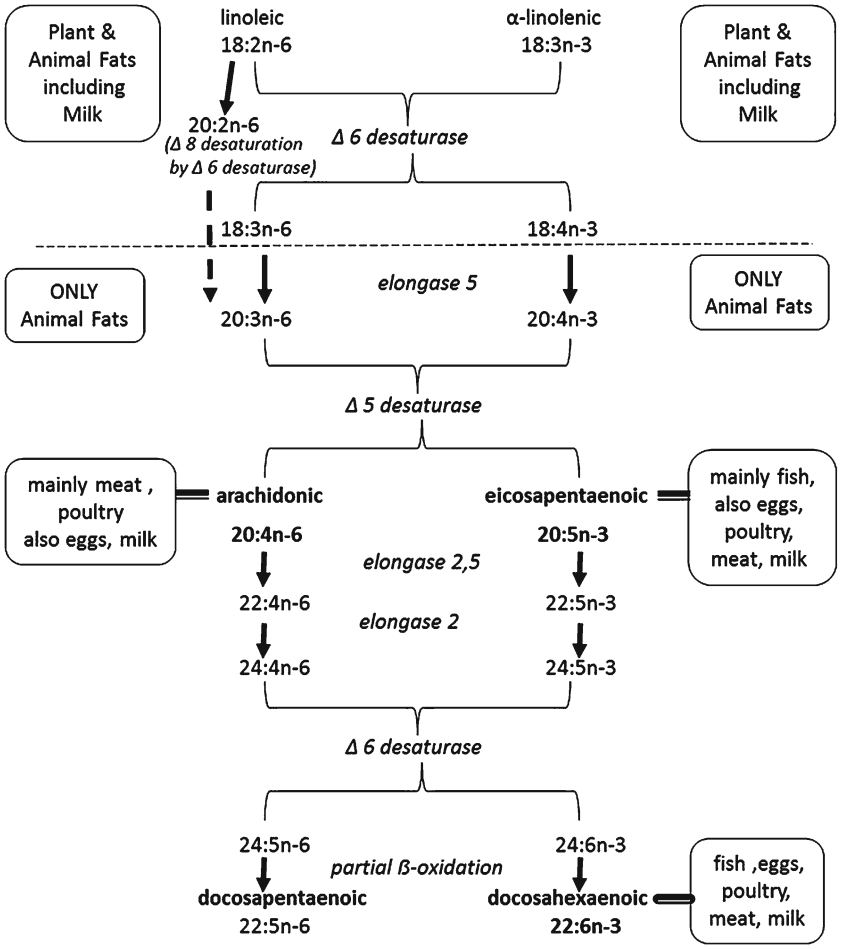

Fig. 1 Schematic of n-6 and n-3 fatty acid desaturation and elongation, illustrating need for Δ 6 desaturase at the beginning and end of the pathway, and food sources of different fatty acids

implicated the liver as the major source of milk 18:1n-9 in the rat [34]. More recent studies on lipid synthesis in the mouse mammary gland, however, have shown higher SCD2 expression in the lactating mammary gland than liver [24, 35–37]. Important species differences are evident. Although the mouse expresses four SCD isoforms, SCD-1, SCD-2, SCD-3, and SCD-4, humans and several other species express two, SCD-1 and SCD-5 [38, 39]. SCD-1 shows almost ubiquitous tissue expression and is increased in liver in response to a high carbohydrate-low fat diet but is deceased by polyunsaturated fatty acids [38–40]. SCD-5, on the other hand, appears to be a unique SCD that arose from gene duplication rather than being an SCD1 isoform, is restricted primarily to the brain and pancreas, and differs from other SCD in being unresponsive to dietary fatty acids [39, 41].

Milk fatty acids of women with genetic defects in lipid metabolism also give insight into the human mammary gland synthesis of 16:0 and 18:1n-9, as well as the intermediate 18:0. Women with familial lipoprotein lipase deficiency, and thus unable to hydrolyze plasma TAG, show a marked increase in milk MCFA, normal 16:0 and 18:0, but considerably reduced 18:1n-9 and 18:2n-6, with undetectable 20:3n-6 or ARA, despite high plasma 18:1n-9, 18:2n-6, and ARA [42, 43]. Phenotypic abetalipoproteinaemia, in which chylomicrons and very low-density lipoproteins are absent, similarly results in large increases in human milk MCFA and 16:0, but greatly reduced 18:1n-9, and virtually absent 18:2n-6 and 18:3n-3 [44]. Overall, it is clear the human mammary gland actively synthesizes MCFA, and probably 16:0 and 18:0, but takes up much of the 18:1n-9, and all of the n-6 and n-3 fatty acids from plasma lipids. This conclusion is consistent with the recently demonstrated decrease in MCFA as 18:1n-9, n-6 and n-3 fatty acids increase in human milk and TAG increase in maternal plasma [45•]. This coordination of endogenous and exogenous fatty acid supplies enables transfer of energy and important milk fat globule membrane components to be maintained, regardless of the maternal dietary fat and carbohydrate balance, or plasma TAG.

The human mammary gland also has unusual pathways of TAG synthesis that differ from other human tissues [46]. Rather than random fatty acid arrangements, human milk TAG show stereospecific fatty acid positioning that results in fairly constant 18-25 % 16:0, with approximately 70 % of the 16:0 acylated at the center (*sn*-2) carbon of the glycerol backbone of TAG destined for secretion in milk. As a result, more than 30 % of human milk TAG have 16:0 esterified at the center position, with 18:1n-9, 18:2n-6, and 18:3n-3 redirected to the TAG outer (*sn*-1 and 3) carbons [7, 46].

There is as yet no information to suggest maternal nutrition or genetic variability alters the unusual TAG structures in human milk.

In sum, human milk contains a complex mix of fatty acids synthesized endogenously and transferred from the maternal diet. Human milk lipid synthesis involves adaptations to balance mammary MCFA synthesis with the supply of monounsaturated, n-6 and n-3 fatty acids from plasma, also maintaining relatively constant 16:0 in unusual TAG structures. Regardless of this counter-balancing between mammary gland fatty acid synthesis and uptake from plasma, there appear to be no particular mechanisms for regulated release of monounsaturated or essential n-6 and n-3 fatty acids in human milk.

Maternal Nutrition and Human Milk Fatty Acids

More than 50 years ago, Insull et al. reported 7.7 % 18:2n-6 in milk from women following their usual diet, which increased to 41.4 % 18:2n-6 within a few days of changing to 40 % dietary energy from corn oil (approximately 52 % 18:2n-6) and to 10.4 % 18:2n-6 when the diet was changed to 40 % energy from lard (approximately 10 % 18:2n-6) [46]. The increase in milk 18:2n-6 on changing to corn oil was accompanied by a 50 % decrease in ARA (measured as tetraenes) from 0.7 % to 0.3 % milk fatty acids and a decrease in DHA (measured as hexaenes) from 0.3 % to trace amounts. These classic studies concluded that dietary fatty acids are rapidly transferred into human milk and that human milk fatty acids can be radically altered within 2–3 days to mimic the quality of the dietary fat, without affecting milk volume or total fat output [46, 47]. Since that time, this concept has been extended and reinforced with numerous studies to show that the secretion of other fatty acids in human milk, including *trans* fatty acids, 18:1n-9, 18:3n-3, EPA, and DHA is largely unregulated, with the amount transferred into milk dependent on the maternal intake of the same fatty acid [7, 23, 32, 47–54]. Stable isotope tracer studies have provided confirmatory evidence for this, showing transfer of 16:0, 18:1n-9, 18:2n-6, and DHA from the maternal diet into human milk, with little or no evidence of selectively among these fatty acids [55–57].

The high concentrations and functional importance of DHA in the brain and retina [26, 27, 57] and knowledge that preformed DHA in milk is more efficient in supporting postnatal brain and retina DHA accretion than 18:3n-3 [58–60] has focused attention on human milk DHA and its dependence on maternal nutrition. DHA, like EPA and ARA, is naturally present in the human diet only in animal lipids but with a much greater abundance of DHA and EPA in fish and seafoods than meats, poultry, and their products [28, 61] (Fig. 1). DHA in human milk from different countries and among women following vegetarian, vegan, or mixed diets ranges from averages of less than 0.1 % in milk from women with vegetarian diets to more than 1.0 % in milk fatty acids from women with high DHA intakes from fish or marine mammals [7, 8, 23, 49, 63–67, 68••]. The typical average DHA in human milk in developed countries with westernized diets is 0.2-0.4 % fatty acids. The dose-dependent increase in human milk DHA with DHA, but not 18:3n-3 supplementation, of lactating mothers reinforces the dependence of human milk DHA on maternal nutrition [23, 53, 54]. This phenomenon is not unique to DHA, but also occurs for18:1n-9, *trans* fatty acids, 18:2n-6, and 18:3n-3 [47–52].

Some insight into how far contemporary fatty acid nutrition has shifted human milk fatty acids may be gleaned from consideration of recent changes to fatty acid nutrition and their impact on human milk. Until the beginning of animal domestication and agriculture some 100,000 years ago, humans followed primarily hunter-gather diets, with bouts of feast and famine [69, 70]. Early domestication of animals, with crops including cereals, roots, and tubers would have had little impact on fatty acid nutrition, with 18:2n-6 necessarily limited to that in animal tissues and plant material, roughly similar amounts of 18:3n-3 and much greater proportions of ARA, EPA and DHA [71–74]. The essentiality of 18:2n-6 and abundance limited mainly to propagative parts of plants might have favored selective retention for use during times of famine. There is no doubt that dramatic changes to fatty acid nutrition have occurred during the past century, resulting in part from the increase of commercial oil-seed processing and use of oil-seed crops as global commodities, recommendations to replace animal fats with vegetable oils to achieve intakes of 10 % energy from 18:2n-6, and changing lifestyles to a greater reliance on processed foods [74, 75]. The available data indicate that while 18:2n-6 contributed less than 2-3 % dietary energy until the late 19th to early 20th century, 80-90 % of all n-6 and n-3 fatty acids in westernized diets are now 18:2n-6, with less than 0.5 % consumed as EPA, DHA, and ARA, and the balance from 18:3n-3 [71, 74–76]. During this same period, human milk 18:2n-6 has doubled from 7-10 % of fatty acids in the 1950s and 1960s to reach 14-18 % or higher in same countries, whereas DHA has decreased [23, 48, 77].

Information, although limited, on human milk fatty acids from populations largely untouched by modern agriculture and industry provide supportive data to suggest that recent changes to maternal fatty acid nutrition have had a major impact on human milk. The Tsimane are a forager-horticulturalist society in the Bolivian Amazonian basin consuming 17 % energy from wild game, 7 % from fresh water fish, 2 % from beef, poultry, and pork, 72 % from locally cultivated staples (rice, plantain, manioc, corn), fruits and nuts, and only 2 % from purchased foods [68••]. Recent work on Tsimane human milk show approximately 10 % 18:2n-6,

similar to human milk in North America and Europe before the influx of 18:2n-6–rich oils [23, 48, 68••, 77]. Tsimane milk not only has higher DHA at 0.7 % fatty acids but also twofold higher ARA and significant EPA compared with the negligible EPA typical of human milk in westernized nations [62–64]. Not only is the high EPA and DHA in Tsimane milk similar to that in women in countries with high fish intakes, but the combination of high EPA and DHA with ARA of approximately 1 % human milk fatty acids resembles human milk from China and Japan [66, 67]. The conclusion follows that human milk unsaturated fatty acids reflect maternal dietary fatty acid quality; whether they reflect an optimal fatty acid supply for the young infant is a different question and not addressed by simply averaging fatty acid levels in human milk from modern-day women.

Maternal Genetics and Human Milk Fatty Acids

Genetic variants contributing to differences in human milk fatty acids have thus far focused largely on qualitative (base-pair changes or single nucleotide polymorphisms, SNP) changes in Δ-6 and Δ-5 fatty acid desaturases (FADS) and very long chain fatty acid elongases (Elovl) required for desaturation and elongation of 18:2n-6 and18:3n-3 (Fig. 1). Several studies have shown an association between SNP in FADS and Elvol and altered plasma, serum, blood cells, and adipose 18:2n-6 and 18:3n-3 and their desaturation-elongation products, although the relevance to human disease remains unclear [78–83]. Studies on human milk fatty acids, on the other hand, have suggested interactions between breastfeeding and genotype, child cognitive development, and risk of allergic disease [84, 85, 86•, 87••, 88].

The Δ-6 and Δ-5 desaturases are encoded by FADS2 and FADS1, respectively, located in head-to head orientation on chromosome 11q, together with FADS3 [89]. FADS3 has not been found to be involved in fatty acid desaturation, and although implicated as a causal candidate gene for hypertriglyceridemia [90, 91], it has not been studied with respect to human milk. The Elovl gene family on chromosome 6 encodes for at least seven elongases with substrate and tissue specific fatty acid elongation [92, 93]. Elovl 1, 3, and 6 are involved in saturated and monounsaturated fatty acid elongation, Elovl 2 and 5 are required for elongation of n-6 and n-3 fatty acids, and Elovl 4 is located in retina photoreceptor cells and involved in DHA synthesis. Recent studies on the ancestry of SNP in the FADS gene cluster have raised the question of whether geographic variance in food resources may have favored mutations for increased n-6 and n-3 fatty acids desaturation in some populations [94••]. Two common, distinct FADS haplotypes involving 28 closely linked SNP in the FADS1/FADS2 gene cluster are strongly associated with, and account for most of the differences in plasma phospholipid ARA, DHA, and the ratios of 18:3n-6/18:2n-6, 18:4n-3/18:3n-3, ARA/20:3n-6 and EPA/20:4n-3. Strong geographic variance, with a high frequency of the haptolotype associated with fatty acid markers of higher Δ6 and Δ5 desaturase activity occur among those of African ancestry with loss of the haplotype in East Asia, Native Americans, and Oceania [94••]. Extrapolation of the evolutionary history of the FADS gene cluster from archaic humans and other primates points to positive selection to favor higher ARA and DHA synthesis after the split from Neanderthals, perhaps favoring desaturation in harsh environments with inconsistent food resources. Although once beneficial, such adaptation may now represent significant maladaptation to modern food supplies where abundant 18:2n-6 would favor ARA synthesis [94••]. Population studies revealing higher ARA and ARA/20:3n-6, indicative of higher Δ6 and Δ5 desaturase activity, among Americans of African compared with European ancestry are consistent with these hypotheses [95].

Early studies linking genetic variants in the FADS gene cluster to child development found a positive advantage of breastfeeding on IQ at 7–13 years of age for children who were carriers of minor allele variants in FADS2, with no effect of maternal genotype, and no advantage of breastfeeding among major allele carrier [84]. Subsequent studies, also involving cohorts born before the addition of DHA and ARA to infant formula contradicted these findings [85, 88] but left open the question of whether maternal genotype modifies the relationship between breastfeeding and child development through effects on milk fatty acids. The first indication that SNP in the FADS gene cluster are associated with human milk fatty acids by Xie and Innis involved a small group of 54 Canadian women of mainly European and Asian descent [96]. Common minor alleles associated with loss of function, also more common in Asians than Europeans, were associated with higher plasma, blood cell, and human milk 18:2n-6 and lower ARA, DHA, and n-6 and n-3 precursor/desaturation product ratios. Although desaturases and elongases are present in mammary cells [97], there is no evidence that the human mammary gland rather than the liver, via plasma delivery, explains the association between human milk n-6 and n-3 fatty acids and genetic variations in FADS or Elovl. Subsequent studies in Dutch women extended these findings, showing that women homozygous for minor alleles in the FADS1/FADS2 gene cluster had lower plasma and human milk DHA, with evidence that genotype also may modify the transfer of dietary DHA into milk lipids [98••]. Similar studies in Germany identified lower ARA and 20:3n-6/ARA in human milk of women who were minor allele carriers of SNP in FADS1/FADS2 [99••]. These associations between minor allele variants in FADS1/FADS2 and lower ARA also have been described for human colostrum [86•]. Maternal genetic variations in n-6 and n-3 fatty acid metabolism reported to date appear to explain only approximately 10 % of the

variability in human milk ARA, EPA, and DHA within a study. Undoubtedly, further developments on genetic variations, including comparative population studies, will provide much needed insight into the variability in human milk fatty acids, the contribution of genetic adaptations to particular food environments, and their implications for the mother-infant dyad.

Conclusions

Human milk has evolved from ancient origins into species-specific milk that provides nutrition, growth-modulating factors, and important immunological protection to the young infant. TAG are one of the largest components of human milk, and their fatty acids are by far one of the most variable. Maternal fatty acid nutrition has an undisputable, strong influence on human milk unsaturated fatty acids, with a smaller but potentially important contribution of maternal genetic variation. Fatty acid nutrition of lactating women has undergone remarkable changes during the past century, and this has radically increased 18:2n-6, and decreased the long chain n-6 and n-3 fatty acids ARA, EPA, and DHA in human milk. The implications for maternal and infant health offer considerable opportunities for research.

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Compliance with Ethics Guidelines

Conflict of Interest Sheila M. Innis has received compensation from Unilever, Pharmavite LLC, and Pronova BioPharma USA for serving on advisory boards; has received compensation from Enzymotec for service as a consultant; has been reimbursed by Pfizer for travel/meeting expenses.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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