Dietary Lipid Quality and Long-Term Outcome

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Abstract
Understanding the importance of dietary fat has grown beyond energy metabolism to recognition of the complex roles of fatty acids, particularly the ω-6 and ω-3 fatty acids in membrane lipids, inter- and intracellular communication and in regulating gene expression. The ω-6 and ω-3 fatty acids accumulated in developing tissues depend on the fatty acids transported across the placenta and secreted in breast milk. These in turn are dependent on maternal fatty acid intakes, which have changed dramatically in the past century with current western diets high in ω-6 linoleic acid and low in ω-3 fatty acids. High intakes of ω-6 fatty acid and low intakes of ω-3 fatty acids compromise long-chain ω-3 fatty acid accumulation in tissues, and this is avoided by dietary docosahexaenoic acid. In addition to the well-known roles in neural development, newer studies are beginning to question the importance of ω-3 fatty acids as a contributor of metabolic development in other organs, with possible implications for the development of feeding behavior and integration of the nutrient energy supply.

Introduction
Considerable evidence has accumulated to show that the nutrient supply in utero and during infancy has long-term implications for later development of cardiometabolic diseases, including obesity, type 2 diabetes and cardiovascular disease [1, 2]. Dietary lipids, specifically fatty acids have effects that extend beyond sources of metabolic and storage energy to central roles in the function of cell membranes, coordination of inter- and intracellular communication and as powerful modulators of gene expression. Central to concerns over the importance of dietary lipids in shaping early development, maternal diet is one of the most important variables contributing to the quality of fatty
acids transferred across the placenta and secreted in mother's milk [3, 4]. This review integrates knowledge of the essential and regulatory functions of fatty acids focusing on the ω-6 and ω-3 fatty acids to consider the importance of dietary fatty acid quality in early metabolic development and long-term outcome.

**Dietary Fatty Acids and Their Sources**

As introduced, maternal diet is one of the most important factors determining the types and amounts of ω-6 and ω-3 fatty acids transferred across the placenta and secreted in human milk. There is no doubt that the absence of Δ12 and Δ15 desaturase enzymes needed to insert a double bond at the ω-6 and ω-3 position, respectively, leads to essentiality of ω-6 and ω-3 fatty acids in humans and other animals. However, which and how much of the different carbon chain C18, C20 and C22 ω-6 and ω-3 fatty acids are needed in the diet remains a subject of uncertainty, further complicated by the impact of the dietary fatty acid composition itself on the metabolism of these fatty acids [5]. From a quantitative standpoint, the major dietary ω-6 and ω-3 fatty acids are the C18 linoleic acid (LA, 18:2ω-6) and α-linolenic acid (ALA, 18:3ω-3), respectively, with the richest source of these fatty acids being vegetable oils. Several of their C20 and C22 metabolites, including arachidonic acid (20:4ω-6, ARA), adrenic acid (22:4ω-6), eicosapentaenoic acid (20:5ω-3, EPA) and docosahexaenoic acid (22:6ω-3, DHA) are of particular interest with respect to neural and visual system development, regulation of gene expression, and inter- and intracellular communications. This includes the eicosanoids synthesized from C20 ω-6 and ω-3 fatty acids and the large family of acyl signal molecules, such as the acylglycines and ethanolamides [5–9]. In animals, including humans, LA and ALA provided in the diet can be converted to C20 and C22 metabolites in a complex pathway that requires Δ6 and Δ5 desaturases and several elongases (fig. 1). Conversion of LA to its metabolites ARA, 22:4ω-6 and 22:5ω-6, and conversion of ALA to EPA and DHA, however, is influenced by the amount and balance of LA and ALA in the diet. This is explained by the dependence of both LA and ALA on the same Δ6 and Δ5 desaturases for metabolism and the relatively low substrate needs of Δ6 and Δ5 desaturases for maximal activity [5]. Modern diets often provide high amounts of LA and are relatively low in ALA due to the types of fats and oils in the food supply [10]. The presence of preformed C20 and C22 ω-6 and ω-3 fatty acids in the diet is also important as the desaturation-elongation pathway is subject to feedback regulation. The C20 and 22 ω-6 and ω-3 fatty acids, mainly ARA, EPA and DHA are present in the diet in animal tissue fats, including human milk. While the amounts and types of ω-6 and ω-3 fatty acids in the diet and its impact on tissue lipid fatty acids is often considered with respect to human nutrition, it is important to
recognize that this also occurs in domesticated animals. Modern agriculture, including grain feeding, results in meats, poultry and eggs that are often high in LA and ARA, but low in ω-3 fatty acids, and this further contributes to higher intakes of ω-6 and loss ω-3 fatty acids [11]. Fish, in contrast to domesticated animals, are rich dietary sources of EPA and DHA, and this is explained by the synthesis of EPA and DHA in phytoplankton and their transfer up the aquatic food chain. Overall, from a practical perspective the types and amounts of vegetable oils and protein food choices (fish, meats, poultry and eggs) dictate the quantity and composition of ω-6 and ω-3 fatty acids in the diet.

Fig. 1. Schematic of major steps of essential fatty acid desaturation and elongation with dietary sources of ω-6 and ω-3 fatty acids.
Dramatic changes in dietary fat quality over the last century contribute to difficulties in understanding the dietary needs for ω-6 and ω-3 fatty acids, particularly of infants and the implication for long-term outcomes. Currently, LA provides 5% or more of dietary energy and 90% of polyunsaturated fatty acids in the average Western diet, while ALA provides about 0.5% of dietary energy [12]. Traditional diets consumed until the last 100–200 years, on the other hand, could have provided no more than 2% energy from LA, likely with equal amounts of ω-6 and ω-3 fatty acids and much higher proportions of total polyunsaturates from ARA, EPA and DHA [10, 11]. Two questions arise: the first is whether or not high intakes of LA flood the desaturation-elongation pathway making it difficult to synthesize long chain ω-3 fatty acids from the small amount of ALA in the modern diet; the second is whether humans have an inherently low capacity to desaturate and elongate ALA and require a dietary intake of preformed DHA, and perhaps EPA, for optimum health. Although it is well-known that vegetarians have lower blood lipid levels of DHA than nonvegetarians, explained by the differences in dietary DHA intakes, blood and human milk levels of ARA do not differ between vegetarians and nonvegetarians [13, 14]. While it is clear that large changes have occurred in the ω-6 and ω-3 fatty acids in human diets, much of the shift in dietary fat intake occurred with an emphasis in reducing elevated serum cholesterol [15] and without consideration of the potential impact of the ω-3 fatty acids or infant development.

**Dietary Lipid Quality in Gestation, Breastfeeding and Early Infancy**

A central question in understanding the physiological importance of maternal fatty acid nutrition for the fetus and breastfed infant is the extent to which selective placental fatty acid transfer or secretion in breast milk protects the infant from inadequate or inappropriate maternal fatty acid intakes. A large body of evidence shows that the quality of unsaturated fatty acids provided via placental transfer and secreted in breast milk is highly dependent on the maternal diet [3, 4], and this in turn impacts the fatty acid composition of fetal and infant tissue lipids [5]. Trans fatty acids are a clear example of the dynamic effect of maternal dietary fat quality on placental fatty acid transfer and secretion in breast milk, with levels as high as 12.8% trans fatty acids in plasma triglycerides of newborn infants and 18.7% trans fatty acids in breast milk of mothers consuming diets high in hydrogenated vegetable oils [16, 17]. Similarly, placental transfer and milk secretion of LA and DHA increase with increasing levels of LA or DHA, respectively, in the maternal diet [3, 4]. The mean levels of LA in breast milk fatty acids have doubled, from about 6–7 to 12–16% milk fatty acids, while DHA appears to have decreased by about 50% to a mean of 0.2–0.3% milk fatty acids in western
countries over the last 50–60 years [4]. However, the levels of DHA in human milk vary widely, both among and within populations, and the major reason for this is the dietary intake of preformed DHA. For example, breastfeeding women following vegetarian diets lacking DHA typically have 0.1% DHA in milk fatty acids, while women with high habitual intakes of fish often have 0.8% or higher DHA in their milk fat [4, 14]. The variability in DHA transfer across the placenta and in human milk has attracted considerable attention with respect to possible implications for infant development, particularly for the developing brain, retina and immune system [5]. However, another important question is whether concurrent exposure to high ω-6 fatty acid interferes with infant ω-3 fatty acid accretion. These two questions form the central dilemma in understanding fatty acid needs for optimal infant development and the implications of the fatty acid quality in current diets. It is well known that dietary ω-3 fatty acid deficiency leads to decreased DHA and a characteristic increase in 22:4ω-6 and 22:5ω-6 in the brain [5].

In this case, the 22:4ω-6 and 22:5ω-6 being formed from LA (fig. 1) [5]. In human infants, Farquharson et al. [18] reported levels of 17.7, 13.4 and 11.6% DHA and 3.2, 4.8, and 7.0% 22:5ω-6 in cerebral cortex phosphatidylethanolamine of infants who had been breastfed or fed formula with 16.0% LA + 1.5% ALA or 14.5% LA + 0.4% ALA. The presence of high and increased 22:5ω-6 in the brain of infants fed formula with very low ALA shows the desaturation-elongation pathway is active and raises the question of the importance of the dietary LA and ALA amounts. To address this question, we studied the effect of a formula diet with 1.2% energy from LA, which meets ω-6 fatty acid requirements for growth, or 10% energy from LA, with constant ALA, on ω-6 and ω-3 fatty acid levels in different organs of piglets [19]. The amount of 10% energy from LA was chosen because this is similar to the amounts of LA in some current infant formulae and also represents the upper end of the current recommended dietary intake range of LA in the US [12]. Comparison to a formula deficient in ALA enabled identification of the importance of ALA in restraining LA metabolism and preventing excess tissue accumulation of ARA. As shown in figure 2, after feeding from birth to 30 days of age, the ω-3 fatty acid (ALA)-deficient formula led to a marked decrease in DHA and increased 22:5ω-6 and 22:4ω-6 in cerebral cortex phosphatidylethanolamine, similar to autopsy data of Farquharson et al. [18], with a decrease in DHA and increase in 22:5ω-6 in the liver and heart. The brain, in contrast to the liver and heart, is characterized by very low amounts of LA. Unlike the brain, ω-3 fatty acid deficiency is permissive for excess LA and ARA in both heart and liver. Importantly, DHA levels in the brain were reduced when the formula LA was increased to 10% energy, even though ALA was held constant at 1.1% energy (fig. 2). The capacity for accretion of very high amounts of EPA in the phospholipids of the heart and liver when the formula diet had a 1:1 balance of LA and ALA is remarkable, as is the loss of EPA when LA was increased to 10% of dietary energy. Overall, the data
Fig. 2. Ethanolamine phosphoglyceride fatty acids in brain, liver and heart of piglets fed formula with, as percent dietary energy, 1.2% LA, 1.1% ALA (balanced LA/ALA); 1.2% LA, <0.1% ALA (ω-3 deficient); 10.7% LA, 1.1% ALA (high LA); 10.7% LA, 1.1% ALA, 0.3% DHA, 0.3% ARA (DHA + ARA supplemented). Values are means ± standard error of 5–7 piglets/diet. Bars with different superscripts are significantly different by ANOVA with Tukey’s test for post-hoc analysis, p < 0.05.
indicate that the desaturase pathways are active and likely require very low amounts of LA and ALA substrate. Because human milk has LA of 6% fatty acids or higher, it seems likely that under practical circumstances; a dietary source of DHA is important for the developing brain. Indeed, as shown in figure 2, small amounts of DHA (0.3% energy) achieve high amounts of DHA in the brain. The importance of high amounts of EPA and the ARA/EPA balance in the liver, heart and potentially other organs still needs to be understood.

**Early and Long-Term Effects of Dietary Lipid Quality**

As discussed in the preceding section, developing infant tissues including those of the brain, liver and other organs are readily altered by the types and amounts of \( \omega-6 \) and \( \omega-3 \) fatty acids in the diet. A large number of studies in pregnant and lactating women, and infants fed formula have addressed the implications of the early DHA supply for visual and neurodevelopmental outcomes in infants. Recent reviews of these studies are available with the general conclusion that beneficial effects of DHA on visual and neurodevelopmental outcomes are more robust in preterm infants, with the findings in term infants inconsistent [20, 21]. A more recent area of interest is the possibility that early fatty acid nutrition, particularly the types and balance of \( \omega-6 \) and \( \omega-3 \) fatty acids may also impact development in other organs, such as the liver, and the potential for early programming of metabolic pathways, predisposing to characteristics of the metabolic syndrome [1, 2]. Two key areas of interest with respect to metabolic programming are the development of hypothalamic circuitry involved in the regulation of feeding behavior and metabolic programming involving altered expression of key genes and proteins regulating metabolic pathways in the liver [1, 2, 22, 23]. During short time windows in development, hormones and key metabolic cues are believed to play important roles in establishing the set point for receptor pathways and control of gene expression. Although it is known that the dietary \( \omega-6 \) and \( \omega-3 \) fatty acids impact \( \omega-6 \) and \( \omega-3 \) fatty acids in the developing brain and liver as shown in figure 2, little is as yet known with regard to the potential programming of neural feeding circuitry or metabolic development in the liver.

Mathai et al. [24] have provided evidence of long-term effects of \( \omega-3 \) fatty acid deficiency in gestation and lactation on feeding behavior in rats. At 16 weeks of age, offspring of animals fed an \( \omega-3 \) fatty acid-deficient diet showed increased food intake following appetite stimulation by food restriction or administration of the glucose antagonist 2-deoxyglucose, suggesting deficits in glucose regulatory appetite networks. Recently, we used 2-D gel proteomics to compare the entire protein complement in brain of embryonic and neonatal offspring of animals fed \( \omega-3 \)-deficient or adequate diets. Among several proteins responsive to \( \omega-3 \) fatty acids, 14-3-3 protein zeta/delta was increased in the \( \omega-3 \) fatty acid-deficient brain in both embryonic and 3-day-old neonates.
Notably, this protein is known to be increased in the brain in response to insulin [25], which in turn plays an important role as an early neurotrophic hormone controlling development of neurons in the hypothalamus [1, 22]. Further studies on the possible role of the early ω-6 and ω-3 fatty acid supply in the development of hypothalamic circuitry involved in feeding behavior are warranted.

It is known that ω-6 and ω-3 fatty acids have unique and important effects on energy substrate metabolism [6]. In the adult, ω-6 and ω-3 fatty acids are known to regulate several transcription factors including peroxisome proliferator-activated receptors, sterol regulatory element-binding protein, liver X receptors and hepatocyte nuclear factor 4, which control expression of genes for lipogenic, lipolytic and glycolytic enzymes [6]. Metabolism at birth is unique as the infant transitions from a low fatty acid supply in utero, representing about 11% total energy at term, to the milk diet which provides about 50% energy from fat, but is also relatively low in protein, representing about 8% of the energy in milk [4]. The transition from prenatal to postnatal life thus demands metabolic adaptation to maintain glucose and amino acids, while promoting fatty acid oxidation [26]. Studies have shown that maternal high-fat diets and protein deficiency alter key enzymes of hepatic glucose and fatty acid metabolism in the fetal and neonatal liver [23, 27], but relatively little is known about the importance of the ω-6 and ω-3 fatty acid supply in isenergetic diets with constant protein, fat and carbohydrate. In recent studies, we used 2-D gel proteomics, together with targeted analysis of gene expression to find out if the maternal supply of ω-3 fatty acids impacts metabolic development in the offspring liver. Higher EPA and DHA in 3-day-old newborn liver was associated with altered expression of proteins and genes not only for enzymes regulating fatty acid metabolism, but also glucose and amino acids; these included higher hepatic mRNA for carnitine palmitoyl transferase (Cpt1a) and acyl CoA oxidase (Acox1) and lower pyruvate kinase (Pklr), higher protein expression for glycerol-3-phosphate dehydrogenase, fructose-1,6-bisphosphatase and serine hydroxymethyltransferase, lower argininosuccinate synthase and higher NADPH, as summarized in figure 3 [28]. The changes in gene and protein expression indicate that ω-3 fatty acids facilitate metabolic transition at birth conserving glucose for the pentose phosphate pathway leading to purine and pyrimidine synthesis and NADPH, sparing of protein from oxidation, and avoiding lipotoxicity by increasing fatty acid oxidation. While the long-term effects of the dietary ω-6 and ω-3 fatty acids on hepatic metabolism during development are not known, recent studies reported that a diet with 35% energy from fat with 18% energy from LA and 0.6% energy from ALA fed over 4 generations led to a gradual increase in fat mass due to combined adipose tissue hyperplasia and hypertrophy, with transgenerational alterations in adipokines, adipose tissue gene expression and hyperinsulinemia [29]. Given the role of ω-3 fatty acids in increasing fatty acid oxidation and of ARA-derived metabolites in adipocyte differentiation
via adipose-specific peroxisome proliferator-activated receptor-γ [30], future studies on the role of current westernized diets and the dietary fatty acid balance in early development of hepatic and adipose tissue development are worthwhile.

**Conclusions**

In summary, we have described the increase in ω-6 fatty acids in Western diets over the last century, and the potential loss of long-chain ω-3 fatty acids from the food supply. Changes in fatty acids in the food supply, including the increase in LA and decrease in ω-3 fatty acids are mirrored in the fatty acids transferred across the placenta and secreted in breast milk, thus impacting
not only the fatty acid nutrition of the mother but also that of the infant. The quality of the fatty acids provided to the fetus and infant impacts fatty acid accretion in multiple tissues, although in an organ-specific manner. While studies in this field are as yet limited, the role of $\omega$-6 and $\omega$-3 fatty acids as key regulators of numerous metabolic pathways, impacting gene expression and protein activities, suggests that further attention should be given to the possibility that the quality of dietary fatty acid in early life has both short- and long-term implications for human health.

References


Discussion

Dr. Puri: I have two questions. The first one is: what should be the ideal ratio of omega-6 to omega-3 in the diet considering that vegetable oil consumption is more pronounced in countries like India? The second question is: is there any literature to support that omega-3 and omega-6 intakes in early infancy affect the manifestation of heart disease later on in life?

Dr. Innis: Following the argument related to the metabolic and biochemical physiology of humans, we know that dietary intakes of n-6 linoleic acid were low, and likely less than 3% of energy, until about 150 years ago. We also know that the amount of linoleic acid needed to saturate the Δ6 desaturase is very low, probably 1–2% of dietary energy. The pertinent question in our opinion is whether the amount of n-6 fatty acid or the n-6:n-3 fatty acid ratio is most important. Given the low needs of the Δ6 desaturase, our opinion is that the quantity of n-6 linoleic acid is more important than the n-6:n-3 fatty acid ratio.

There is no direct evidence in humans linking n-6 and n-3 fatty acids in early infancy to later heart disease. This is also difficult to assess because of the large changes in dietary fatty acids over the last half-century, with changes also including saturated and trans fatty acids. One of the major current concerns worldwide is the increase in metabolic syndrome. The n-3 fatty acids are associated with lower triglycerides, blood glucose, inflammatory mediators and possibly improved glucose tolerance. Whether this occurs in infants and can be programmed by the early n-6 and n-3 fatty acid supply is not known.
Dr. Gottrand: You nicely showed us that low omega-3 intake in the mother has an impact on the brain and on the liver. Are you aware of any work published on the gut in such models?

Dr. Innis: Yes. Studies from our laboratory have shown that the early fatty acid supply impacts intestinal development and susceptibility to later inflammatory responses [1, 2].

Dr. van Goudoever: Since DHA has such an effect on brain development and there is a wide variation in DHA in breast milk among different populations around the world, does that affect brain development around the world? Are there any data that the DHA content of breast milk impacts infant development?

Dr. Innis: Yes. Observational studies have linked higher DHA in milk or gestation to better child development [3–6]. However, there are no data to indicate that babies breastfed by vegetarian mothers have poorer outcome.

Dr. van Goudoever: The second question is related to my own field, neonatology, and it relates to your remark on DHA and the effect on neuronal migration. Preterm infants' neurons migrate from gestational age of about 24 to about 32 weeks, that's when all the neurons migrate. Should there be a role for DHA in that phase? What is your opinion?

Dr. Innis: Based on what we know about DHA and brain development, specifically the role in neurogenesis, it is reasonable to expect that the preterm infant will be much more susceptible to the effects of n-3 fatty acid deficiency than an infant born at term. Whether or not they need more DHA is a different question and we do not have that data.

Dr. Simmer: I wanted to make some comments about LA in humans. I think you suggested that in your piglets, reducing LA improved the DHA status. That's not what we find in preterm infants, and if we just talk about intravenously fed preterm infants where many people feed 100% soy oil, you can dilute that with olive oil or other oils, and you have absolutely no effect on DHA status of the preterm infant, so I am not sure the piglet is a great model. Then the other comment is about LA in the diets in general. I was at a fatty acid conference in Maastricht earlier this year, and there was a debate on the American Heart Association recommendation that you reduce LA in the diet, and it was the most aggressive unpleasant debate that I ever heard, and people are really strongly divided. I think it might be quite premature on a population basis suggest that you reduce vegetable oils or LA in the diet. I think we need a lot more human randomized data before we can make recommendations about that.

Dr. Innis: In our piglet studies, we reduced LA to 1.2% of dietary energy, and with a similar amount of ALA, we showed that DHA was accumulated in the brain. Whether or not this would occur in humans consuming low amounts of LA, less than 3% dietary energy, is not known. Measurements of blood lipid fatty acids in premature infants supported by intravenous lipids are difficult to extrapolate to the brain; obviously, the fatty acids from the intravenous lipids exchange with the fatty acids in blood cells, and depending on when the blood sample is drawn, the plasma lipids may reflect the infusate if sufficient time for clearance is not allowed. You are correct, there is currently considerable debate regarding recommendations for linoleic acid. There is no doubt that it could be difficult, if not impossible, to consume 6–10% energy from polyunsaturated fatty acids without refined vegetable oils. We are of the opinion that if there is debate, there is no scientific consensus.

Dr. Klish: You already addressed part of my question in terms of the ratio of omega-3 and omega-6 fatty acid. Because our population has become so dependent on plant oils, it's very hard to decrease the omega-6 component of that ratio. However, in infant formula, the solution has been to increase the DHA content rather than decrease the omega-6 or linoleic acid content. What would be more appropriate, knowing that
human milk is composed primarily of saturated fat and has only a small amount of unsaturated fat?

**Dr. Innis:** First, we do agree with the addition of DHA to formula. Infant formulas contain linoleic and α-linolenic acid, and now arachidonic and docosahexaenoic acid. Human milk, however, has more than two n-6 fatty acids and more than two n-3 fatty acids. Understanding of the importance of fatty acids such as 20:3n-6, 22:4n-6 and 20:5n-3 in organs other than the brain is limited. We would not recommend reducing linoleic acid in current formulas until more is known.

**Dr. Fasano:** I would like to pick your brain about a strong debate far to be settled concerning maternal nutrition and, therefore, fetal development and mental performance. I was very intrigued by the data you mentioned about the maternal high-fat diet and metagenomics and methylation of genes. You state that dopamine definitely affects behavior; share with us your thoughts about that, and what you really think this would imply in terms of performance in young kids based on fetal nutrition.

**Dr. Innis:** In our experience of working with pregnant women, poor dietary n-3 fatty acids, specifically DHA intakes, are often associated with different dietary patterns and lower intakes of several nutrients than in women with high intakes of DHA. In addition, DHA is associated with protein in foods, since it is found only in animal tissue lipids. It seems likely that the association between low DHA or fish intake in pregnancy and child outcome is complex, and may well involve several nutrients. Animal studies have shown maternal nutrition impairs gene methylation in the offspring; these studies, however, are primarily high fat/low fat (low carbohydrate/high carbohydrate) comparisons.

**Dr. Fasano:** Actually, what I was getting at was, do you think that poor nutrition, either malnourishment, undernourishment or unbalanced nutrition, puts the kids in disadvantage in terms of social intellectual performance.

**Dr. Innis:** Yes, but this will depend on the severity, duration, the nutrient in question and timing in pregnancy when the nutrient deficiency or excess occurs.

**Dr. Kleinman:** What is the maximum ratio of linoleic to linolenic acid, before you inhibit the desaturases?

**Dr. Innis:** Based on what we know about the desaturases, these enzymes appear to be fully saturated with substrate. As I mentioned earlier, it seems more likely that the quantity of fatty acid is important; changing a ratio from say 10:1 to 4:1 would not make any difference if the enzyme is saturated.

**Dr. Kleinman:** Infant formula appears to meet that optimal ratio. Thus, if desaturases are active even in fetal life, it’s a little hard to imagine why there would be a need to supplement that system with polyunsaturated long-chain fatty acids. I also have a comment about these functional cognitive and behavioral outcomes. As I interpret the published data, differences in outcomes converge over time, so that by the age of 8 or 10 years you can’t separate the groups by type of early infant diet. Even during the periods of time when there are outcome differences, as you pointed out, these are inconsistent and are often present at different time points, in different studies. Thus, there doesn’t seem to be a lasting effect or potentially even a short-term benefit that leads to a lasting effect from early fatty acid supplementation. This conclusion seems also to be supported by the wide variation in the concentrations of long-chain polyunsaturates in breast milk. There is as much as a 20- to 40-fold difference in concentration of omega-3 long-chain fatty acids from one country or region to another. Thus, it doesn’t seem to follow, from an evolutionary perspective, that it would be necessary to supplement either the pregnant mother or the young full-term infant with LC-PUFAs.

**Dr. Innis:** It is correct that current studies relating fatty acids to infant outcome are inconsistent, and that long-term outcome data are lacking. A main point of our piglet study was the demonstration that taking a diet with high linoleic acid and adding
arachidonic acid and docosahexaenoic acid does not give the same tissue lipids, for example in the heart, as a diet with low linoleic acid. We agree that there is still much to be learned about the desaturases and pathways of phospholipid fatty acid acylation.

**Dr. Simmer:** Having written the Cochrane reviews on this, I think you are absolutely right for the term infant, but for the preterm infant a big Australian trial which was published in *JAMA* last year showed that high-dose DHA did reduce disability, but it was looking at the question of in utero supply compared with the breast milk supply for babies <30 weeks gestation, which is a different scenario.

**Dr. Mace:** When we talk about the benefit of omega-3 fatty acid, we always talk about DHA. What about α-linolenic per se? What are the known biological effects of α-linolenic acid?

**Dr. Innis:** This is a very important point. It is simplistic to think about n-3 fatty acid requirements simply from the perspective of DHA. For example, α-linolenic acid with a low linoleic acid diet clearly functions to restrain linoleic acid metabolism. Whether or not α-linolenic acid has unique functions is unclear; however, given the very different metabolism for α-linolenic acid, this is a reasonable hypothesis.

**Dr. Shreffler:** I was intrigued by the systems biology/proteomics approach you took, and would like you to defend that a little bit. How do you go about validating those targets and choose which outcomes to correlate targets to? Do you see consistent changes, for example with omega-3 deficiency that you do with maybe omega-6 excess, etc? And do changes on the transcriptional level corroborate your protein changes?

**Dr. Innis:** We chose the systems biology approach because little is known about the developing liver, and the infant milk diet with 50% energy from fat is clearly very different from the adult diet. For the proteins that showed a change in abundance, we mapped these onto metabolic pathways and then looked for corroborating evidence to indicate that pathways were altered through gene expression using real-time PCR. For example, we have looked at carnitine palmitoyl transferase and acyl-CoA oxidase (fatty acid oxidation) as well as pyruvate kinase (glycolysis).

**Dr. Shreffler:** And if I could add, how well do changes correlate for example with varying those ratios, you know where you see other similar outcomes?

**Dr. Innis:** We have not yet looked at hepatic protein or gene expression with ade- quate n-3 but varying n-6 fatty acids.

**Dr. Guandalini:** I was intrigued by the data you presented on maternal high-fat diet influencing the choices of food in the offspring via programming of opioid receptors. Now, if that is true in the humans as well, that would really be important in terms of health policy. It seems to me that we are creating a vicious cycle by having a high-fat diet during pregnancy thus predisposing these children to our choices which are unhealthy in terms of excess fat. What is your comment on this?

**Dr. Innis:** This is a fascinating hypothesis. Studies are currently underway in our lab to look at this.

**Dr. Zlotkin:** This question is more for Dr. Kleinman and Dr. Simmer. In South Asia, a third of the infants born are low birthweight, some of those are preterm and some of them are low birthweight, non-preterm. Most of the data in the Cochrane reviews are from developed countries on both preterm and full-term infants. I would agree with your conclusion in terms of the functional impact. For the infants born for example in south Asia who are low birthweight, do you think that the conclusions that have been drawn from the meta-analysis of studies in the developing world would also fit?

**Dr. Innis:** I would agree, it is not correct to extrapolate from current meta-analysis to low-birthweight infants in South Asia.

**Dr. Kleinman:** I think that when you are dealing with a population that begins with a diet that is severely restricted in calories, protein, fat and quality of fat and very
far from what we know to be optimal and you are also dealing with an infant who is born very prematurely, it makes biological sense that you need to support that in different ways than what we have been talking about. There aren't a lot of data though to help us answer this question.

Dr. Stathatos: You mentioned that high fat diet decreases the methylation process. I would like to know whether the high fat in your studies was unsaturated fat or saturated fat. And the second question is: what is the effect of saturated fat on long-term health processes?

Dr. Innis: The studies showing altered methylation in animals fed different diets are important because they show the biological plausibility and importance of methylation in developmental programming. However, studies thus far linking diet in animals to methylation are difficult to extrapolate to humans. For example, 40% energy from fat and high saturated fat is unphysiological for rodents. Very little has been done on saturated fat and early human development. As we know, human milk is 20–25% palmitate, representing about 10–12% of the infant's energy intake. We have no indication that the saturated fat in human milk has any untoward effect.

Dr. Kleinman: Could I ask a follow-up question on the opioid receptors and the increased perception of sweet taste? We know that we are born with an innate preference for sweet and salt. Can diet enhance those preferences or influence the way they change over time?

Dr. Mennella: You don't have to teach children to like sweet or salt; that, as you said, is innate; they are born preferring a much more intense sweetness, and this doesn't decline until around mid- to late adolescence. There is some work to suggest that when the sweet preference starts decreasing, it is like the closing of the epiphysis. So, there may be some metabolic factors signaling back to the taste receptors. There are individual differences, and one of the things that we have been looking at is the brain pathways related to the actions of drugs and abuse which basically just co-act with the pathways that were designed for sweet, so the opioids and the dopamine. We find that children who have a family history of alcoholism or addiction prefer a much higher level intensity of sweetness as young as 5 years of age. So, one way of looking at it is, the mouth is really an indicator of how the brain is processing these hedonic signals. Some of Liem's and Gerry Beauchamp's work are showing that what children learn is the context of what food should be sweet. That's the cultural learning, how sweet should something be. There has been no work for both salt and sweet to show how one can shift them downward. Whether lowering the sodium content of the diet will lead to a global shift downward in preference is a good question. No one knows. When we think about sweet for children, it's not just the tasting, sweets make children feel good. It's a very powerful stimulus that we have now in our environment, where we have refined sugars, we have intensities of sweetness that we probably have never encountered before. These are all important questions that really haven't been looked at.

References