Membrane Composition and Cellular Responses to Fatty Acid Intakes and Factors Explaining the Variation in Response

Carlo Agostoni\textsuperscript{a} · Patrizia Risé\textsuperscript{b} · Franca Marangoni\textsuperscript{c}

\textsuperscript{a}Pediatric Clinic 2, Fondazione IRCCS Ca Granda – Ospedale Maggiore Policlinico, Department of Clinical Sciences and Community Health, and \textsuperscript{b}DiSFeB, Department of Pharmacological and Biomolecular Sciences, University of Milan, \textsuperscript{c}Franca Marangoni: NFI, Nutrition Foundation of Italy, Milan, Italy

Abstract

The lipid membrane bilayer undergoes continuous changes, and its lipid composition is both adaptive and highly varied, with substantial molecular variety. The balance of dietary fats, namely saturated versus unsaturated, and polyunsaturated fatty acids (PUFA) of the n-6 series versus those of the n-3 series, may change membrane composition thus affecting membrane order, intracellular signaling processes, and gene expression. As a consequence, changes in the production of both lipid and peptide mediators influencing the individual adaptive responses take place. More than that of all the other fatty acids, the n-3 PUFA composition of cell membranes depends on their dietary intake. n-3 PUFA levels in cell membranes are related to both inflammatory and immune diseases, possibly by downregulating the expression of genes involved in their synthesis and maybe the pathogenesis of processes associated with the disease itself. The inter-individual variability of DNA sequences involved in the synthesis of long-chain PUFA may explain differences in responses to their dietary contribution in regulating the risk of disease. Lifestyle factors (such as smoking and alcohol consumption) may in turn negatively impact PUFA metabolism. Accordingly, different amounts of dietary PUFA may be necessary to meet the requirements for these nutrients in development and disease prevention on an individual basis.

Copyright © 2013 Nestec Ltd., Vevey/S. Karger AG, Basel
**Introduction**

The importance of dietary fats is primarily due to the fact that they provide the backbone of membranes, an essential component of all living organisms. If DNA can be described as an ‘eternal’ molecule of life, then membranes can be described as an ‘eternal’ structure of life as existing membranes are formed from preexisting membranes [1]. However, the lipid membrane bilayer, which provides a dynamic environment in which much of the important metabolic chemistry of life occurs, undergoes continuous changes, and its lipid composition is both adaptive and highly varied, with substantial molecular variety. Here, we will concentrate on the types of dietary fats, their influence on membrane composition and consequently on membrane function and cellular responses, with emphasis on the high variability of responses within the human race. While environmental factors have been recognized to be at the origin of some variability, recent studies have clarified the decisive role of genetics to the point that, whenever possible, the genetic characterization should now enter the trials on the functional roles of dietary fats.

**Fatty Acid Intakes, Membrane Composition and Cellular Responses**

Applying the classical physiological conformer-regulator paradigm to quantify the influence of dietary fats on membrane lipid composition (i.e. where the membrane variable is plotted against the same variable in the environment – in this case dietary fats), membrane lipid composition appears as an almost regulated parameter. Indeed, membranes remain relatively constant in their saturated (SFA) and monounsaturated (MUFA) fatty acid content over a wide range of dietary variation for these fatty acids. On the other hand, their composition has been found to be more responsive to n-6 and n-3 polyunsaturated fatty acid (PUFA) levels in the diet. In particular, the esterification degree of n-3 PUFA in cell membrane, and consequently the n-3/n-6 ratio, is most sensitive. These differential responses are probably due to the fact that the precursors of both n-6 and n-3 PUFA classes cannot be synthesized de novo by mammals. As a consequence, the relative abundance of PUFA in the diet has a major influence on the composition of membrane bilayers. Moreover, the association between membrane composition and the intake of n-6 and n-3 PUFA with the diet and especially the dietary n-3/n-6 ratio may be attributed to the capacity of PUFA belonging to the two metabolic series to substitute for each other [1].

n-6 and n-3 PUFA are the biologically active fatty acids. Within the n-6 series, linoleic acid (LA), the firstly recognized essential fatty acid, gives origin by de-
satisfaction and elongation processes to arachidonic acid (AA), precursor of the eicosanoid families, and therefore one of the most essential components for life. The compound of the n-3 family with a shorter chain and lower unsaturation degree α-linolenic acid (ALA) can be converted to the more biologically active n-3 long-chain PUFA (LC-PUFA) eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). Also in this case, the process mainly occurs by a series of desaturation and elongation reactions.

Within this context, by increasing the contents of EPA and DHA in membranes, it is possible to modify the pattern of production of different lipid mediators [2]. In general, the multiple actions of PUFA appear to involve multiple mechanisms that connect the cell membrane, the cytosol, and the nucleus. For some actions, PUFA – in particular the n-3, which are the most studied – appear to act via receptors or sensors, thus regulating signaling processes that influence patterns of gene expression, while other n-3 effects seem to directly involve changes in cell membrane fatty acid composition. Accordingly, these changes can affect membrane order, intracellular signaling processes, and, most important, the gene expression, leading to changes in the production of both lipid and peptide mediators. Neurocognitive performance, visual development, immune function, inflammatory reactions and surrogates of the metabolic picture connected with the cardiovascular health (i.e. arterial blood pressure, cardiac rhythm, insulin sensitivity, overweight and obesity development) represent the main functional outcomes, at least in children and neonates. They share similar biological mechanisms and have been evaluated in relation to changes in fatty acid intake to explain the association with dietary fatty acids. Among them, we will mainly focus on the immune and inflammatory processes.

**Functional Outcomes and Cellular Responses**

The timescale of the increased prevalence of atopic disease matches the period over which large changes in the type of fat consumed have occurred in western populations, represented by a decrease in the intake of SFA and an increase in the intake of PUFA of the n-6 series, mainly LA. LA is by far the main PUFA, and particularly the main n-6 PUFA, in the Western diet, and is the precursor of AA, the principal substrate for the synthesis of eicosanoids, particularly of 2-series prostaglandins and thromboxanes (via cyclooxygenase enzymes) and of 4-series leukotrienes (via 5-lipoxygenase) [3]. The 4-series leukotrienes are recognized mediators of allergic inflammation. In addition to proinflammatory effects such as induction of fever, pain and vascular per-
meability, prostaglandin E₂ exerts effects on the Th1/Th2 balance. It decreases the production of the Th1 type cytokines IFN-γ and IL-2, enhances the production of Th2 type cytokines IL-4 and IL-5, and promotes immunoglobulin E synthesis by B cells. On this basis, it has been hypothesized that increased intake of the n-6 PUFA LA is related to increased prevalence of allergic disease via enhanced AA and prostaglandin E₂ production [4]. Conversely, increased consumption of n-3 LC-PUFA results in their incorporation into immune cells, which occurs largely at the expense of AA, thus decreasing the availability of the substrate for prostaglandin E₂ biosynthesis. Indeed, the dietary intake of n-3 LC-PUFA has been shown to result in decreased production of prostaglandin E₂ and other eicosanoids, including 4-series leukotrienes, by human monocytes and neutrophils. Thus, it has been proposed that n-3 LC-PUFA protects against allergic diseases. Recent studies have demonstrated that EPA and DHA also give rise to a novel family of eicosanoid-like mediators, termed E and D resolvins, respectively, that have been shown to be anti-inflammatory and inflammation resolving in cell culture and animal models. Changes in the fatty acid composition of immune cells also affect phagocytosis, T cell signaling and antigen presentation capability. These effects appear to be mediated at the membrane level, suggesting important roles of fatty acids in membrane order, lipid structure and function, and membrane trafficking. Thus, the function of human immune cells is affected by their fatty acid composition, mainly by the cell membrane contents of AA, EPA and DHA [5]. Emerging evidence suggests that fatty acids can additionally act as second messengers, regulators of signal-transducing molecules or transcription factors. Acylation with long-chain fatty acids can occur on a variety of signaling molecules and can affect their membrane translocation and functions. Dietary fatty acids can alter functional properties of lipid mediators by changing the composition of acyl moieties of these molecules. It has been demonstrated that LC-PUFA and their metabolites bind and activate peroxisome proliferator-activated receptors (PPARs). PPARs are nuclear hormone receptors and transcription factors that regulate the expression of broad arrays of genes involved not only in lipid and glucose metabolism, but also in immune and inflammatory responses. PPARs may therefore be important cellular targets that mediate modulation of immune responses by dietary fatty acids [6]. The binding of PUFA to PPARα results in rapid changes in the expression of genes involved in lipid oxidation, with n-3 LC-PUFA being potent activators of PPARα [7]. PPARα genetic variations modulate the degree of the association between PUFA intake, specifically n-6 and long-chain n-3 fatty acid intakes, and different lipid parameters [7]. Also sterol regulatory element-binding proteins (SREBPs) are involved in the regulation of lipid
Fatty Acid Intakes and Variation in Response 115

metabolism; in the liver, SREBP-1c activates fatty acid synthase, fatty acid desaturases, elongases, etc. The tissue content of LC-PUFA such as AA and DHA is maintained in a narrow range by feedback regulation of synthesis. Delta-6 desaturase catalyzes the first and rate-limiting step of the HUFA synthesis. Moreover, the same SRE (sterol regulatory element) also mediates the suppression of the delta-6 desaturase gene by LC-PUFA. The identification of SREBP-1c as a key regulator of delta-6 desaturase suggests that the major physiological function of SREBP-1c in the liver may be the regulation of phospholipid synthesis rather than triglyceride synthesis [8]. Therefore, current data suggest that the intake of PUFA, particularly of the n-3 series, can modulate immune and inflammatory responses, although some discrepancies are still present. Besides genetic variation, other factors such as health status, disease, immune response stage and possibly age (and gender), all contribute to the responsiveness of the immune function to PUFA supplementation [9] and need to be separately considered. At present, information on genetic variation has largely overcome the preexisting knowledge on variation associated with environmental and lifestyle factors.

Variation in Response: Genetics

In the last years, the prominent role of the interindividual genetic variability in metabolism, incorporation, synthesis of biochemical intermediates and even effects on gene expression has been shown to be closely connected with the individual asset of the apolotypes, including single-nucleotide polymorphisms (SNPs), associated with PUFA metabolism. Indeed, in addition to diet, common polymorphisms in the fatty acid desaturase (FADS) gene cluster have very marked effects on human PUFA and LC-PUFA status. In addition to FADS1 and FADS2, the first to be identified, the gene product of FADS3 is associated with desaturating activity too. With the same dietary intake of LA and ALA, their respective health effects may differ due to genetic differences in metabolism. Delta-5 and delta-6 desaturases, FADS1 and FADS2, respectively, influence the serum, plasma and membrane phospholipid levels of LA, ALA and LC-PUFA during pregnancy, lactation, and may affect infant’s IQ, atopy and coronary heart disease (CHD) risk. At low intakes of EPA and DHA, polymorphisms at the 5-lipoxygenase level increase the CHD risk, whereas polymorphisms at cyclooxygenase-2 increase the risk for prostate cancer. High dietary levels of LA have been associated with increased risk for breast cancer. In intervention studies on the biological effects of LA, ALA and LC-PUFA, and the effects of genetic variants in FADS1 and FADS2, 5-Lipoxygenase and cyclooxygenase-2 should
be taken into consideration when determining both nutritional requirements and chronic disease risk [10].

In a preliminary study devoted to explore whether FADS1 and FADS2 gene cluster polymorphisms influence PUFA contents in serum phospholipids of human adults, the size effect of FADS SNPs was very high since genetic variants explained as much as 28.5% of the variation in serum AA contents in this cohort of free-living individuals with considerable variation in lifestyle and dietary habits. The reconstructed haplotypes predicted some 12 and 10% of the variation in the AA precursors. No association was found between genetic variants and DHA variance, supporting the concept that little DHA is synthesized endogenously and DHA serum concentrations are primarily determined by the dietary supply of preformed DHA from fish and other sources [11]. The association between FADS polymorphisms and manifestations of respiratory and allergic disorders was also explored. There were no associations with total or specific immunoglobulin E levels, but less than half the risk for allergic rhinitis and atopic eczema has been found in subjects carrying minor alleles of several SNPs, even though statistical significance was lost after correcting for multiple testing. Evidence suggests that also the activity of stearoyl-CoA desaturase 1 (SCD1), which is rate limiting for the conversion of SFA to MUFA, may be positively associated with inflammation. Positive associations were identified between CRP levels and 16:0, 16:1 and the SCD1 index (18:1/18:0) in European and Asian females, while 18:0 was inversely associated with CRP in the same groups. Ten SNPs in SCD1 were genotyped in all subjects. One SNP (rs2060792) was associated with 16:0 and 18:0 levels in females of European descent. This same SNP was also associated with CRP levels in both groups of females. Overall, SCD1 activity and genetic variation have an important role in modulating the relationship between fatty acids and inflammation in young adults [12]. Based on this genetic variation, individuals may require different amounts of dietary PUFA or LC-PUFA to achieve comparable biological effects.

New data have become available to show that FADS SNPs also modulate DHA status in pregnancy as well as LC-PUFA levels in children and in human milk. There are indications that FADS SNPs modulate the risk for allergic disorders and eczema [13], as well as the effect of breastfeeding on asthma symptoms [14] and later cognitive development [15]. Based on these observations in human-based research, two take-home messages may be derived: (1) the genetic variability may have a trans-generational effect via breastfeeding, and (2) the genetic variation in human desaturase genes affects enzyme activity and, consequently, disease risk factors. Accordingly, interindividual variation in desaturase function may be attributed mainly to genetic components, besides lifestyle determinants. As such, population-based research regarding the
role of desaturases in disease risk is challenged by this complex gene-lifestyle paradigm. Unraveling the contribution of each component is paramount for understanding the interindividual variation that exists in plasma lipid profiles, and will provide crucial information to develop personalized strategies and in particular dietary intakes to improve health interventions [16].

Despite the fact that multiple studies have shown statistically significant interactions between n-3 PUFA and certain genetic variants in intermediate and disease phenotypes, the individual level of evidence is still insufficient on increasing or even reducing the intake of n-3 or n-6 PUFA based on each individual’s genotype. Indeed, phenotypic indicators are still lacking. More studies are then required to explore the effects of FADS gene variants in populations with different ethnic backgrounds, lifestyles and dietary habits, and to investigate in greater depth the interaction of gene variants, diet and clinical end points, including immune response and developmental outcomes. Analyses of FADS gene variants should be included into all sizeable cohort and intervention studies addressing biological effects of PUFA and LC-PUFA in order to consider these important confounders, and to enhance study sensitivity and precision [17]. Also elongases 2, 4 and 5, encoded by genes ELOVL2, ELOVL4 and ELOVL5, have a key role in the biosynthesis of LC-PUFA. To date, few studies have investigated the associations between elongase polymorphisms and cardiovascular health, and the investigation of possible associations between ELOVL polymorphisms and adipose tissue fatty acids, serum lipids, inflammation has allowed to assess the lack of correlations with common genetic variants in elongases [18].

**Variation in Response: Lifestyle**

Smoking and alcohol consumption may influence the absorption, biosynthesis, or metabolism of serum fatty acids. The reduction in total fats and DHA levels in milk of smoking versus nonsmoking mothers [19] has been associated with lower plasma DHA in infants born to smoking mothers [20]. In vitro studies have confirmed the negative effects of cigarette smoke on PUFA metabolism and AA and DHA production through a decreased conversion of LA and ALA, and presumably depressed desaturase activities, in mammary cell lines [21]. The negative effects of smoking observed in the maternal-fetal dyad are summarized in table 1. In general, levels of the major LC-PUFA (AA, EPA and DHA) are lower in plasma of smokers than nonsmokers, and serum levels of phospholipid DHA and cholesterol ester and phospholipid AA were in-
versely associated with smoking [22]. Pawlosky et al. [23] have also demonstrated a decrease in delta-5 desaturase activity in smokers. In a recent case-control study, an inverse correlation has been found between smoking habits and omega-3 index in patients with myocardial infarction [24]. Concerning alcohol consumption, it has been observed that, as the number of alcoholic drinks per week increase, there is a parallel increase in palmitic acid (16:0) and oleic acid (18:1) in cholesterol ester and phospholipids, myristic acid (14:0) in cholesterol esters, and palmitoleic acid (16:1), adrenic acid (22:4), and n-9 eicosatrienoic acid (20:3) in phospholipids. In contrast, levels of cholesterol ester and phospholipid LA, phospholipid stearic acid (18:0) and the serum PUFA:SFA ratio decrease [22]. In a population-based study focused on alcohol consumption and dietary habits, male heavy drinkers had less favorable nutritional intake than moderate drinkers and abstainers. In fact, male heavy drinkers assumed lower fiber, retinol, calcium and iron, and moderate/heavy drinkers had higher vitamin D intake than nondrinkers. In men, moderate drinkers had lower fruit intake and heavy drinkers had lower milk intake than nondrinkers. Moderate drinkers had higher energy intake from total fats and MUFA than nondrinkers, and, almost surprisingly, fish intake was higher among female moderate drinkers and male moderate/heavy drinkers than nondrinkers [25]. Matching these data with observations on fatty acid composition, it may be derived that the fatty acid metabolism may be deranged in alcohol consumers, as observed in smokers. The effects of ethanol consumption on the supply of fatty acids from the maternal compartment to the fetus has also been investigated [26]. The perfusion of ethanol through the placenta resulted in the reduction in the absolute rate of transfer of ALA and DHA and in the availability of PUFA to the developing fetus.

**Table 1. Effects of smoking on PUFA metabolism observed in the maternal-fetal dyad**

| In mothers: higher plasma lipid levels and lower milk total fat and DHA content in the first months of lactation [19] |
| In mammary gland cells: exposure to cigarette smoke negatively affects the synthesis of n-3 LC-PUFA from the precursor [21] |
| In infants: reduction in LC-PUFA pools, particularly of the n-3 series, in infants born to smoking mothers in spite of lack of differences in maternal dietary intakes vs. nonsmokers [20] in association with reduced fetal growth [27] |
| Speculative associations: in breastfed infants, a lower total fat content of human milk is negatively associated with developmental indices at 12 months [28]; in adults: negative relationship between maternal smoking during the third trimester and offspring adult intelligence [29] |
Conclusions

Fatty acid composition of the cell membrane reflects both environmental and pathophysiological factors. More than any other fatty acids, the n-3 PUFA depend on the dietary intake. n-3 PUFA levels in cell membranes are related to both inflammatory and immune diseases, possibly by downregulating the expressions of genes involved in processes associated with the disease itself. The differences in PUFA metabolism associated with variants in human genes and environmental and lifestyle factors suggest that different amounts of dietary PUFA may be necessary in order to meet the requirements for these nutrients in development and disease prevention on an individual basis, but individual phenotypic indicators are still lacking.

Disclosure Statement

The authors have nothing to declare.

References


