



Epigenetic Effects of N-3 Polyunsaturated Fatty Acids

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Abstract

N-3 polyunsaturated fatty acids (PUFAs) are essential components of the cell membrane. They display a wide range of effects, acting on brain functions and cardiovascular physiology. N-3 long-chain PUFAs have to be ingested from the diet and are nowadays quasi restricted to fish or seafood and are thus consumed in unsatisfactory low proportions compared to n-6 PUFAs. Experimental animal models have shown that n-3 PUFAs could act through epigenetic effects. For instance, maternal n-3 uptakes correlate negatively with offspring body weight in rodents. Such an association has been debated in humans. Yet, there is a negative correlation with n-3 maternal intakes and child's sensitivity to allergies or asthma. In experimental animals or in vitro, n-3 supplementation or incubation can

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V. B. Patel, V. R. Preedy (eds.), *Handbook of Nutrition, Diet, and Epigenetics*,
https://doi.org/10.1007/978-3-319-55530-0_45

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modify DNA methylation state, either globally or locally on some gene promoters. The promoter methylation of fatty acid desaturase-2 (FADS2), a desaturase involved in PUFA synthesis, is increased by DHA. The transcriptome of micro-RNAs (miR) is also modified by n-3 PUFAs. The compounds derived from n-3 PUFAs, such as ResolvinD1, are also able to alter miR synthesis to resolve inflammation. Therefore, these still fragmentary observations open the road to deepen the knowledge of the varied molecular mechanisms of the n-3 PUFAs.

Keywords

N-3 polyunsaturated fatty acids · Linoleic acid · Alpha-linolenic acid · Arachidonic acid · EPA · DHA · Resolvin D1 · FADS2 · Brain · Neurodevelopment · Offspring · Maternal · miR · DNA methylation

List of Abbreviations

AA	Arachidonic acid
ALA	Alpha-linolenic acid
BDNF	Brain-derived neurotrophic factor
BMI	Body mass index
DHA	Docosahexaenoic acid
ELOVL5	Fatty acid elongase 5
EPA	Eicosapentaenoic acid
FADS1	Fatty acid desaturase-1
FADS2	Fatty acid desaturase-2
LA	Linoleic acid
n-3 PUFAs	N-3 polyunsaturated fatty acids
RvD1	Resolvin D1

Introduction

The essential n-3 polyunsaturated fatty acids (n-3 PUFAs) are cell membrane constituents and are derived from diet. They have been the focus of numerous studies for their diverse beneficial roles in brain and cardiovascular physiology, or in the prevention of colon cancer, for instance. This diversity of actions is paralleled by a myriad of effects on several cell compartments: as membrane components they can modify the presence of proteins in the lipid rafts or the membrane properties such as membrane fluidity (Langelier et al. 2010). They can bind to membrane and nuclear receptors, and regulate gene expression. They are also metabolized in active compounds such as autacoids and others, as we will describe below (Piomelli et al. 2007). Besides this already large complexity, another aspect of their abilities is now emerging with the possibility that they could also play a role in epigenetics.

Before gathering the experimental evidences, we will briefly address the origin and metabolism of n-3 PUFAs and explain the importance of these dietary constituents.

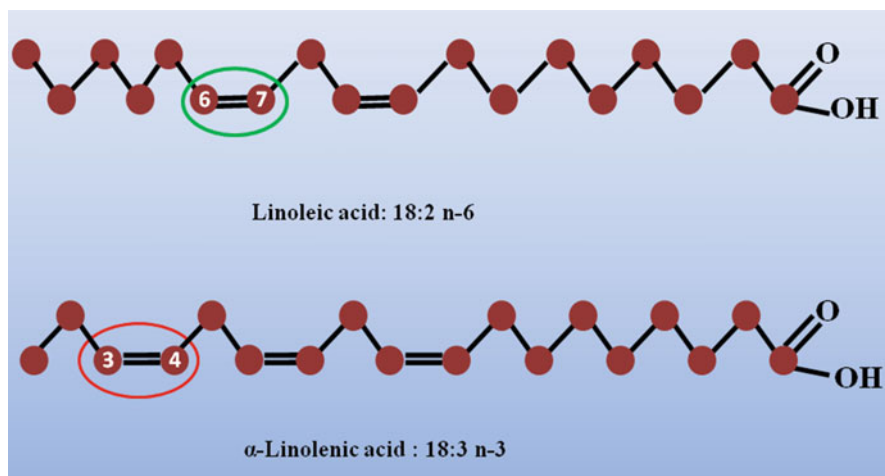


Fig. 1 Chemical structures of the precursors of the n-6 (or ω 6) and the n-3 (or ω 3) families. The double bond after which the family is named has been circled

Polyunsaturated Families

The most abundant PUFAs in the cell membrane phospholipids belong to two families, named n-6 and n-3. This denomination comes from the place of the first double bond in the aliphatic chain, i.e., carbon 6 and carbon 3 for the n-6 and n-3 family, respectively (Fig. 1).

Although these families are distinct molecularly and functionally, they share common characteristics. The precursors of both families cannot be synthesized by mammals and have to be ingested from the diet; thus they are essential. Both precursors are metabolized by the same set of enzymes and as a consequence compete with each other. Linoleic acid (LA, C18:2 n-6) is the precursor of the n-6 PUFAs and α -linolenic acid (ALA, C18:3 n-3) the precursor of the n-3 (Fig. 2).

They are metabolized through a succession of elongases and desaturases (Fig. 2) in the liver with two key enzymes, the fatty acid desaturase-2 (FADS2) and fatty acid desaturase-1 (FADS1), also named Δ 6 and Δ 5 desaturase, respectively. These enzymes are considered to determine the rate of conversion (Baker et al. 2016).

LA and ALA are converted into long-chain PUFAs, which are the active compounds of the families: Arachidonic acid (AA, C20:4 n-6), eicosapentaenoic acid (EPA, C20:5 n-3), and docosahexaenoic acid (DHA, C22:6 n-3) are the most active among long-chain PUFAs. The rate of conversion into long-chain PUFAs is low, and consequently, n-3 long-chain PUFAs have to be ingested from the diet to be present and stored in significant amounts in the cell phospholipids. A balanced nutrition demands a certain proportion of n-3 in the diet, and nutritional studies show that western diet is unfortunately characterized by a lack of n-3 PUFAs. The origin of n-3 PUFAs in modern diet comes almost exclusively from seafood and fish, and

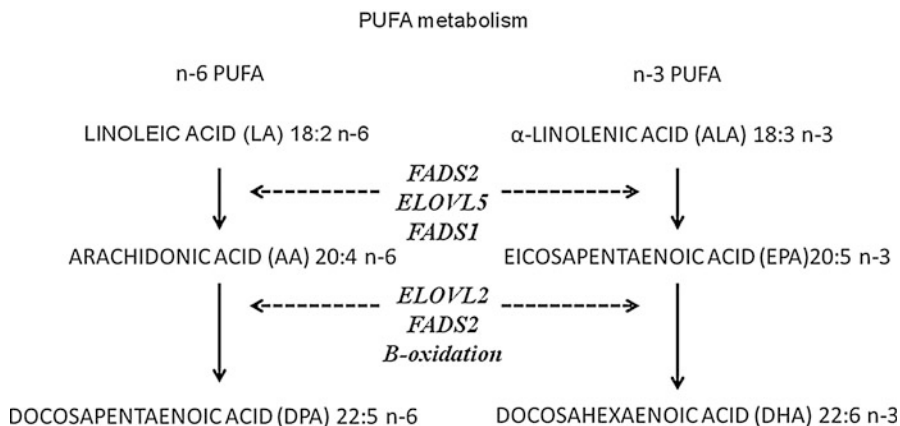


Fig. 2 Metabolism of the polyunsaturated fatty acids. The two families share the same set of enzymes, which explains the competition between them

therefore, the resulting ratio n-6/n-3 is close to 15, while 5 would be an ideal one (Baker et al. 2016).

Brain and retina are the organs in which the highest proportions of EPA and DHA can be found. A low abundance of these PUFAs in the cell membrane promotes several disorders in brain functions, and conversely, n-3 PUFAs abundance is favorable to brain development and physiology. Experimental animal models have amply demonstrated that impaired structure establishment and delayed cell migration in the brain are associated with a deficiency in DHA (Coti Bertrand et al. 2006). In humans, a recent study has shown that the maternal n-6/n-3 ratio had direct consequences on the child neurodevelopment (Bernard et al. 2013) and that higher DHA levels associated with low LA were favorable for preterm babies, particularly for the retina and visual acuity (Tam et al. 2016, Liu et al. 2010).

Heritable Effects of Maternal Exposure to N-3

The idea that n-3 could display epigenetic effects came from animal experiments mainly.

The fetus relies on maternal PUFA supply since its bioconversion of long-chain PUFAs is very limited (Haggarty 2010). The access through the placenta of different levels of n-3 PUFAs can directly affect molecule and protein synthesis, and processes involved in neurodevelopment, which could explain the delay in the fetal neurodevelopment of deficient pups already mentioned. Yet there exist other long-term consequences in the offspring which persist during adulthood and cannot be explained by the direct perinatal effects of these compounds.

Referring to experimental animal models, multiple examples can be cited. Hirabara et al. (2013) showed that feeding rats with fish oil for 2 generations resulted in a lower basal glycemia and an increase in insulin sensitivity in the G2 generation.

The maternal n-3 PUFA intake is correlated negatively with the offspring body weight in rodents and a higher n-3 maternal intake correlated positively with an alleviation of the metabolic syndrome (Kasbi-Chadli et al. 2014) although this association has not been confirmed in humans (Vidakovic et al. 2016). In piglets, maternal DHA favored offspring social behavior after weaning and improved sickness behavior after an inflammatory challenge (Clouard et al. 2015).

Similarly, we showed that in vitro cultures of neural stem cells (NSC) from newborn rats were deeply and durably modified by a maternal diet rich in DHA and EPA, functionally and molecularly (Goustard-Langelier et al. 2013): pups NSC from n-3-supplemented mothers proliferate more rapidly than cells from deficient animals (Fig. 3), but their differentiation was not enhanced by addition of DHA or AA in the culture medium, while the differentiation of deficient cells was increased, as assessed by the measurement of neurite lengths (Figs 4 and 5).

These observations point to the existence of persistent effects of n-3 PUFAs and, therefore, suggest an epigenetic mechanism of action. In humans, the evidence is less unequivocal, since observational or interventional studies have reached inconsistent results. While there seems to finally be no connection with maternal n-3 PUFA ingestion levels and child body mass index (BMI), fat deposition, or obesity in humans (Vidakovic et al. 2016), there seems to be a beneficial effect of maternal n-3 PUFAs on the outcome of allergic diseases and asthma in childhood (Best et al. 2016). A study also showed that ingestion of n-3 PUFAs during pregnancy correlated with lower systolic blood pressure, and a reduction of aortic stiffness in children, unrelated to the children's own n-3 PUFAs consumption (Bryant et al. 2015).

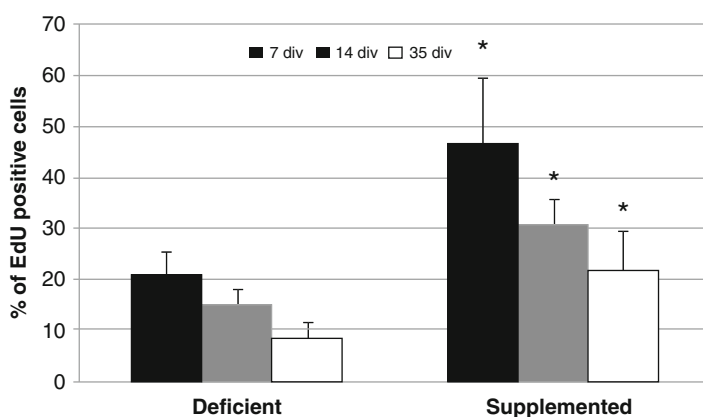


Fig. 3 Proliferation of neural stem cells derived either from n-3-deficient or n-3-supplemented rat pups. The dividing cells were labeled with 10 μ M EdU for 6 h, allowed to adhere on glass coverslips coated with polyornithine and laminin, and fixed with paraformaldehyde. EdU labeling was realized per manufacturer's instructions, and the cells were counterstained with DAPI. Cells positive for EdU were counted, and the numbers were expressed as percentage of total cell counts. The cells were counted using Image J; at least 500 cells were counted for each coverslip. DIV, days in vitro (Reprinted with permission from Elsevier from Goustard-Langelier et al. *J. Nutr. Biochem.* 24 (2013) 380–387)

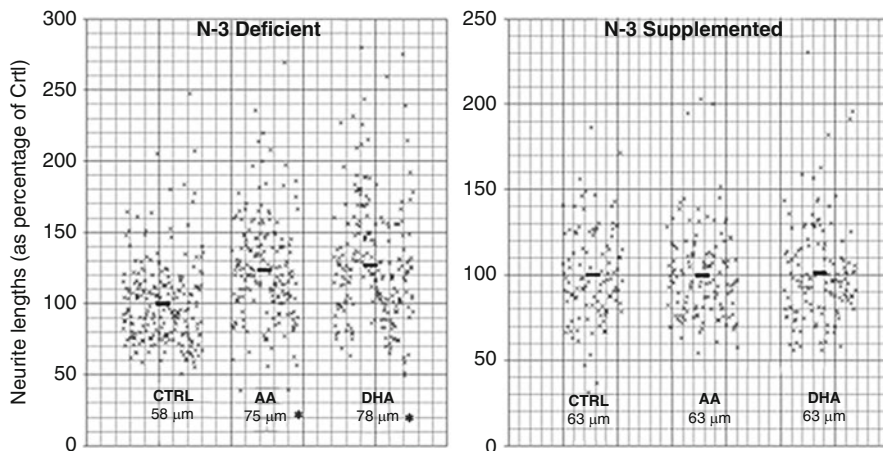


Fig. 4 Expansions of neurites in differentiated neurons from deficient or supplemented rat pups, supplemented or not with 5 μM AA or DHA. The cells from deficient or supplemented pups were allowed to differentiate on P/L glass coverslips for 8 days in the absence of growth factors. The cells were fixed with 4% paraformaldehyde and immunolabeled with β-III tubulin antibody, and the nuclei were counterstained with DAPI. The neurite lengths were measured using Image J. The figure compiles the results from cells that were examined from 20 to 35 days in vitro. The numbers at the bottom of the figure express the average length in μm recorded for cells in each treatment condition. The asterisk denotes a significant difference at $P < 0.05$ (Reprinted with permission from Elsevier from Goustard-Langelier et al. *J. Nutr. Biochem.* 24 (2013) 380–387)

The discrepancy of outcomes from experimental animal models and human observational studies may come from the fact that human diet is more complex than experimental diets for animal models and presents a variability in terms of quality, quantities, and ingestion pattern that can blur the effects of PUFAs. Also, as we will develop below, genetic variants in humans can influence the end result of PUFAs' actions.

In spite of the inconsistent and indefinite results obtained in humans, that have to be confirmed, a few studies have turned to the analysis of epigenetic effects induced by n-3 PUFAs.

Since this topic is just emerging, we will address the two domains that have been mainly developed so far, i.e., DNA methylation and micro-RNA synthesis.

N-3 PUFAs and DNA Methylation

There are several studies showing that n-3 PUFAs can modify global DNA methylation, either in vivo or in vitro, or the methylation state of the promoter of particular genes.

An association study in 2014 showed that in Alaska native Yup'ik people, 27 differentially methylated CpG sites correlated with n-3 PUFA intakes (Aslibekyan et al. 2014). The study was realized on 185 individuals, from both

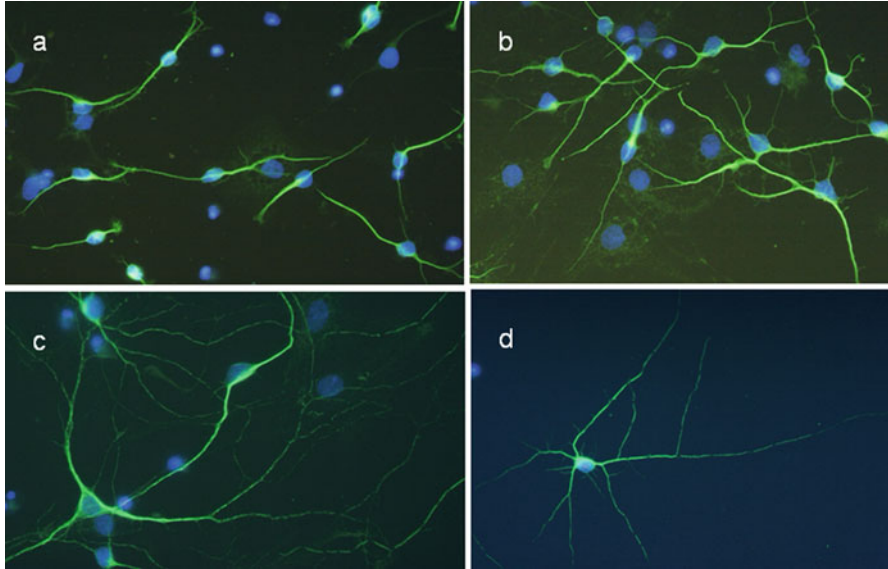


Fig. 5 Cells from deficient or supplemented rat pups differentiated in the presence of 5 μM DHA. (a) Neurons from n-3-deficient NSC cultures differentiated in the absence of 5 μM DHA. (b) Neurons from n-3-deficient NSC cultures differentiated in the presence of 5 μM DHA. (c) Neurons from n-3-supplemented NSC cultures differentiated in the absence of 5 μM DHA. (d) Neurons from n-3-supplemented NSC cultures differentiated in the presence of 5 μM DHA (*Unpublished (C. Heberden)*)

sex, and a total of approximately 470,000 CpG sites were examined. N-3 PUFAs modified the DNA methylation state in cord blood monocytes when the mothers had been supplemented with 400 mg/day DHA during the pregnancy from 8 to 12 weeks of gestation until parturition. The methylation levels of the promoters of T-relevant genes were modified by n-3 PUFA supplementation, and the Th1/Th2 balance in infants was modified as well (Lee et al. 2013).

In the same vein, in the rat fed a diet rich in fat and supplemented or not with n-3 PUFAs, n-3 PUFAs could modify the global methylation state and prevent the decrease of methylation induced by the high fat diet in Pparg2 promoter (Amaral et al. 2014).

Hirabara et al. (2013) showed that in mice supplemented with fish oil for 2 generations, the global DNA methylation was decreased.

The modifications of the methylation are more often studied on specific genes. For instance, in patients with renal diseases from either gender, 4 g per day of n-3 PUFAs for 8 weeks modified FADS2 and ELOVL5 methylation promoters in peripheral blood monocytes. The modifications were significant but different according to the sex. These modifications correlated with the modulation of the synthesis of PUFAs (Hoile et al. 2014).

Experimental animal models have also shown that supplementation with n-3 PUFAs can modify the methylation level of specific genes: when dams were

supplemented with ALA during gestation and lactation, the maternal liver FADS2 gene promoter and intron I were hypermethylated, and a negative association was noted between hypermethylation and FADS2 expression. Similarly, the FADS2 promoter was hypermethylated in the offspring livers, to an extent comparable to the mothers' (Niculescu et al. 2013).

He et al. (2014) showed that in the rat daily supplementation of ALA during gestation and lactation modified the expressions of several genes in brain offspring and decreased the methylation of the promoter of methyl-CpG binding protein 2. In Sprague Dawley rats, n-3 PUFA supplementation reduced the methylation of the *BDNF* exon IV promoter induced by western diet. This methylation was associated with anxiety like in behavior in open arms equipment and correlated with gene expression and protein content (Tyagi et al. 2015).

In vitro as well, the impact of n-3 PUFAs on DNA methylation has been described: Ceccarelli et al. (2011) demonstrated that an incubation of leukemia U937 cells with EPA induces a demethylation of a single CpG site, and this demethylation correlates with a decrease in cell proliferation. More recently, it has been shown that EPA could, in the same cells, induce the demethylation of intron 1 of *HRas* (Ceccarelli et al. 2014).

If experimental models and in vitro studies confirm the existence of the effect of n-3 PUFAs on DNA methylation, the conclusions are less clear-cut in human studies. A recent study has pointed out the intricacy of the question by investigating the effects of n-3 PUFAs on the methylation of interleukin-6 promoter (IL6) (Ma et al. 2016). They showed that the effect of n-3 PUFAs was dependent on genetic variants: in heterozygotes for an allele of IL6, higher n-3 PUFAs correlated with lower methylation at the IL6 promoter, but did not in the homozygotes. Therefore, the effects of n-3 PUFAs could be modified by single-nucleotide polymorphisms.

The complexity of the human situation, either because of its genetics or its nutrition, results in somewhat contradictory results, but should not mask or exclude the possibility that n-3 PUFAs can act on DNA methylation state.

N-3 PUFAs and miR

The miR can regulate the transcriptome and consequently cell physiology. In view of the wide range of effects of fatty acids, the hypothesis that these components could affect miR expression has been tested on several models covering different fields. The next paragraph will sum up a few of the recent studies, and Table 1 recapitulates the identities of the miR involved and the putative functions that have been ascribed to them.

In vivo, Shah et al. (2011) demonstrated that a diet supplemented with fish oil had chemoprotective properties in the rat colon and that the diet had a specific miR signature. In obese Sprague Dawley rats, a diet enriched in marine alga oil, rich in DHA, was shown to reduce the amounts of miR-33 and miR-122, both involved in the enhancement of lipid metabolism genes related with obesity (Baselga-Escudero et al. 2013). Recently, it was shown that brown thermogenesis, beneficial against

Table 1 N-3 PUFAs and miR expressions in the literature

Ref	Organism	Treatment	miR	Function
Shah et al. 2011	Rat colon	Diet, fish oil	↑18a, 27b, 93, 200c, 497 ↓21	Chemoprevention
Baselga-Escudero et al. 2013	Obese rat liver	Marine alga oil	↓33, 122	Lipid metabolism
Goustard-Langelier et al. 2013	Newborn rat NSC cultures (ex vivo)	Diet during pregnancy, fish oil	↑210	Cell proliferation, BDNF?
Kim et al. 2016	Mice	Diet, fish oil	↑30c	thermogenesis
Farago et al. 2011	Glioma	DHA	↑20b, 17, 26a, 29c	apoptosis
Mandal et al. 2012	Breast cancer	DHA	↓21	Induction of PTEN and decrease of CSF1
Gil-Zamorano et al. 2014	Caco2	DHA	↑192, 30c	Lipid metabolism
Antal et al. 2014	Glioma	DHA + ionization	↑146, 181	Apoptosis
Daimiel-Ruiz et al. 2015	Caco2	DHA	↑107	Circadian rhythms
Yao et al. 2015	Human cholangiocarcinoma cells	DHA	↓26 a 26 b	↑15 PGDH

obesity and promoted by EPA, was induced through increased binding of EPA to the free fatty acid receptor 4 (FFAR4) leading to miR-30b and miR378 upregulation. The *in vitro* association was confirmed *in vivo* in fish-oil-fed mice (Kim et al. 2016).

In neural stem cell cultures derived from rat newborns born to deficient or supplemented n-3 PUFA mothers, we observed a significant increase in miR-210 expression. This miR is involved in cell proliferation, a parameter that was indeed increased in the supplemented-derived cultures (Goustard-Langelier et al. 2013).

In vitro also, Farago et al. showed that the cytotoxic action of AA and DHA on glioma cells was the result of specific changes of miR, the putative targets of which were apoptosis related. DHA has been often studied for its anticarcinogenic properties: in breast cancer cells, DHA treatment downregulates miR-21, which in turn upregulates the phosphatase and tensin homolog (PTEN) and which results in the decrease of colony-stimulating factor 1 (Mandal et al. 2012). In Caco2 cells, DHA upregulates miR associated with lipid metabolism and circadian rhythms (Gil-Zamorano et al. 2014; Daimiel-Ruiz et al. 2015). In human cholangiocarcinoma

cells, DHA downregulates miR-26a and miR-26b, which consequently promotes the expression of 15-hydroxyprostaglandin dehydrogenase (*15 PGDH*), the enzyme which catalyzes the synthesis of prostaglandin E2 (Yao et al. 2015).

The PUFAs can act either on their own or in association with another treatment: for instance, PUFA associated with ionizing radiations induced the overexpression of miR-146 in human glioma cells in vitro (Antal et al. 2014). Also, PUFAs associated with fermentable fibers can synergize to play a protective role in colon cancer and modify the expression of several miR (Shah et al. 2011). Table 1 recapitulates the different miR modifications.

Unlike the work by Kim et al. cited above and related to brown thermogenesis, it has been not always determined whether the PUFAs act themselves by binding to their specific receptors or through their multiple derived compounds. Yet in the field of inflammation, it has been now demonstrated that n-3 PUFA derivatives are acting as mediators and are able to modify the panel of miR. Acute inflammation is indeed terminated by effective resolution which then allows tissue repair. If the resolution process is inefficient, a chronic inflammation takes place and leads to cellular damages. This process is coordinated by anti-inflammatory and pro-resolving mediators. Resolvins, protectins, or lipoxins are specialized mediators which are derived from n-3 PUFAs and are actively involved in the disruption of persistent inflammation. The history of the identification of resolvins has been retraced by Serhan et al. (2004), and for deeper knowledge, the reader is invited to refer to this excellent review. Resolvin D1 (7*S*,8,17*R*-Trihydroxy-docosa-4*Z*,9*E*,11*E*,13*Z*,15*E*,19*Z*-hexaenoic acid, RvD1) was identified in resolution exudates of mice treated with aspirin and DHA. Recchiuti et al. (2011) showed that an injection of 300 ng/Kg in mice could upregulate a set of 6 specific miR, the targets of which were involved in pro-inflammatory pathways. The results were reproduced in vitro on human macrophage cultures. RvD1 binds to two G-protein-coupled receptors, and the same laboratory (Krishnamoorthy et al. 2012) showed that through the binding to one of the GPCR, RvD1 was able to upregulate miR-208a, an miR upregulating the anti-inflammatory cytokine IL10 in human macrophages. In the rat, an intravitreal injection of RvD1 induced a downregulation of specific micro-RNAs related to the synthesis of Sirtuin1 (Rossi et al. 2015).

In the obesity field, a recent article has shown that in mice developing non-alcoholic steatohepatitis (NASH), RvD1 coupled to calorie restriction could improve multiple parameters deteriorated in NASH and induce a specific miR signature.

Thus through direct action or their derivatives, n-3 PUFAs can modify miR transcriptome and, therefore, modulate numerous target mRNAs and proteins.

Conclusion

In many ways, the epigenetic field is still in its infancy, and more work is needed to define its overall influence in physiology. Although the classical actions already described and recognized cannot be disregarded, epigenetic seems to be part of their abilities, and the effects of PUFAs are multiple. It is obvious that PUFAs represent more than mere membrane constituents. The molecular mechanisms of the PUFAs

need to be better described and understood, since they are brought by the diet but also are consumed as supplements which are available over the counter. Therefore, the pioneering studies cited in this chapter deserve to be thoroughly dealt with and open the road to many more studies related to these essential food constituents.

Dictionary of Terms

- **Autacoids** – Substances released locally that can act as either messengers or hormones.
- **Body mass index** – Value obtained by dividing the weight of an individual by the square of his height. For an adult, a BMI lower than 25 kg/m² is normal, while higher than is considered overweight, and higher than 30 is considered obese.
- **CpG sites** – In the DNA sequence, a cytosine (C) immediately followed by a guanine (G). These sites are susceptible to methylation.
- **Desaturase** – Enzyme that creates a double bond by removing two carbon atoms from the aliphatic chain of a fatty acid.
- **Elongase** – Enzyme that adds two carbon atoms at the end of the aliphatic chain of a fatty acid.
- **Lipid rafts** – Microdomains of the plasma membrane, characterized by their lipid compositions. Compared to the rest of the plasma membrane, the lipid rafts are abundant in cholesterol and sphingolipids. Due to the lipid composition, these domains tend to be less fluid than the rest of the membrane. They serve as signaling platforms.
- **Polyunsaturated fatty acids** – Fatty acids with more than one double bond in their chemical structure. Fatty acids are divided in two categories: saturated, without double bond (e.g., palmitic acid, C16:0) or unsaturated. The unsaturated fatty acids can be monounsaturated (one double bond, e.g., oleic acid C18:1 n-9) or polyunsaturated (more than one double bond, e.g., linoleic acid, C18:2 n-6).

Key Facts

Key facts of linoleic and α -linolenic fatty acids

- Abundant in vegetables, not synthesized by mammals.
- Metabolized by the same set of enzymes.
- Esterified in membrane phospholipids.
- Linoleic acid is abundant in peanuts and sunflower.
- α -linolenic abundant in rapeseed and soybean.

Key facts of arachidonic acid

- Most abundant derivative of linoleic acid metabolism
- Ubiquitous

- Released by phospholipase A2
- Metabolized in eicosanoids: prostaglandins, leukotrienes
- Mainly involved in pro-inflammatory processes

Key facts of docosahexaenoic acid

- Most abundant derivative of α -linolenic acid metabolism
- Not ubiquitous, abundant in CNS
- Metabolized in resolvin, protectin, etc.
- Involved in anti-inflammatory processes
- Mainly present in seafood and pumpkin seeds

Key facts of neural stem cells

- Neural stem cells are present in defined areas of the brain: the subventricular zone, the hippocampus, and the hypothalamus.
- They are dividing during the whole life although at a slower pace with age.
- They are localized in proximity of ventricles or capillaries, which make them sensitive to the signals of the environment.
- They have different functions, related to the niche to which they belong: In the subventricular zone, they give rise to olfactory neurons; in the hippocampus, the new neurons impact memory, learning abilities, and mood; and in the hypothalamus, food intake and energy homeostasis.
- Nutrition can modulate adult neurogenesis by modifying neural stem cell properties.

Summary Points

1. Long-chain PUFAs belong to two different families, the n-6 and n-3 families.
2. N-3 long-chain PUFAs are inefficiently metabolized from ALA.
3. In the western diet, n-6 PUFA ingestion overrides n-3 PUFAs.
4. The main dietary source of long-chain n-3 PUFAs is fish and seafood.
5. The effects of dietary n-3 PUFAs are abundant and rely on varied molecular mechanisms.
6. Experimental models demonstrate that some effects are heritable in offspring.
7. In humans, heritable effects have been shown as well, such as aortic stiffness in children, related with n-3 maternal levels.
8. N-3 PUFAs modify the DNA methylation of the FADS2 gene promoter, a key enzyme for PUFA synthesis.
9. EPA and DHA can regulate miR synthesis in different organs.
10. Resolvin D1, a DHA derivative, acts through miR modifications to terminate inflammation.

References

- Amaral CL, Crisma AR, Masi LN et al (2014) DNA methylation changes induced by a high-fat diet and fish oil supplementation in the skeletal muscle of mice. *J Nutrigenet Nutrigenomics* 7:314–326
- Antal O, Hackler L Jr, Shen J et al. (2014) Combination of unsaturated fatty acids and ionizing radiation on human glioma cells: cellular, biochemical and gene expression analysis. *Lipids Health Dis* 13:article142
- Aslibekyan S, Wiener HW, Havel PJ et al (2014) DNA methylation associated with n-3 fatty acids uptake in Yup'ik people. *J Nutr* 144:425–430
- Baker EJ, Miles EA, Burdge GC et al (2016) Metabolism and functional effects of plant-derived omega-3 fatty acids in humans. *Prog Lipid Res* 60:30–56
- Baselga-Escudero L, Arola-Arnal A, Pascual-Serrano A et al (2013) Chronic administration of proanthocyanidins or docosahexaenoic acid reverses the increase of miR-33a and miR-122 in dyslipidemic obese rats. *PLoS One* 8:e69817
- Bernard JY, De Agostini M, Forhan A et al (2013) The dietary n-6: n-3 fatty acid ratio during pregnancy is inversely associated with child neurodevelopment in the EDEN mother-child cohort. *J Nutr* 143:1484–1488
- Best KP, Gold M, Kennedy D et al (2016) Omega-3 long-chain PUFA intake during pregnancy and allergic disease outcomes in the offspring: a systematic review and meta-analysis of observational studies and randomized controlled trials. *Am J Clin Nutr* 103:128–143
- Bryant J, Hanson M, Peebles C et al (2015) Higher oily fish consumption in late pregnancy is associated with reduced aortic stiffness in the child at age 9 years. *Circ Res* 116:1202–1205
- Ceccarelli V, Racanicchi S, Martelli MP et al (2011) Eicosapentaenoic acid demethylates a single CpG that mediates expression of tumor suppressor CCAAT/enhancer-binding protein delta in U937 leukemia cells. *J Biol Chem* 286:27092–27102
- Ceccarelli V, Nocentini G, Billi M et al (2014) Eicosapentaenoic acid activates RAS/ERK/C/EBP β pathway through H-Ras intron 1 CpG island demethylation in U937 leukemia cells. *PLoS One* 9:e85025
- Clouard C, Souza AS, Gerrits WJJ et al (2015) Maternal fish oil supplementation affects the social behavior, brain fatty acid profile, and sickness response of piglets. *J Nutr* 145: 2176–2184
- Coti Bertrand P, O'Kusky JR, Innis SM (2006) Maternal dietary (n-3) fatty acid deficiency alters neurogenesis in the embryonic rat brain. *J Nutr* 136:1570–1575
- Daimiel-Ruiz L, Klett-Mingo M, Konstantinidou V et al (2015) Dietary lipids modulate the expression of miR-107, an miRNA that regulates the circadian system. *Mol Nutr Food Res* 59:552–565
- Farago N, Feher LZ, Kitajka K et al (2011) MicroRNA profile of polyunsaturated fatty acid treated glioma cells reveal apoptosis-specific expression changes. *Lipids Health Dis* 10. Article Number: 173
- Gil-Zamorano J, Martin R, Daimiel L et al (2014) Docosahexaenoic acid modulates the enterocyte Caco-2 cell expression of microTNAs involved in lipid metabolism. *J Nutr* 144:575–585
- Goustard-Langelier B, Koch M, Laviolle M et al (2013) Rat neural stem cell proliferation and differentiation are durably altered by the in utero polyunsaturated fatty acid supply. *J Nutr Biochem* 24:380–387
- Haggarty P (2010) Fatty acid supply to the human fetus. *Annu Rev Nutr* 30:237–255
- He F, Lupu DS, Niculescu MD (2014) Perinatal α -linolenic acid availability alters the expression of genes related to memory and to epigenetic machinery, and the Mecp2 DNA methylation in the whole brain of mouse offspring. *Int J Dev Neurosci* 36:38–44
- Hirabara SM, Folador A, Fiamoncini J et al (2013) Fish oil supplementation for two generations increases insulin sensitivity in rats. *J Nutr Biochem* 24:1136–1145
- Hoile SP, Clarke-Harris R, Huang RC et al (2014) Supplementation with N-3 long-chain polyunsaturated fatty acids or olive oil in men and women with renal disease induces differential

- changes in the DNA methylation of FADS2 and ELOVL5 in peripheral blood mononuclear cells. *PLoS One* 17:e109896
- Kasbi-Chadli F, Boquien CY, Simard G et al (2014) Maternal supplementation with n-3 long chain polyunsaturated fatty acids during perinatal period alleviates the metabolic syndrome disturbances in adult hamster pups fed a high-fat diet after weaning. *J Nutr Biochem* 25:726–733
- Kim J, Okla M, Erickson A et al (2016) Eicosapentaenoic acid potentiates Brown thermogenesis through FFAR4-dependent up-regulation of miR-30b and miR-378. *J Biol Chem* 291:20551–20562
- Krishnamoorthy S, Recchiuti A, Chiang N et al (2012) Resolvin D1 receptor Stereoselectivity and regulation of inflammation and Proresolving MicroRNAs. *Am J Pathol* 180:2018–2027
- Langelier B, Linard A, Bordat C et al (2010) Long chain-polyunsaturated fatty acids modulate membrane phospholipid composition and protein localization in lipid rafts of neural stem cell cultures. *J Cell Biochem* 110:1356–1366
- Lee HS, Barraza-Villarreal A, Hernandez-Vargas H et al (2013) Modulation of DNA methylation states and infant immune system by dietary supplementation with ω -3 PUFA during pregnancy in an intervention study. *Am J Clin Nutr* 98:480–487
- Liu A, Chang J, Lin Y, Shen Z, Bernstein PS (2010) Long-chain and very long-chain polyunsaturated fatty acids in ocular aging and age-related macular degeneration. *J Lipid Res* 51:3217–3229
- Ma Y, Smith CE, Lai CQ et al (2016) The effects of omega-3 polyunsaturated fatty acids and genetic variants on methylation levels of the interleukin-6 gene promoter. *Mol Nutr Food Res* 60:410–419
- Mandal CC, Ghosh-Choudhury T, Dey N et al (2012) miR-21 is targeted by omega-3 polyunsaturated fatty acid to regulate breast tumor CSF-1 expression. *Carcinogenesis* 33:1897–1908
- Niculescu MD, Lupu DS, Craciunescu CN (2013) Perinatal manipulation of α -linolenic acid intake induces epigenetic changes in maternal and offspring livers. *FASEB J* 27:350–358
- Piomelli D, Astarita G, Rapaka R (2007) A neuroscientist's guide to lipidomics. *Nat Rev Neurosci* 8:743–754
- Recchiuti A, Krishnamoorthy S, Fredman G et al (2011) MicroRNAs in resolution of acute inflammation: identification of novel resolvin D1-miRNA circuits. *FASEB J* 25:544–560
- Rossi S, Di Filippo C, Gesualdo C et al (2015) Interplay between intravitreal RvD1 and local endogenous Sirtuin-1 in the protection from endotoxin-induced uveitis in rats. *Mediat Inflamm* 2015:126408
- Serhan CN, Gotlinger K, Hong S et al (2004) Resolvins, docosatrienes, and neuroprotectins, novel omega-3-derived mediators, and their aspirin-triggered endogenous epimers: an overview of their protective roles in catabasis. *Prostaglandins Other Lipid Mediat* 73:155–172
- Shah MS, Schwartz SL, Zhao C et al (2011) Integrated microRNA and mRNA expression profiling in a rat colon carcinogenesis model: effect of a chemo-preventive diet. *Physiol Genomics* 43:640–654
- Tam EWY, Chau V, Barkovich AJ et al (2016) Early postnatal docosahexaenoic acid levels and improved preterm brain development. *Pediatr Res* 79:723–730
- Tyagi E, Zhuang Y, Agrawal R et al (2015) Interactive actions of Bdnf methylation and cell metabolism for building neural resilience under the influence of diet. *Neurobiol Dis* 73:307–318
- Vidakovic AJ, Gishti O, Voortman T et al (2016) Maternal plasma PUFA concentrations during pregnancy and childhood adiposity: the generation R study. *Am J Clin Nutr* 103:1017–1025
- Yao L, Han C, Song K et al (2015) Omega-3 polyunsaturated fatty acids upregulate 15-PGDH expression in cholangiocarcinoma cells by inhibiting miR-26a/b expression. *Cancer Res* 75:1388–1398