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Regulation of gene expression in brain and liver by marine n-3 polyunsaturated fatty acids

Progress in Nutrition
Vol. 12, N. 1, 24-28, 2010

Titolo
Regolazione dell'espressione genica nel cervello e nel fegato da parte di acidi grassi n-3 polinsaturi di origine marina

Key words
Docosahexaenoic acid (DHA), docosapentaenoic acid (DPA), eicosapentaenoic acid (EPA), n-3 polyunsaturated fatty acids (PUFA), gene expression

Parole chiave
Acido docosaesaenoico (DHA), acido docosapentaenoico (DPA), acido eicosapentaenoico (EPA), acidi grassi polinsaturi n-3 (PUFA), espressione genica

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Summary
Polyunsaturated fatty acids (PUFA) can affect gene expression through changes in membrane composition and signalling, eicosanoid production, oxidant stress, nuclear receptor activation or covalent modification of specific transcription factors. This paper considers the effects of marine n-3 PUFA on expression of genes involved in various pathways and in brain and liver. Increasing the n-3 PUFA content of the diet of rats induces changes in the expression of more than 100 genes in the brain, involved in synaptic plasticity, cytoskeleton, signal transduction, ion channel formation, energy metabolism and regulatory proteins. Further work has revealed an interaction between zinc and docosahexaenoic acid (DHA) in brain (*in vivo* & *in vitro*). In the liver, studies suggest that marine n-3 PUFA are involved in the suppression of glycolytic and lipogenic genes, and as activators of fatty acid oxidation at the level of gene expression to control mitochondrial and peroxisomal lipid metabolism.

Riassunto
Gli acidi grassi polinsaturi (PUFA) possono influenzare l'espressione genica modificando la composizione di membrana e la trasmissione del segnale: produzione di eicosanoidi, stress ossidativo, attivazione di recettori nucleari, modifica covalente di specifici fattori di trascrizione. Questo studio considera gli effetti di n-3 PUFA di origine marina sull'espressione di geni coinvolti nei diversi pathways, nel cervello e nel fegato. Aumentare il contenuto di n-3 PUFA nella dieta dei ratti induce cambiamenti nell'espressione di oltre 100 geni nel cervello, coinvolti nella plasticità sinaptica, citoscheletro, trasduzione del segnale, formazione di canali ionici, metabolismo energetico e proteine regolatrici. Ulteriori studi hanno rivelato una interazione tra zinco e acido docosaesaenoico (DHA) nel cervello (*in vivo* e *in vitro*). Nel fegato, gli studi suggeriscono che n-3 PUFA di origine marina sono coinvolti nella sopressione della lipogenesi, e come attivatori dell'ossidazione di acidi grassi a livello di espressione genica nel controllo del metabolismo lipidico mitocondriale e perossisomiale.

Introduction

Fatty acids are energy rich molecules that play important metabolic roles. Fatty acids can be categorised into three groups depending on the number of double bonds namely: saturated, mono unsaturated and polyunsaturated fatty acids (PUFA). Alpha-linolenic acid (ALA) (n-3) is an essential PUFA which can be metabolized *in vivo* by desaturation and elongation enzymes to form a series of long chain more unsaturated n-3 PUFA. The major products of this pathway are eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid, (DHA) (1). Fatty acids, especially n-3 PUFA, have been implicated in modulation of various biochemical pathways including the ability to affect gene expression. PUFA are known to regulate the gene expression in various cell types like liver (2), adipose (3), muscle (4), brain (5), heart (6) and colon (7). In rodents, PUFA rich diets repress various lipogenic genes and increase the expression of genes involved in beta-oxidation (2, 8-10). It is believed that PUFA can potentially affect gene expression through changes in membrane composition and signalling, eicosanoid production, oxidant stress, nuclear receptor activation or covalent modification of specific transcription factors. This report will review the effects of marine n-3 PUFA on expression of genes involved in various pathways in brain and liver. It was initially thought that fatty acids affect cellular metabolism solely through indirect mechanisms such as changing membrane phospholipid concentrations or producing signalling intermediates like eicosanoids. However, actions of fatty acids on gene expression occur within hours of feeding animals diets rich in PUFA suggesting a more direct mode of action. The discovery of Gottlicher et al of nuclear receptors capable of binding fatty acids to modulate gene expression established a direct role for fatty acids at nuclear level (11). The main receptors that interact with PUFA to regulate gene expression are peroxisome proliferator-activated receptor (PPAR), liver X receptor (LXR) and Hepatic nuclear factor - 4α (HNF-4α). PUFA also regulate gene expression by regulating the transcription factors like sterol regulatory element binding protein (SREBP) and carbohydrate response element binding protein (ChREBP) (12).

The **brain** is a lipid rich organ, infact it has the second highest concentration of lipids in body after adipose. Brain phosphoglycerides are rich in DHA and arachidonic acid (AA) and contain smaller proportions of DPA and docosatetraenoic acid (n-6) (13). The n-3 PUFA regulate the expression of several genes in brain. Kitajaka et al reported that rats fed throughout their life with either a vegetable oil (rich in ALA) or fish oil containing EPA, DPA and DHA showed alterations in approximately 100 genes in brain, 55 of which were upregulated and 47 were down-regulated, relative to control rats (14). The genes altered were mainly involved in synaptic plasticity, cytoskeleton, signal transduction, ion channel formation, energy metabolism and regulatory proteins. They also found that diets rich in n-3 PUFA (ALA and the marine n-3 PUFA) significantly affected neural energy metabolism and ATP of gene expression. In a separate experiment they observed that genes encoding for alpha- and gamma-synuclein were over expressed in young rats fed with fish oil (marine n-3 PUFA) for one month (15, 16). Synucleins are associated with synaptosomes and play a role in neural plasticity and learning.

Many mood disorders like neuroinflammation and depression are associated with excessive production of cytokines like IL-1beta, IL-12, IL-6 and TNF-α as well as chemokines like vascular cell adhesion molecule-1 (VCAM-1) and intracellular cell adhesion molecule – 1 (ICAM) (for review see (13, 17)). Furthermore, the marine n-3 PUFA are well known to

inhibit the expression of these cytokines, via effects in reducing the production of proinflammatory prostaglandins (PGE2) and leukotrienes (LTB4) (18, 19). Brain derived neurotropic factor (BNDF) is a potent trophic factor which combined with PGE2 regulates the synaptic plasticity and induces long-term potentiation. It has been reported that feeding rats with n-3 PUFA deficient diet alters the expression of BNDF, cyclic AMP response element binding protein (CREB) and p38 mitogen activated protein kinase (MAPK) expression and activity (20). In contrast, feeding marine n-3 PUFA normalizes BDNF levels that are reduced with brain injury (20).

Marine n-3 PUFA also regulate the expression of zinc transporters (ZnT) in brain. Zinc is regarded as an important nutrient because it plays an essential role in biological systems as catalytic or structural cofactor in numerous zinc-dependent enzymes, in signal transduction and as component of transcription factors. The uptake of Zn from extracellular environment to cytoplasm is mediated by ZnT. Jayasooriya et al reported that the expression of ZnT3 is upregulated in rats raised on n-3 PUFA deficient diet, compared with rats given n-3 sufficient diets (21). It was also observed that there was a decrease in plasma zinc levels and increase in brain zinc levels. The results suggest that over expression of ZnT3 due to a perinatal omega-3 PUFA deficiency caused abnormal zinc metabolism in the brain. Neuronal zinc is reported to be involved in the formation of amyloid plaques which is character of AD during adulthood. Also low plasma levels of Zn have been associated with incidence of AD in humans. Thus, the influence of dietary omega-3 PUFA on brain zinc metabolism could explain the observation made in population studies that the consumption of fish is associated with a reduced risk of dementia and Alzheimer's disease (21). It has been recently shown that zinc and DHA have opposing effects on the expression levels of histones H3 and H4 in human neuronal cells. Both histones were downregulated by Zn in the absence of DHA (Zn effect) and upregulated by DHA (DHA effect) in the presence of Zn (physiological condition). Such novel information provides possible clues to the molecular basis of the opposing effects of zinc and DHA on neuroprotection (22).

The **liver** plays a central role in whole body lipid metabolism and dietary fat has significant impact on hepatic lipid metabolism. Studies on primary hepatocytes have revealed several major metabolic pathways that are targeted by PUFA in liver. Each pathway involves changes in gene expression. First, n-3 PUFA induction of microsomal and β-oxidation (mitochondrial and peroxisomal) requires PPARα (23). Second, PUFA suppression of glycolitic and lipogenic genes like liver pyruvate kinase and fatty acid synthase involves three transcription factors, SREBP-1, ChREBP and MLX (24-26). Third, PUFA suppression of the glycolytic enzyme, l-pyruvate kinase, does not involve PPARα, SREBP-1 or LXRα (27-29), but involves ChREBP and MLX heterodimer (24, 25, 30). Fourth, PUFA suppression of PUFA synthesis lowers levels of fatty acid elongase-5 (Elovl-5), Δ^5desaturase (Δ^5D) and Δ^6desaturase (Δ^6D). PUFA control of SREBP-1 nuclear abundance explains part of this mechanism (24, 30, 31). These studies suggest that n-3 PUFA function as feed-forward activators of fatty acid oxidation at the level of gene expression to control mitochondrial and peroxisomal lipid metabolism. Marine n-3 PUFA are also reported to increase the mean size of mitochondria as well as increase the expression of genes involved in fatty acid oxidation like CPT-II (32) and UCP-2 (23) in hepatocytes. Marine n-3 PUFA also function as feedback inhibitors of glycolysis, *de novo* lipogenesis, mono- and polyunsaturated fatty acid synthesis to con-

trol the production and cellular content of saturated, mono- and polyunsaturated fatty acids. These regulatory schemes not only reduce overall hepatic lipid content and VLDL secretion, but may also eliminate excessive marine n-3 PUFA that may promote oxidant stress or impair membrane integrity (33).

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