Critical Review

Advances in n-3 polyunsaturated fatty acid nutrition

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There is conclusive evidence to demonstrate the role of omega-3 polyunsaturated fatty acids (n-3 PUFA) in human development and growth, vision, and cell membrane fluidity (membrane order). N-3 PUFA also contribute to human health maintenance through correction of arrhythmias, inhibition of platelet aggregation and prolongation of clotting time, lowering blood pressure, lowering serum triglycerides and plasma homocysteine, being anti-inflammatory and immunomodulatory, being cardio-protective, increasing insulin sensitivity in Asians, and decreasing the risk of breast and colorectal cancers. This understanding of a wide spectrum of biological effects attributable to n-3 PUFA has been unsettled by a systematic review of randomized clinical intervention trials (RCTs) which has reported that n-3 PUFA have negligible or no effect on all-cause or cardiovascular mortality. Here, possible reasons for the inconsistencies in regard to n-3 PUFA and cardiovascular diseases, along with the implications for their broader biology, are considered.

Key Words: n-3 PUFA, randomized clinical intervention trials, cohort, cardiovascular disease, biological function

Controversy

The Cochrane Library online published a 743-page article on "Omega-3 fatty acids for the primary and secondary prevention of cardiovascular disease (Review)", authored by Dr Lee Hooper from the University of East Anglia, UK, on July 18, 2018.[1] The article included 79 randomized clinical intervention trials (RCTs) involving 112,059 volunteers with an intervention period of 1-6 years, published before April 2017. The main findings were : high quality evidence showed that increased long-chain omega-3 polyunsaturated fatty acids (LC n-3 PUFA) eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) status leads to slight or no effect on all-cause mortality; moderate quality evidence suggested a slight decrease or no effect on cardiovascular death, death or onset of coronary heart disease, and stroke or arrhythmia. High-quality evidence suggested that EPA and DHA can slightly lower serum triglycerides and increase high-density lipoprotein cholesterol (HDL-C). Medium/low quality evidence suggested that excessive intake of α-linolenic acid (octadecatrienoic acid, ALA) has little or no effect on all-cause or cardiovascular death or coronary artery disease, but may slightly reduce the incidence of cardiovascular disease, coronary heart disease mortality and arrhythmia. The impact of ALA on stroke is currently unclear because of the low quality of evidence. Evidence suggested that taking n-3 PUFA capsules does not decrease but eating fish can slightly reduce the risk of heart disease and stroke. Although EPA and DHA reduce triglycerides, supplementation with n-3 PUFA may have no significant effect on the prevention or treatment of heart and circulatory diseases. However, increasing ALA from plants may have a slight protective effect on some heart and circulatory diseases. The Cochrane article attracted the attention of health institutions, professionals, enterprises and the media. It challenges our understanding of n-3 PUFA biology and health relevance developed over some 50 years. Back then, the morbidity and mortality from cardiovascular diseases among Eskimos in Greenland, Denmark, who were predominantly marine mammal eaters, was found to be appreciably lower than for other populations.[2-4] This has been confirmed in several epidemiological cohort studies.[5-7] Why is the Cochrane Library report inconsistent with the cohort studies? There are various possible reasons as discussed below.

Study design and comparators

The difference between cohort study and RCT

Although RCT is the gold standard for studying the effects of nutrients or functional components of food on disease and disease risk factors, any RCT has its limitations for observing clinical outcomes, especially mortality and disease morbidity. RCT cannot be carried out for many years or even decades due to factors such as research funding and compliance; the different genetic

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background, age, gender, and health status of the subjects (healthy population or patients, course of disease and complications etc.); different comparators used in the interventions, such as olive oil, n-6 PUFA vegetable oil (safflower oil, sunflower oil, corn oil, soybean oil, etc.), liquid paraffin and carbohydrates; the different intervention period (1-6 years); the use of dietary supplements such as deep sea fish oil, multivitamin mineral tablets and other natural products; the purity of n-3 PUFA used in the intervention, such as standardised deep sea fish oil (30% long-chain n-3 PUFAs, i.e. 180 mg of EPA and 120 mg of DHA per gram) or highly purified fish oil (EPA plus DHA greater than 50%); the different source and quality of n-3 PUFA, such as salmon oil which is high in EPA, tuna oil which is high in DHA, or seal oil which is relatively high in DPA; and the difference in acid value and peroxide value of n-3 PUFA. In addition, some of studies are called RCT, but the investigators do not provide n-3 PUFA for subjects, they only instruct the subjects to purchase specific brand of fish oil or recommend subjects to consume a certain amount of fish per week. In addition, most studies included in meta-analyses of long chain n-3 PUFA do not measure the blood levels of subjects before the start of the intervention, and at the conclusion. Therefore, neither it is not possible to know if all subjects started at the same (hopefully low) blood n-3 value nor whether their blood n-3 values increased as a result of the treatment. Indeed, even in subjects who have consumed similar intakes of fish oil, there is a large variation in the blood n-3 value after supplementation, indicating significant between-subject responses to supplementation. The failure to measure blood n-3 values significantly limits the findings of any studies not reporting such blood parameters. Due to the lack of a standardized RCT method for investigating the effects of n-3 PUFA on morbidity and mortality of cardiovascular diseases (or other diseases), there is great heterogeneity between RCTs, which is therefore likely to produce different results (positive, negative or no effects). It is no surprise that a meta-analysis of dozens of such different RCTs yields results that have little or no impact.

Fish, oils and nutrients

Due to the increased awareness of the health effects of n-3 PUFAs and fish, the intake of fish has been dramatically increasing globally.[8] Furthermore, a significant portion of adults in western countries have taken deep-sea fish oil for up to 40 years, resulting in increased sales year-on-year.[9] N-3 PUFA may be consumed from multiple sources, as a food (fish or fish oil), a supplement, a nutraceutical, and also as a drug. Further adding to the demand for these n-3 PUFA sources, has been an awareness of the unhealthy effects of saturated fatty acids. Food in restaurants was often deep fried in lard, and the beef and lamb fat which leaked from the barbecue onto the sieve tray, later re-used or used as a spread. Thirty years ago, many families liked to use animal fat for cooking. Modern human cell membrane phospholipid fatty acid composition and structure have undergone tremendous change over some 30 years to be more optimised (n-3 PUFA composition has significantly increased, and that for saturated fatty acids significantly reduced). Global life expectancy was 58 years 30 years ago, and 72 years in 2016.[10] Therefore, findings about fatty acid intake and health outcomes 30 years ago are unlikely to be repeated in modern populations; it would not be surprising if little or no effect of n-3 PUFA on cardiovascular diseases were now in evidence on meta-analysis and systematic review.

Food source and n-3 PUFA type

N-3 PUFA mainly exist in fish and certain plants. ALA and linoleic acid (octadecadienoic acid, n-6 PUFA) are two essential fatty acids for humans. ALA is mainly found in plant nuts and seeds, such as flaxseed, perilla seed, chia seed, walnut and hemp seeds. LC n-3 PUFA EPA and DHA are mainly found in fish and other marine products. They can also be converted from ALA to DHA by desaturation, chain elongation and peroxisomal beta-oxidation catalyzed by δ-6 and δ-5 desaturases, chain elongation enzymes (elongases) and oxidase. However, the conversion rate of ALA to DHA in humans is very low. The conversion rate is 3-8% for vegetarians, while it is only 1-3% for omnivores, so DHA is also called a conditionally essential fatty acid.[11]

It is of interest that recent data on n-3 fatty acid intakes of pregnant women from three geographical regions of China (coastal, inland, and river/lake) reported low total intakes of LC n-3 PUFA, and high intakes of linoleic acid (main dietary n-6 PUFA).[12] The imbalance of these two classes of PUFA (n-6 to n-3) is a potential problem for pregnant women in China in terms of adequate supply of DHA to the developing infant.[13]

Human cell membrane fatty acids

Fatty acids are the main components of phospholipids in human cell membranes. LC n-3 and n-6 PUFA play an important role in maintaining the structure and function of cell membranes. DHA is extremely high in the human retina and brain.[14,15] DHA is an indispensable substance for maintaining visual and brain function. Because it plays an important role in the fluidity of cell membranes, it affects cell membrane receptors (such as rhodopsin) by regulating the fluidity of membranes, thereby regulating activities of membrane-bound enzymes (such as Na/K-dependent adenosine triphosphatase) and membrane G-protein ion channels, and can affect the transmission of signals through the action of inositol phosphate, diacyglycerol and protein kinase C. DHA can directly affect the biosynthesis of neurotransmitters, the transmission of signals, the absorption of serotonin, the binding of β-adrenergic receptors and serotonin-activated receptors, the activity of monoamine oxidase[15] and the formation of N-docosahexaenoylethanolamine (synaptamide, a promoter of neurogenesis) from DHA-rich phosphatidyl serine.[14,16]

Gene expression

N-3 PUFA can alter gene expression in the brain and other tissues and act as precursors for lipid mediators such as resolvins, maresins and neuroprotectins.[16] N-3 PUFA can down-regulate the activities of proteoglycan decomposing enzymes, inflammatory factors (interleukin-1 and TNF), COX-2, fatty acid synthase, acetyl coenzyme A carboxylase, S14 protein and stearyl coenzyme A desaturase.[11]

At the same time, n-3 PUFA can also up-regulate the activities of lipoprotein lipase fatty acid binding protein, acetyl coenzyme A synthase, carnitine palmitoyltransferase 1, acetyl coenzyme A dehydrogenase, acetyl coenzyme A oxidase, cytochrome P-450 4A2 and peroxidsome proliferator-activated receptor-α.[11]

Ethnicity, single nucleotide polymorhisms (SNPs) and n-3 PUFA function

N-3 PUFA also have many other beneficial functions for human health. For example, n-3 PUFA can reduce insulin resistance, increase insulin sensitivity and regulate glucose metabolism.[17] Geographic and ethnic differences have a significant impact on the intake of n-3 PUFAs and the risk of type 2 diabetes, so we have proposed that Caucasian and Asian type 2 diabetic patients respond differently to n-3 PUFA.[18] Only in Asian populations was the intake of LC n-3 PUFA and fish negatively correlated with the risk of type 2 diabetes mellitus, and the levels of DHA and total n-3 PUFA in tissues were significantly lower in type 2 diabetic patients than in healthy subjects.[19] In Caucasian populations, the SNP loci rs7645550 and rs1183319 on the PIK3CA-KCNMB3 region all had significant interaction with dietary n-3:n-6 PUFA, when the ratio of n-3:n-6 was low (<0.11), rs7645550 allele TT homozygotes have lower insulin resistance (HOMA-IR) levels relative to C allele carriers, while rs1183319 allele G carriers have higher HOMA-IR levels; when the ratio of n-3:n-6 PUFA was higher than 0.11, rs7645550 and rs1183319 had no significant relationship with HOMA-IR. In the same population, the SNP loci rs4795413 and rs8065443 on the PSMD3 gene interact with n-3 PUFA or the ratio of n-3:n-6 PUFA and co-regulate the HOMA-IR level; when n-3 PUFA is low (<0.68% energy), the HOMA-IR levels of the T allele carriers of rs4975413 were significantly higher than that of CC homozygotes; when the ratio of n-3:n-6 PUFA is low (<0.68%), the HOMA-IR is higher in A allele carriers of rs8065443 than in the other genotype carriers. Meanwhile, the relationship between rs7578326 and rs2943641 on the IRS1 gene and insulin resistance was also regulated by dietary fat in type 2 diabetes.[20]

In Chinese populations, the PEPD gene and n-3 PUFA have a significant interaction, which affects the risk of type 2 diabetes mellitus. The positive correlation between the SNP locus rs3786897 risk allele on the PEPD gene and type 2 diabetes was only observed when the erythrocyte membrane n-3 PUFA content was low. There was no significant relationship when n-3 PUFA levels were high, that is, n-3 PUFA could eliminate the adverse effect of PEPD risk allele on type 2 diabetes mellitus.[21] Therefore, there are different patterns of interaction between n-3 PUFA and genes in Western and Asian populations. Genetic polymorphisms may explain the inconsistent relationship between n-3 PUFA and insulin resistance or type 2 diabetes in different populations. The RCT results in China have shown that n-3 PUFA has a positive effect on the prevention of type 2 diabetes, and they have a beneficial effect on the risk factors of type 2 diabetes such as fasting blood glucose and insulin. Fish oil intervention can significantly up-regulate the mRNA expression of key genes in the insulin-signaling pathway, such as Insr, Irs1, Akt1 and Glut4.[22] The effects of n-3 PUFA on blood lipids in type 2 diabetic patients may vary according to the genetic variations of CD36, NOS3 and PPAR-gamma genes, and personalized dietary recommendations based on certain genetic components to improve blood lipid profile may be extremely effective for n-3 PUFA intake.[23] After 6 months of intervention with purified deep-sea fish oil (EPA plus DHA = 60%), the content of 3-carboxy-4-methyl-5-propyl-2-furanpropanoate (CMPF), a metabolite of n-3 PUFA in plasma, was significantly increased. The changes of EPA in plasma phosphatidylcholine, EPA and DHA in plasma were significantly positively correlated with CMPF, while change of serum triacylglycerol was negatively corrected with CMPF.[24] We hypothesized that CMPF may be a biomarker for the intake of fish and fish oil, and elevated plasma CMPF may increase insulin sensitivity in patients with type 2 diabetes.

Blood pressure

LC n-3 PUFA can reduce systolic and diastolic blood pressure. In a RCT conducted in Inner Mongolia, China, increasing dietary LC n-3 PUFA intake significantly reduced cardiovascular risk factors in hypertensive patients, which may be related to dietary n-3 PUFA correcting the imbalance of renin-angiotensin system and inhibiting the expression of downstream levels of MAPK phosphorylated protein.[25]

Inflammation and immunomodulation

The n-3 PUFA also have anti-inflammatory and immunomodulatory activities. One of the roles of EPA and DHA in cells is to regulate the production of bioactive substances. Eicosanoids derived from arachidonic acid (eicosatetraenoic acid, AA, an n-6 PUFA) as a substrate, and its regulation mechanism is to produce various eicosanoid bioactive substances through competition between EPA and AA, such as 3-series prostaglandins, prostacyclin and thromboxane, 5-series leukotrienes (LT) and lipoxins etc. EPA and DHA also regulate ion flux in cardiomyocytes. AA also promotes the formation of other inflammatory substances such as cytokines, tumor necrosis factor- α (TNF- α), interleukin-6, and reactive oxygen species. EPA and DHA compete with AA at the COX and lipoxygenase levels, resulting in a decrease in the inflammatory eicosanoids, cytokines, interleukin-6, and reactive oxygen species converted from AA. The 5 series LT produced from EPA is inactive compared with the 4 series LT converted from AA.[26]

Platelet function

Platelet aggregation is initiated by thromboxane A_2 (TXA_2), a potent platelet aggregation factor and vasoconstrictor produced by AA in the platelet cell membrane. EPA and AA released from platelet cell membrane phospholipids, which competitively bind COX, resulting in an alternative form of TXA_3, but is relatively inactive or less active in platelet aggregation and vasoconstriction, thus reducing the production of TXA_2, thereby reducing the tendency to form a thrombus. A diet with a high ratio of n-6 to n-3 PUFA can cause an increase in the ratio of n-6 to n-3 PUFA in the tissue (e.g., a higher ratio of AA to EPA) and will also promote TXA_2 production, this leads

to an increased tendency to thrombosis. Dietary intervention trials on humans and animals demonstrated that LC n-3 PUFAs obtained from marine and plant-derived n-3 PUFAs reduced TXA_2 production.[11]

Non-alcoholic fatty liver

Dietary n-3 PUFA have the effect of preventing and improving non-alcoholic fatty liver. The mechanism may be due to the enhanced activity of PPARγ by n-3 PUFA, which in turn increases the transport capacity of hepatocytes to triglycerides and inhibits fat accumulation in the liver. In addition, dietary n-3 PUFA can also improve the risk factors of metabolic diseases by regulating other series of gene transcription factors (PPARγ, SREBP1 etc.), and can also significantly enhance the β-oxidation of fatty acids and reduce endogenous lipid accumulation in liver cells. The reduction of the expression and production of pro-inflammatory factors may be another mechanism by which n-3 PUFAs prevent and ameliorate the effects of non-alcoholic fatty liver.[26]

Neoplasia, the brain and multifunctionality

In addition, n-3 PUFA can also increase heart rate and may prevent some cancers. LC n-3 PUFA can significantly reduce the risk of breast cancer with a significant dose-effect relationship.[27] N-3 PUFA also shown some benefits on attention deficient hyperactivity disorder (ADHD), schizophrenia and depression.[28-30] N-3 PUFA, especially DHA, can reduce plasma homocysteine concentration, which may have an role in preventing cognitive decline and type 2 diabetes.[31,32] A recently updated Cochrane review reported that preterm birth <37 weeks and early preterm birth <34 weeks were reduced in women who had received LC n-3 PUFA compared with no omega 3.[33]

Cautionary note

Readers are reminded that it is important to look at details of published papers, rather than relying on only the Abstract. A case in point is a recent paper by Manson et al on marine n-3 fatty acids and prevention of cardiovascular disease and cancer.[34] In the Abstract it was stated that "Supplementation with n-3 fatty acids did not result in a lower incidence of major cardiovascular events or cancer than placebo", however looking at the results it is clear that, compared with placebo, n-3 PUFA significantly reduced total myocardial infarction with an HR 0.72 (95% CI, 0.59 to 0.90) (Abstract and Table 2); percutaneous coronary intervention (PCI) 0.78 (0.63-0.95); total coronary heart disease 0.83 (0.71-0.97); death from myocardial infarction 0.50 (0.26-0.97) (Table 2). In addition, the authors classified "myocardial infarction" as a primary end point in the Methods, however, this was changed to a secondary end point in the Results of the Abstract section. Further inspection of this paper, revealed no details of the substances used in n-3 PUFA placebo from any published VITAL paper or other reference so far as can be seen.

Conclusion

In conclusion, a recent RCT systematic review suggests that n-3 PUFA have only a slight or no significant reduction in mortality and cardiovascular disease morbidity. However, a more comprehensive understanding of the global trends in n-3 PUFA nutritional status, their multifunctionality, population differentials and underlying nutrigenomics supports the continuing emphasis on their availability and consumption. Specifically, a knowledge of blood n-3 fatty acid status before and after the studies is a requisite for dependable policy for their use. Important benefits of n-3 PUFA intake obtain for many aspects of human health, in prevention and clinical settings.

AUTHOR DISCLOSURES

The authors declare no conflicts of interest.

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