# DHAid™ – The vegetarian source

Ulla FREITAS[1]
Marie LEBLANC[2]

[1] *Lonza Ltd, Muenchensteinerstr. 38, 4002 Basel, Switzerland*
[2] *Lonza France Sarl, 55 rue Aristide Briand, 92309 Levallois-Perret Cedex, France*
<*marie.leblanc@lonza.com*>

***Abstract:*** *In humans, DHA occurs naturally as a cell membrane fatty acid in the brain, retina, testes and sperm, and has been reported to be essential in the development of these organs and cells. There it is crucial for the functioning of embedded proteins, i.e. rhodopsin for vision and postsynaptic receptors for neurotransmission. In phospholipids in general, DHA contributes to membrane properties such as fluidity, flexibility and permeability. A deficiency in DHA can lead to memory loss, learning disabilities and impaired visual acuity. Limited storage of DHA in adipose tissue suggests that a continuous supply is needed. These facts clearly demonstrate the physiological importance of DHA for humans and have resulted, for example, in the recommendation of increasing dietary intake of DHA during pregnancy and lactation. Also in the maintenance of cardiovascular health, DHA plays an important role. DHAid™ is a pure vegetarian source of omega-3 docosahexaenoic acid (DHA). It is produced from microalgae in a controlled process in fermentation vessels by the Swiss life-science company Lonza. Due to its renewable sources, DHAid™ is environmentally friendly. DHAid™ is allergen free and is free of potential contaminants that are discussed for seafood.*

***Key words:*** *docosahexaenoic acid, DHA, microalgal oil, omega-3, polyunsaturated fatty acids*

## What is DHA (docosahexaenoic acid)?

Fatty acids are classified according to their degree of saturation (number of double bonds), into saturated fatty acids, monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs). PUFAs can further be classified into two different series that cannot be converted into each other: omega-3 and omega-6 PUFAs [1].

There are distinct types of omega-3 fatty acids that are ingested with the diet and used by the body. These are docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) as well as alpha-linolenic acid (ALA). Whereas ALA can be found most abundantly in foods, it has become clear that DHA is the most important omega-3 fatty acid for human health, particularly in the areas of brain, heart and eye health. It occurs naturally as a building block of cell membranes [1, 2] and contributes to membrane properties such as fluidity, flexibility and permeability [3].

## Sources of DHA

DHA in the body is derived mainly from intake of fish and seafood. Fatty fish, such as herring, mackerel, tuna or wild salmon, is the most substantial source of DHA [4]. *Table 1* gives an overview of the DHA contents in fish and seafood.

Nevertheless it is well-known that dietary DHA intake with a typical Western diet is well below recommended values. It is also clear that vegetarians and those who do not eat fish get very little DHA with their diet [5]. Therefore, the consumption of dietary supplements and functional food enriched with DHAid™ constitutes an attractive option in order to achieve the recommended intake. It is important to note that DHAid™ represents an allergen-free and vegetarian source of DHA that comes from renewable resources and does not contribute to the common problem of overfishing of the sea.

## Conversion between different omega-3 fatty acids

Nature has foreseen a pathway to produce DHA from the precursor omega-3 fatty acid ALA in the human body. During this process, ALA is enzymatically converted to EPA and further to DHA. An important question is whether dietary intake of ALA, can provide sufficient amounts of EPA and DHA by conversion through the omega-3 PUFA elongation-desaturation pathway. ALA is present in marked amounts in plant sources, including green leafy vegetables and commonly-consumed oils such as rape-seed and soybean oils, so that increased intake of this fatty acid would be easier to achieve than an increase in fish consumption.

Table 1. *DHA content of fish and seafood [4].*

| Fish species | DHA (mg/100 g) |
|---|---|
| Atlantic salmon | 1,457 |
| Pacific mackerel | 1,195 |
| Atlantic herring | 1,105 |
| Tuna | 223 |
| Haddock | 162 |
| Shrimps | 144 |
| Alaska king crab | 118 |

However, it has become clear that the dietary intake of the precursor ALA cannot make up for the low dietary intake of DHA. Humans are very poor DHA synthesizers from precursor omega-3 fatty acids [6]. Aging, illness and stress, as well as excessive amounts of omega-6 rich oils (corn, safflower, sunflower, cotton seed) can all compromise conversion [7]. Various human supplementation studies have addressed the question of the bioconversion process and have concluded that conversion of ALA to EPA is limited and conversion further to DHA is extremely low [8-10]. Aging, illness and stress contribute to this limited conversion process as well as the excessive intake of omega-6 rich oils due to competition for the same enzymes [7].

In addition, there is also a certain degree of retro-conversion from DHA back to EPA [11]. EPA but not DHA concentrations in plasma were observed to increase in response to dietary EPA intake.

In respect of this background, uptake of dietary DHA might be critical for maintaining adequate membrane DHA concentrations [12].

doi: 10.1684/ocl.2008.0204

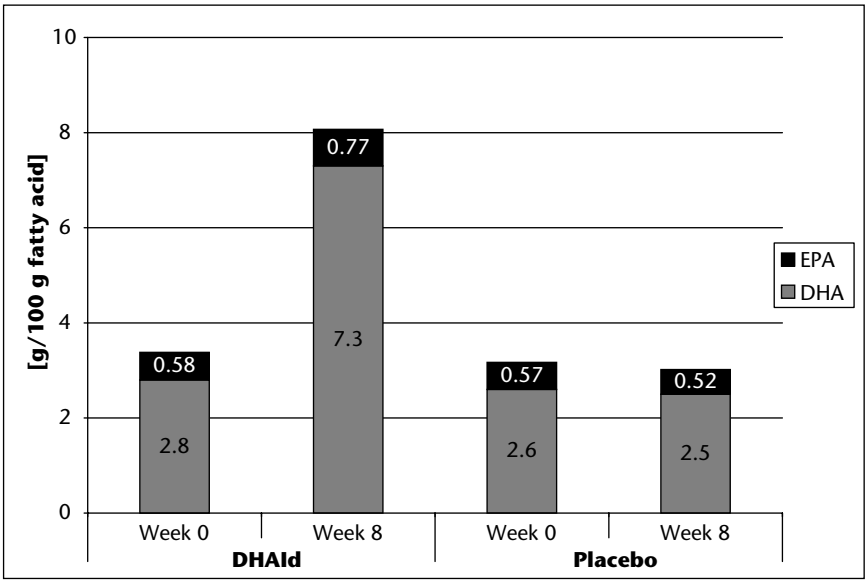


Figure 1. *Changes in DHA and EPA content of plasma phospholipids before and after 8 weeks of supplementation with DHAid™ (g/100 g fatty acid) [17].*

Therefore, Health Organisations throughout the world have made specific recommendations to increase DHA levels [13-16].

In a recent study including more than 100 healthy vegetarians, 8 weeks of DHAid™ supplementation was found to significantly increase their DHA and EPA plasma levels compared with placebo *(figure 1)* [17]. It is worth mentioning that exclusive DHA supplementation also increased EPA plasma phospholipid levels, which may be explained by increased retro-conversion from DHA to EPA.

## Benefits from DHAid™

### *Pregnancy*

Under the present dietary conditions, maternal intake of omega-3 fatty acids is insufficient to keep up with the increased demand during pregnancy [18]. Especially in the last trimester, the period during which much of the fetus' brain, eye and nervous system development occurs, maternal DHA levels decline significantly [19]. Maternal and infant DHA status becomes reduced after each following pregnancy, which is especially important in pregnancies spaced at short intervals or with multiple births [20].

Most national and international authorities therefore recommend increasing DHA intake during pregnancy and lactation to at least 200 mg/day [13, 15, 21].

After birth, the newborn baby continues to obtain DHA from the mother via breast milk [22]. The DHA content of the breast milk is directly related to the dietary DHA intake of the mother, supplementation with DHA increases the DHA content in human milk [23, 24]. Prenatal and early postnatal positive DHA status is thought to have important consequences on the growth and function of the central nervous system (CNS) and, consequently, on neurological and cognitive development of the child [22, 25-27].

In addition, human pregnancy supplementation trials with omega-3 fatty acids have shown a significant reduction in the incidence of premature delivery [28, 29], and also of post-natal depressions of the mother [30, 31].

### *Brain health*

DHA is a major structural and functional building block of the brain – about 30% of the structural lipids of the grey matter are DHA. A change in the fatty acid composition of synaptic membranes can affect the functions of neuronal membrane receptors, ion channels and enzymes [32]. DHA is required during fetal and child development for the growth and functional development of the brain [33]. Hence it is not surprising that DHA deficiency in early life has been associated with a variety of learning and cognitive disorders [18, 22]. DHA is required during *fetal and child development* for the growth and functional development of the brain and eye [34, 35]. The significant positive association between maternal docosahexaenoic acid intake during pregnancy and the children's mental processing scores at 4 years of age suggest that optimization of the docosahexaenoic acid status of expectant women may offer long-term developmental benefits to their children [18, 22].

In *adolescents*, DHA supplementation was found to prevent aggression enhancement during times of mental stress, such as in exam periods [36]. In *adults*, DHA maintains normal brain function, and scientific evidence links reduced DHA levels to a number of mental disorders including depression, dementia, schizophrenia and Alzheimer's disease [2, 37-42].

Depression and other mental diseases in *elderly subjects* are associated with significantly lower omega-3 fatty acid levels than in same age control subjects [7, 43-45].

Psychological stress in humans induces the production of proinflammatory cytokines of the omega-6 series which can be increased by an imbalance of omega-6 to omega-3 fatty acids in the blood. There is evidence that such an overproduction is involved in the pathophysiology of major depression [7]. DHAid™ may contribute to a healthy brain throughout life.

### *Visual function*

The importance of DHA in retinal function is reflected by the high concentration of DHA especially in the retinal photoreceptor. About 30-40% of the fatty acid composition of the rod photoreceptor outer segments of the retina is DHA. Increased dietary intake of omega-3 fatty acids increases the omega-3 fatty acid content of the rod outer segments. Biophysical and biochemical properties of DHA may affect photoreceptor membrane function by altering permeability, fluidity, thickness and lipid phase properties [26]. The tissue DHA status affects retinal cell signalling mechanisms involved in signal transduction [46].

DHA is involved in the intercellular signalling pathway that transforms light signals to neuronal activity. Thus, DHA plays an important role in eye health during the prenatal phase and in the first years of life as well as in the elderly [26, 46].

Several human studies support the importance of adequate maternal DHA consumption during pregnancy for the maturation of the visual system of infants [18, 47]. Various clinical studies in infants and meta-analyses suggest that greater visual acuity in infancy is associated with increased intake of long-chain PUFAs. Reduced visual acuity has consistently been observed in primate and rodent offspring subjected to dietary conditions during gestation that result in significant reductions in retinal concentrations of DHA. Further it has been observed that functions of the retina mature earlier when infants are supplemented with DHA [18, 34].

In addition, diets high in omega-3 fatty acids and especially DHA may act in a protective role against age-associated pathology to the vascular and neuronal retina in the elderly [48, 49].

### *Cardiovascular health*

Although a traditional Eskimo diet contains much more fat than commonly recommended, Eskimo populations seem to be immune to heart disease [6, 50]. It is now recognized that this is thanks to the intake of large amounts of fish and marine mammals, which are rich in omega-3 PUFAs [51]. Similar studies in Japan, comparing inhabitants from fishing villages with farming villagers, also showed that a higher intake of oily fish resulted in a reduced risk of heart disease.

Various scientific studies clearly demonstrate that a high intake of omega-3 fatty acids, especially DHA, correlates with heart health [52, 53]. Interestingly, the dietary precursor essential fatty acid of EPA and DHA, alpha-linolenic acid (ALA), has not consistently been found to have beneficial effects on cardiovascular health [54, 55].

The heart health effects of DHA include effects on triglycerides *(figure 2)*, high-density lipoprotein cholesterol, platelet function, endothelial and vascular function, blood pressure, cardiac excitability, measures of oxidative stress as well as pro- and anti-inflammatory cytokines [17, 56-62].

Increased consumption of dietary omega-3 PUFA increases the concentration of omega-3 PUFA in plasma phospholipids, which is associated with a protective effect on cardiovascular diseases and lower plasma homocystein levels [63].

Two large intervention studies have shown that fish or fish oil consumption have a significant protective effect against fatal cardiovascular disease. The DART-Study (Diet and Reinfarcion Trial) demonstrated that relatively low dosages of omega-3 fatty acids reduced the risk of a secondary coronary event and resulted in a 30% reduction in mortality attributable to a reduction in CVD death [60].

In the GISSI Prevenzione Trial, which included more than 11'000 subjects that had survived a heart attack, the risk of cardiovascular death was significantly lowered by 17% after 3 months of supplementation with DHA/EPA [64, 65].

A low (≤ 4%) red blood cell membrane content of EPA + DHA (omega-3 index) has recently been identified as an indicator for increased risk of death from coronary heart disease, whereas an omega-3 index ≥ 8% was associated with the greatest cardio-protection [66]. In a double-blind, placebo-controlled intervention study with 114 healthy vegetarian subjects, supplementation with DHAid™ during 8 weeks could significantly increase the omega-3 index [17].

Mori and colleagues concluded from a study with 59 mildly hyperlipidemic but otherwise healthy men that DHA supplementation is the principal omega-3 fatty acid in fish, since DHA but not EPA was found to lower blood pressure and heart rate in humans [67].

Current dietary intakes of DHA in North America and Europe are well below those recommended by the American Heart Association for the management of patients with coronary heart disease [68]. Dietary supplements and food containing DHAid™ therefore can be regarded as an ideal way to increase DHA levels and thus increase cardiovascular health.

## DHAid™ production process

Using naturally occurring microalgae, Lonza's innovative technology allows DHA oils to be produced with superior quality.

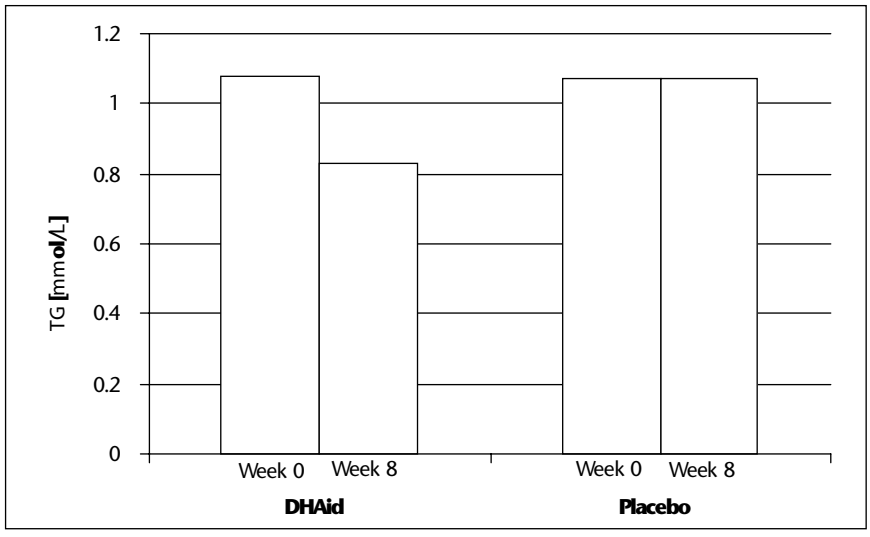


Figure 2. *Reduction in blood triglyceride levels following 8 weeks of DHAid™ supplementation as compared with the placebo group [61].*

During the unique production process in fermentation vessels, microalgae are grown in large volumes and accumulate significant quantities of DHA. Then, DHA is extracted from the dried microalgae and refined in processes that are very similar to those used in the production of conventional vegetable oils.

### *DHAid™ quality & safety*

DHAid™ quality at a glance:

– Accordance with HACCP and GMP standards for food products.
– Production ISO 9001 certified.
– Production based on renewable resources.
– Full traceability.
– Free of any materials of animal origin.
– Free of any genetically modified organisms (GMOs).
– Allergen free.
– Vegetarian source of DHA.
– High concentration of DHA.
– Clean taste.

DHAid™ safety at a glance:

– Self-affirmed GRAS.
– Solvent free production process.
– Approved according to Novel Food in EU, Australia and New Zealand.
– Free of any potential contaminants that are discussed for seafood [69].
– Multi-step fermentation process of DHAid™ uses a non-toxigenic and non-pathogenic marine protist, Ulkenia sp.
– Extensive human, animal and *in vitro* Tox studies confirm the safety of DHAid™ from microalgae [70-72].

REFERENCES


1. TRAUTWEIN EA. N-3 fatty acids – physiological and technical aspects for their use in foods. *Eur J Lipid Sci Technol* 2001 ; 103 : 45-55.
2. HORROCKS LA, YEO YK. Health benefits of docosahexaenoic acid (DHA). *Pharmacol Res* 1999 ; 40(3) : 211-25.
3. ALESSANDRI JM, POUMES-BALLIHAUT C, LANGELIER B, *ET AL.* Incorporation of docosahexaenoic acid into nerve membrane phospholipids : bridging the gap between animals and cultured cells. *Am J Clin Nutr* 2003 ; 78 : 702-10.
4. US DEPARTMENT OF AGRICULTURE. (2005). USDA Nutrient Database for Standard Reference, http://www.health.gov/dietaryguidelines/dga 2005.
5. DAVIS BC, KRIS-ETHERTON PM. Achieving optimal essential fatty acid status in vegetarians : current knowledge and practical implications. *Am J Clin Nutr* 2003 ; 78 (3 Suppl) : 640S-646S.
6. GERSTER H. Can adults adequately convert alpha-linolenic acid (18 : 3n-3) to eicosapentaenoic acid (20 : 5n-3) and docosahexaenoic acid (22 : 6n-3)? *Int J Vitam Nutr Res* 1998 ; 68(3) : 159-73.

7. LOGAN AC. Omega-3 fatty acids and major depression : A primer for the mental health professional. *Lipids Health Dis* 2004 ; 3(1) : 25-32.
8. GOYENS PL, SPILKER ME, ZOCK PL, KATAN MB, MENSINK RP. Conversion of ALA in humans is influenced by the absolute amounts of ALA and LA in the diet and not by their ratio. *Am J Clin Nutr* 2006 ; 84 (1) : 44-53.
9. WILLIAMS CM, BURDGE CG. Long-chain n-3 PUFA : plant v. marine sources. *Proc Nutr Soc* 2006 ; 65(1) : 42-50.
10. BURDGE GC. Metabolism of alpha-linolenic acid in humans. *Prostaglandins Leukot Essent Fatty Acids* 2006 ; 75 : 161-8.
11. FISCHER S, VISCHER A, PREAC-MURSIC V, WEBER PC. Dietary Docosahexaenoic acid is retroconverted in man to Eicosapentaenoic acid, which can be quickly transformed to prostaglandin I3. *Prostaglandins* 1987 ; 34 (3) : 367-75.
12. BURDGE CG, JONES AE. Eicosapentaenoic and docosapentaenoic acids are the principal products of alpha-linolenic acid metabolism in young men. *Br J Nutr* 2002 ; 88 (4) : 355-63.
13. SIMOPOULOS AP, LEAF A, SALEM JR. N. Workshop statement on the essentiality of and recommended dietary intakes for Omega-6 and Omega-3 fatty acids. *Prostaglandins Leukot Essent Fatty Acids* 2000 ; 63 (3) : 119-21.
14. KRIS-ETHERTON PM, HARRIS WS, APPEL LJ, AMERICAN HEART ASSOCIATION. Nutrition Committee. AHA Scientific Statement. Fish consumption, fish oil, omega-3 fatty acids and cardiovascular disease. *Circulation* 2002 ; 106 : 2747-57.
15. AGENCE FRANÇAISE DE SÉCURITÉ SANITAIRE DES ALIMENTS (AFSSA). http ://www.afssa.fr.
16. SCIENTIFIC ADVISORY COMMITTEE ON NUTRITION. (2004). Advice on fish consumption :benefits&risks. http ://www.sacn.gov.uk/reports.
17. GEPPERT J, KRAFT V, DEMMELMAIR H, KOLETZKO B. Docosahexaenoic acid supplementation in vegetarians effectively increases omega-3 index : A randomized trial. *Lipids* 2005 ; 40 (8) : 807-14.
18. DECSI T, KOLETZKO B. n-3 fatty acids and pregnancy outcomes. *Curr Opin Clin Nutr Metab Care* 2005 ; 8 (2) : 161-6.
19. AL MD, VAN HOUWELINGEN AC, HORNSTRA G. Long-chain polyunsaturated fatty acids, pregnancy, and pregnancy outcome. *Am J Clin Nutr* 2000 ; 71 (suppl) : 285S-291S.
20. AL MD, VAN HOUWELINGEN AC, HORNSTRA G. Relation between birth order and the maternal and neonatal docosahexaenoic acid status. *Eur J Clin Nutr* 1997 ; 51 (8) : 548-53.
21. KOLETZKO B, CETIN I, BRENNA JT, *ET AL.* Dietary fat intakes for pregnant and lactation women. *Br J Nutr* 2007 (10) : 1-5.
22. HELLAND IB, SMITH L, SAAREM K, SAUGSTAD OD, DREVON CA. Maternal Supplementation with very-long-chain n-3 fatty acids during pregnancy and lactation augments children's IQ at 4 years of age. *Pediatrics* 2003 ; 111 (1) : e39-e44.
23. FIDLER N, SAUERWALD T, POHL A, DEMMELMAIR H, KOLETZKO B. Docosahexaenoic acid transfer into human milk after dietary supplementation : a randomized clinical trial. *J Lipid Res* 2000 ; 41 : 1376-83.
24. JENSEN CL, MAUDE M, ANDERSON RE, HEIRD WC. Effect of docosahexaenoic acid supplementation of lactating women on the fatty acid composition of breast milk lipids and maternal and infant plasma phospholipids. *Am J Clin Nutr* 2000 ; 71 : 292S-299S.
25. CHERUKU SR, MONTGOMERY-DOWNS HE, FARKAS SL, THOMAN EB, LAMMI-KEEFE CJ. Higher maternal plasma docosahexaenoic acid during pregnancy is associated with more mature neonatal sleep-state patterning. *Am J Clin Nutr* 2002 (76) : 608-13.
26. ALESSANDRI JM, GUESNET P, VANCASSEL S, *ET AL.* Polyunsaturated fatty acids in the central nervous system : evolution of concepts and nutritional implications throughout life. *Reprod Nutr Dev* 2004 ; 44 (6) : 509-38.
27. CETIN I, KOLETZKO B. Long-chain omega-3 fatty acid supply in pregnancy and lactation. *Curr Opin Clin Nutr Metab Care* 2008 ; 11 (3) : 297-302.
28. SMUTS CM, HUANG M, MUNDY D, PLASSE T, MAJOR S, CARLSON SE. A randomized trial of docosahexaenoic acid supplementation during the third trimester of pregnancy. *Obstet Gynecol* 2003 ; 101 (3) : 469-79.
29. ALLEN KG, HARRIS MA. The role of n-3 fatty acids in gestation and parturition. *Exp Biol Med* 2001 ; 226 (6) : 498-506 ; (Maywood).
30. OTTO SJ, DE GROOT RH, HORNSTRA G. Increased risk of postpartum depressive symptoms is associated with slower normalization after pregnancy of the functional docosahexaenoic acid status. *Prostaglandins Leukot Essent Fatty Acids* 2003 ; 69 (4) : 237-43.
31. HIBBELN JR. Seafood consumption, the DHA content of mothers' milk and prevalence rates of postpartum depression : a cross-national, ecological analysis. *J Affect Disord* 2002 ; 69 (1-3) : 15-29.
32. HAAG M. Essential fatty acids and the brain. *Can J Psychartry* 2003 ; 48 (3) : 195-203.
33. HORWOOD LJ, FERGUSSON DM. Breastfeeding and later cognitive and academic outomes. *Pediatrics* 1998 ; 101 (1) : 1-7.
34. BIRCH EE, CASTAÑEDA YS, WHEATON DH, BIRCH DG, UAUY RD, HOFFMAN DR. Visual maturation of term infants fed long-chain polyunsaturated fatty acid-supplemented or control formula for 12 mo. *Am J Clin Nutr* 2005 ; 81 (4) : 871-9.
35. INNIS SM. Dietary (n-3) fatty acids and brain development. *J Nutr* 2007 ; 137 (4) : 855-9.
36. HAMAZAKI T, SAWAZAKI S, ITOMURA M, *ET AL.* The effect of docosahexaenoic acid on aggression in young adults. *J Clin Invest* 1996 ; 97 (4) : 1129-34.
37. YOUNG G, CONQUER J. Omega-3 fatty acids and neuropsychiatric disorders. *Reprod Nutr Dev* 2005 ; 45 (1) : 1-28.
38. PEET M, STOKES C. Omega-3 fatty acids in the treatment of psychiatric disorders. *Drugs* 2005 ; 65 (8) : 1051-9.
39. JOHNSON EJ, SCHAEFER EJ. Potential role of dietary n-3 fatty acids in the prevention of dementia and macular degeneration. *Am J Clin Nutr* 2006 ; 83 : 1494S-1498S.
40. SIMOPOULOS AP. Omega-3 fatty acids in inflammation and autoimmune diseases. *J Am Coll Nutr* 2002 ; 21 (6) : 495-505.
41. PARKER G, GIBSON NA, BROTCHIE H, HERUC G, REES AM, HADZI-PAVLOVIC D. Omega-3 fatty acids and mood disorders. *Am J Psychiatry* 2006 ; 163 : 969-78.
42. SINCLAIR AJ, BEGG D, MATHAI M, WEISINGER RS. Omega-3 fatty acids and the brain : Review of studies in depression. *Asia Pac J Clin Nutr* 2007 ; 16 (Suppl 1) : 391-7.
43. TIEMEIER H, VAN TUIJL HR, HOFMAN A, KILIAAN AJ, BRETELER MM. Plasma fatty acid composition and depression are associated in the elderly : the Rotterdam Study. *Am J Clin Nutr* 2003 ; 78 (1) : 40-6.
44. FREEMANTLE E, VANDAL M, TREMBLAY-MERCIER J*ET AL.* Omega-3 fatty acids, energy substrates, and brain function during aging. *Prostaglandins Leukot Essent Fatty Acids* 2006 ; 75 (3) : 213-20.
45. WHALLEY LJ, FOX HC, WAHLE KW, STARR JM, DEARY IJ. Cognitive aging, childhood intelligence, and the use of food supplements : possible involvement of n-3 fatty acids. *Am J Clin Nutr* 2004 ; 80 : 1650-7.
46. UAUY R, HOFFMAN DR, PEIRANO P, BIRCH DG, BIRCH EE. Essential fatty acids in visual and brain development. *Lipids* 2001 ; 36 (9) : 885-95.
47. JUDGE MP, HAREL O, LAMMI-KEEFE CJ. A docosahexaenoic acid-functional food during pregnancy benefits infant visual acuity at four but not at six months of age. *Lipids* 2007 (42) : 117-22.
48. SANGIOVANNI JP, CHEW EY, CLEMONS TE, *ET AL.* The relation of dietary lipid intake and age-related macular degeneration in a case-control study : AREDS Report No. 20. *Arch Ophthalmol* 2007 (125) : 671-9.
49. SANGIOVANNI JP, CHEW EY. The role of omega-3 long chain polyunsaturated fatty acids in health and disease of the retina. *Prog Retin Eye Res* 2005 (24) : 87-138.

50. O'KEEFE JR. JH, Harris WS. From Inuit to implementation : omega-3 fatty acids come of age. *Mayo Clin Proc* 2000 ; 75 (6) : 607-14.

51. DEWAILLY E, BLANCHET C, LEMIEUX S, *ET AL.* n-3 Fatty acids and cardiovascular disease risk factors among the Inuit of Nunavik. *Am J Clin Nutr* 2001 ; 74 : 464-73.

52. VON SCHACKY C. Omega-3 fatty acids and cardiovascular disease. *Curr Opin Clin Nutr Metab Care* 2004 ; 7 (2) : 131-6.

53. HARRIS WS, POSTON WC, HADDOCK CK. Tissue n-3 and n-6 fatty acids and risk for coronary heart disease events. *Atherosclerosis* 2007 ; 193 (1) : 1-10.

54. SANDERSON P, FINNEGAN YE, WILLIAMS CM, *ET AL.* UK Food Standards Agency. Alpha-linolenic acid workshop report. *Br J Nutr* 2002 ; 88 (5) : 573-9.

55. WANG C, HARRIS WS, CHUNG M, *ET AL.* n-3 fatty acids from fish or fish oil supplements, but not alpha-linolenic acid, benefit cardiovascular disease outcomes in primary- and secondary prevention studies : a systematic review. *Am J Clin Nutr* 2006 ; 84 (1) : 5-17.

56. MORI TA, WATTS GF, BURKE V, HILME E, PUDDEY IB, BEILIN LJ. Differential effects of eicosapentaenoic acid and docosahexaenoic acid on vascular reactivity of the forearm microcirculation in hyperlipidemic, overweight men. *Circulation* 2000 ; 102 : 1264-9.

57. BUCHER HC, HENGSTLER P, SCHINDLER C, MEIER G. n-3 polyunsaturated fatty acids in coronary heart disease : a meta-analysis of randomized controlled trials. *Am J Med* 2002 ; 112 (4) : 298-304.

58. HU FB, BRONNER L, WILLET WC, *ET AL.* Fish and omega-3 fatty acid intake and risk of coronary heart disease in women. *JAMA* 2002 ; 287 (14) : 1815-21.

59. ENGLER MM, ENGLER MB. Omega-3 fatty acids : role in cardiovascular health and disease. *J Cardiovasc Nurs* 2006 ; 21 (1) : 17-24.

60. BURR ML, GILBERT JF, HOLLIDAY RM, *ET AL.* Effects o changes in fat, fish and fibre intakes on the death and myocardial reinfarction : Diet and reinfarction trial (DART). *Lancet* 1989 ; 2 (8666) : 757-61.

61. GEPPERT J, KRAFT V, DEMMELMAIR H, KOLETZKO B. Microalgal docosahexaenoic acid decreases plasma triacylglycerol in normolipidaemic vegetarians : a randomised trial. *Br J Nutr* 2006 ; 95 (4) : 779-86.

62. KELLEY DS, SIEGEL D, VEMURI M, CHUNG GH, MACKEY BE. Docosahexaenoic acid supplementation decreases remnant-like particle-cholesterol and increases the (n-3) index in hypertriglyceridemic men. *J Nutr* 2008 ; 138 (1) : 30-5.

63. LI D, MANN NJ, SINCLAIR AJ. A significant inverse relationship between concentrations of plasma homocysteine and phospholipid docosahexaenoic acid in healthy male subjects. *Lipids* 2006 ; 41 (1) : 85-9.

64. MARCHIOLI R, GISSI PREVENZIONE INVESTIGATORS. Early protection against sudden death by n-3 polyunsaturated fatty acids after myocardial infarction : time-course analysis of the results of the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico (GISSI)-Prevenzione. *Circulation* 2002 ; 105 (16) : 1897-903.

65. MARCHIOLI R, GISSI PREVENZIONE INVESTIGATORS. Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction : results of the GISSI-Prevenzione trial. Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico. *Lancet* 1999 ; 354 (9177) : 447-55.

66. HARRIS WS, VON SCHACKY C. The omega-3 index : a new risk factor for death from coronary heart disease? *Prev Med* 2004 ; 39 : 212-20.

67. MORI TA, BAO DQ, BURKE V, PUDDEY IB, BEILIN LJ. Docosahexaenoic acid but not eicosapentaenoic acid lowers ambulatory blood pressure and heart rate in humans. *Hypertension* 1999 ; 34 : 253-60.

68. HOLUB DJ, HOLUB BJ. Omega-3 fatty acids from fish oils and cardiovascular disease. *Mol Cell Biochem* 2004 ; 263 (1-2) : 217-25.

69. HITES RA, FORAN JA, CARPENTER DO, HAMILTON MC, KNUTH BA, SCHWAGER SJ. Global assessment of organic contaminants in farmed salmon. *Science* 2004 ; 303 (5655) : 226-9.

70. KROES R, SCHAEFER EJ, SQUIRE RA, WILLIAMS GM. A review of the safety of DHA45-oil. *Food Chem Toxicol* 2003 (41) : 1433-6.

71. BLUM R, KIY T, WAALKENS-BERENDSEN I, WONG AW, ROBERTS A. One-generation reproductive toxicity study of DHA-rich oil in rats. *Regul Toxicol Pharmacol* 2007 ; 49 (3) : 260-70.

72. BLUM R, KIY T, TANAKA S, WONG AW, ROBERTS A. Genotoxicity and subchronic toxicity studies of DHA-rich oil in rats. *Regul Toxicol Pharmacol* 2007 ; 49 (3) : 271-84.