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# Prostaglandins, Leukotrienes and Essential Fatty Acids

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## Docosahexaenoic acid (DHA) and cardiovascular disease risk factors

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### A B S T R A C T

Numerous epidemiological and controlled interventional trials have supported the health benefits of long-chain omega-3 fatty acids in the form of docosahexaenoic acid (DHA, 22:6n-3) plus eicosapentaenoic acid (EPA, 20:5n-3) from fish and fish oils as well as from algal sources. The beneficial effects on cardiovascular disease and related mortality including various risk factors: HDL-cholesterol ratio) have been observed in the absence of any concomitant blood cholesterol lowering. With appropriate dosages, consistent reductions in both fasting and postprandial triglyceride levels and moderate increases in fasting HDL-cholesterol levels have been observed with algal DHA in the majority of trials. These results are similar to findings for fish oils containing DHA and EPA. Related to greater fish intake, higher levels of DHA in circulating blood biomarkers (such as serum phospholipid) have been associated with reduced risks for the progression of coronary atherosclerosis and lowered risk from sudden cardiac death. Controlled clinical trials have also indicated the potential for algal DHA supplementation to have moderate beneficial effects on other cardiovascular disease risk factors including blood pressures and resting heart rates. Recommended intakes of DHA+EPA from numerous international groups for the prevention and management of cardiovascular disease have been forthcoming, although most have not offered specific recommendations for the optimal individual intake of DHA and EPA.

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### 1. Introduction

Numerous population (epidemiological) and controlled interventional trials have supported highly beneficial effects of docosahexaenoic acid (DHA, 22:6n-3) plus eicosapentaenoic acid (EPA, 20:5n-3) from fish and fish oils in decreasing cardiovascular disease (CVD) and cardiac mortality and favorably modifying numerous CVD risk factors independent of blood cholesterol lowering [1–3].

Cardiovascular disease is responsible for approximately 50% of all mortality in North America. By the age of 60, approximately half of the population has some form of clinical or pre-clinical cardiovascular disorder including various stages of atherosclerosis. Death rates from coronary heart disease in North America are markedly higher than corresponding rates in Japan despite only moderately higher levels of blood cholesterol in the North American population [4]. Thus, much of the dramatic difference in CVD rates in North Americans relative to the Japanese are due to blood cholesterol-independent risk factors. There is mounting evidence that DHA plus EPA from fish/fish oil can significantly reduce CVD and mortality independently of lowering LDL-

cholesterol levels [3,4]. Many of the CVD risk parameters which are favorably affected by DHA and EPA are not routinely measured in the North American health care system. Interestingly, the intake of fish and DHA and EPA per day in Japan is much higher than that for North Americans [5].

The published epidemiological studies of Daviglus et al. [6] indicated that Americans who consumed at least 34 g or more of fish per day on average had a 40–50% lower risk of myocardial infarction and much lower risk of coronary heart disease (CHD), CVD, and all-cause mortality. This intake (34 g fish/day) is the equivalent of approximately two fatty fish servings per week and can be expected to deliver approximately 250–500 mg of DHA and EPA (combined) per day on average. It is noted that the predominant source of DHA plus EPA in the Western-type diet is fish/seafood [7]. Direct assessment has indicated DHA and EPA intakes to average 82 and 35 mg per day, respectively, with a DHA:EPA ratio of 2.3/1 [8]. This reflects an average fish intake of almost one serving per 7–10 days.

The 10-year MRFIT study [9] correlated increased intakes of DHA and EPA (omega-3 fatty acids) as consumed by various sectors of the population with the relative risk of heart-related mortality. Increased consumptions of DHA and EPA up to 664 mg/day were associated with an approximate 40% reduction in CVD, CHD, and a significant reduction in all-cause mortality. An intake of 664 mg DHA and EPA per day would require approximately 3–5

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fatty fish servings per week depending on the type/source. Interestingly, current North American intakes of DHA and EPA (combined) represent approximately 130–150 mg/day or one-fourth of the 664 mg/day [4,8].

An overall review and meta-analysis of the various studies correlating fish consumption with CHD mortality has been published by He et al. [10]. Inclusion of the various cohort studies represents an evaluation of over 220,000 subjects followed over an average period of 11.8 years. Increased fish consumption per week was associated with a significant progressive reduction in CHD mortality. The highest apparent level of protection was observed at fish intakes of five or more servings per week which resulted in an estimated 35–45% reduction in the risk of CHD mortality. It is noteworthy that such a level of fish consumption is approximately equivalent to 650 mg/day or more of DHA and EPA (combined). This latter intake (650 mg/day) is essentially the target recommended by the 1999 NIH Workshop in Bethesda as supported by ISSFAL (International Society for the Study of Fatty Acids and Lipids) for optimal health and cardio care in normal healthy subjects [11].

Recent studies have focused on the potential for fish oil supplements enriched with DHA and EPA to modify 'hard' clinical endpoints such as cardiac mortality in patients and at-risk subjects. The 1999 GISSI-Prevenzione trial results from Italy [12] indicated a highly beneficial effect of DHA and EPA supplementation in patients who had experienced a myocardial infarction relative to controls. The 11,324 patients who had experienced a myocardial infarction were assigned to supplemental interventions following the introduction of a Mediterranean-type diet (which included moderate fish consumption) as well as aggressive treatment with various pharmaceutical agents for cardiovascular care. Approximately half the patients received omega-3 supplementation providing 850–882 mg/day of DHA plus EPA (combined) or placebo. Over the follow-up interval of 3.5 years, the individuals who were given EPA and DHA were found to exhibit an approximate 30% reduction in overall cardiovascular deaths and a reduction in sudden cardiac death of about 45%. A review of recent omega-3 trials indicated reductions of CHD mortality of 36% and total mortality of 17% with evidence for greater benefits in secondary prevention than primary prevention in populations consuming low amounts of omega-3 fatty acids [13].

## 2. Blood triglyceride and lipoprotein risk factors

Much of the traditional focus on the modification of circulating lipids/lipoprotein factors for the primary prevention and secondary management of CVD has emphasized a reduction in total cholesterol and LDL-cholesterol levels because of the established relationship between these latter two risk parameters and serious CVD end-points. More recently, evidence suggests that fasting plasma (serum) triglycerides are an important and independent risk factor for CHD [14]. For example, a progressive increase in the incidence of major coronary events over an 8-year period in men was associated with progressively increased levels of fasting triglyceride levels (from  $<1.2$  up to  $>1.9$  mmol/L) over 8 years of follow-up [15]. Recently, a prospective study based on a population with prevalent metabolic syndrome (as seen with increasing occurrence in our societies today) supported plasma triglyceride as an independent predictor of CVD with even moderate elevations (1.13–1.49 mmol/L) increasing the risk for fatal plus non-fatal CVD [16].

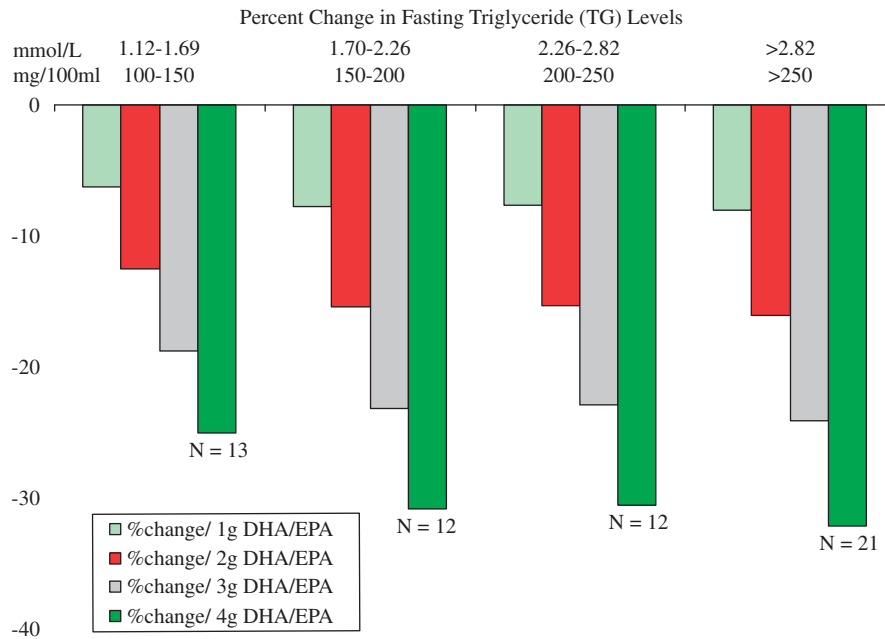
The cardioprotective effect of higher circulating levels of HDL-cholesterol has long been recognized. More recently, the ratio of fasting triglyceride:HDL-cholesterol levels is thought to be a potentially stronger predictor of myocardial infarction than even

the total cholesterol:HDL-cholesterol or LDL-cholesterol:HDL-cholesterol ratios [17,18]. A pattern of high circulating triglyceride levels together with low HDL-cholesterol concentrations has been related to a more aggressive transition from atheroma to atherothrombosis. Furthermore, the ratio of triglyceride:HDL-cholesterol has been found to be closely correlated with the small, dense LDL particles which are regarded as highly atherogenic [19]. Higher ratios of triglyceride:HDL-cholesterol are related inversely to LDL size and positively with particle concentration. Thus, lowering circulating triglyceride levels as well as the triglyceride:HDL-cholesterol ratio is becoming of increased importance in reducing the risk of CVD and hard end-points (non-fatal and fatal myocardial infarctions). Recognizing that lowering of fasting triglyceride levels often leads to a moderate reduction (rise) in the calculated LDL-cholesterol concentrations (because of the indirect use of the Friedwald equation for indirectly assessing LDL-cholesterol levels via calculations) and that cholesterol associated with lower density lipoprotein particles in addition to LDL-cholesterol represents a risk factor for CHD. The non-HDL-cholesterol levels are of considerable interest in risk assessment and appear to be at least as useful as LDL-cholesterol measurements for risk assessment and, for some populations and sectors, may be of greater predictability [20].

The ability of supplementation with fish oil and derived concentrates containing DHA and EPA omega-3 fatty acids to lower fasting triglyceride levels has long been recognized, and appropriate review articles on this topic are available [3,21]. Fig. 1 gives the average expected percentage reduction in fasting triglyceride levels as estimated from a review of various published trials by Harris [21]. The expected reductions for each gram of DHA and EPA (combined) as consumed daily over a period of at least a few weeks (in amounts ranging from 1 g up to 4 g DHA and EPA daily) are given in relation to the fasting baseline serum (plasma) triglyceride levels in the target populations. It is apparent that the reductions in fasting triglyceride levels range from ~20% to 35% for dosages of 3–4 g of DHA and EPA (combined) per day.

## 3. Effect of algal DHA supplementation on circulating plasma lipids and lipoproteins

A number of studies have been performed which have evaluated the effect of using algal DHA devoid of EPA on circulating plasma lipids and lipoproteins in controlled human trials. While the vast majority of the literature (as outlined above) has employed fish-oil-based mixtures of EPA+DHA in the numerous lipid-lowering studies, a few studies have directly compared DHA with EPA concentrates or DHA from an algal source with DHA+EPA from fish oil. Highly purified DHA appears to have similar triglyceride-lowering effects when compared with EPA [22]. Furthermore, modest dosing with algal DHA (1000 mg/day) over 8 weeks was found to provide a significant decrease in fasting triglyceride levels (an average of 21.8%) as compared to a very similar lowering (18.3%) in the DHA+EPA group providing similar amounts of long-chain omega-3 fatty acids [23]. The latter authors concluded that there does not appear to be significant differences in triglyceride-lowering between DHA only and DHA+EPA combination products when the dosing is performed based on the amount of DHA. It is noted that a portion of dietary DHA (approximately 10% based on the net rise in circulating EPA levels in relationship to circulating DHA plus EPA elevations when algal DHA is fed) is converted into EPA within human subjects [24,25]. Thus, the vast majority of the triglyceride-lowering observed with algal DHA is most likely due to DHA itself with a very small contribution from any resulting

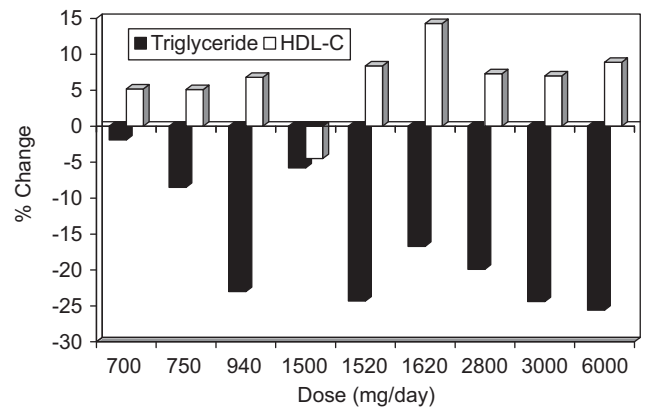


**Fig. 1.** Average expected % change in fasting triglyceride (TG) levels based on differing baseline triglyceride status in various cross-over and parallel studies (modified with permission from [www.dhaomega3.org](http://www.dhaomega3.org)).

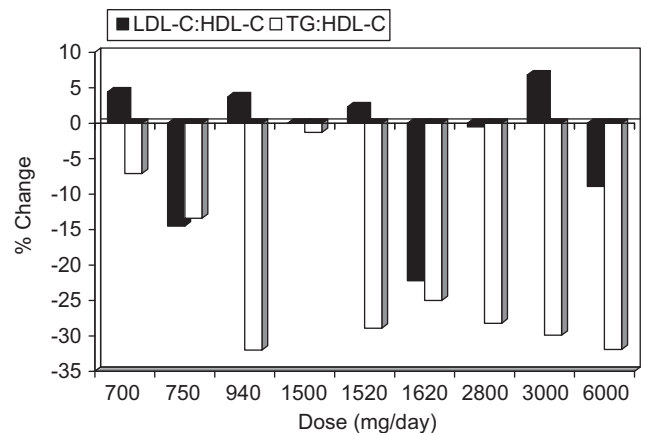
generation of EPA via metabolism. It is also noted that bioavailability studies by Arterburn et al. [26] have found similar bioequivalencies for DHA from different algal oils in capsules as compared to DHA-fortified food based on the resulting elevations in DHA levels in plasma phospholipid and erythrocyte membranes following 28 days of supplementation.

With the availability of the algal DHA ('veggie DHA') more than 12 years ago, we conducted and published a clinical trial [24] in vegetarian subjects who had normal fasting triglyceride levels (mean values of 0.81 mmol/L). An encapsulated placebo or supplemental DHA providing 1.62 g/day for a 6-week period was evaluated with blood sampling and corresponding lipid/lipoprotein measures at day 0, day 21, and day 42. The key findings from this trial included no effect of DHA on total LDL-cholesterol levels, a significant elevation in HDL-cholesterol levels at week 3 (by 13% overall) and week 6 (by 17% overall) along with a significant reduction in the LDL-cholesterol:HDL-cholesterol ratio at week 3 (by 11%) and at week 6 (by 22%). Furthermore, a significant reduction in fasting triglyceride levels at week 3 (by 22%) and at week 7 (by 17%) was observed with algal DHA supplementation. While not reported in the original publication in 1996, calculations from the latter group indicated an overall 8.2% reduction in the levels of non-HDL cholesterol and a substantial reduction (by 25%) in the mean fasting triglyceride:HDL-cholesterol ratio upon termination of the trial at day 42.

Figs. 2 and 3 provide the calculated % change in circulating lipid parameters based on mean values at entry and following supplementation with varying doses of DHA for varying durations upon review of 8 published studies using DHA-containing algal oil [24,27–32] and the publication by Conquer and Holub [33] where two different dosages of DHA were given (750 mg or 1500 mg daily) for a 42 day period. A summary of the percent changes in fasting triglyceride and HDL-cholesterol levels from these studies are given in Fig. 2. Depending upon the dose of DHA employed/day and the baseline lipids/lipoprotein values for the test subjects, the overall reductions in fasting triglyceride and the triglyceride:HDL-cholesterol ratios for the studies summarized ranged from 1.9% to 25.6% and 1.3% to 32.0%, respectively



**Fig. 2.** Effects of supplementation with algal DHA on fasting triglyceride (TG) and HDL-cholesterol (HDL-C) levels from clinical trials.



**Fig. 3.** Effects of supplementation with algal DHA on LDL-cholesterol (LDL-C):HDL-cholesterol (HDL-C) and TG:HDL-C ratios from clinical trials.

(Figs. 2 and 3). The mean fasting triglyceride and the triglyceride:HDL-cholesterol levels were lower following DHA supplementation (relative to initial levels) in 9/9 trials (Figs. 2 and 3). Higher overall HDL-cholesterol levels following supplementation were found in 8/9 trials and in 6/9 trials in the case of LDL-cholesterol. The LDL-cholesterol:HDL-cholesterol ratio exhibited inconsistent shifts across the trials (Fig. 3). The mean dose (all studies) was 2092 mg DHA/day and the average changes (following supplementation relative to entry) in the plasma lipids/lipoprotein levels were as follows: triglyceride (−16.7%), HDL-cholesterol (+6.5%), LDL-cholesterol (+4.1%), LDL-cholesterol:HDL-cholesterol ratio (−3.2%), and the mean triglyceride:HDL-cholesterol ratio (−22.0%). If normalized to a per 1000 mg dose basis, the mean corresponding changes upon DHA supplementation can be calculated to be −8.0%, +3.1%, +2.0%, +1.5%, and −10.5%, respectively.

Prospective studies have indicated that surges in non-fasting or postprandial triglyceride levels following a meal containing simple carbohydrates and fat represents a strong and independent predictor of atherosclerosis and cardiovascular disease [34]. A number of studies using EPA+DHA in the form of supplemental fish oil have shown their capacity to significantly reduce both fasting and postprandial triglyceride elevations via inhibition of hepatic triglyceride synthesis and/or enhancement of triglyceride clearance from the circulation in the form of chylomicron particles and very-low-density lipoproteins [2,21]. The early work of Agren et al. [35] indicated that a DHA-oil providing 1.68 g/day of DHA over a 14 week period resulted in a significant reduction in both fasting and postprandial triglyceride levels. Very recently, Kelley and colleagues [32] reported upon daily supplementation with 3000 mg DHA/day from an algal source over a 45 day period which provided a 24% reduction in fasting triglyceride concentrations from baseline, and, in addition, the effect of DHA on postprandial triglyceride concentrations was found to mimic its effect on the fasting levels. These authors also reported marked decreases in the concentrations of fasting large very-low-density lipoproteins, intermediate-density lipoproteins, and the mean diameter of the former particles with similar changes being observed for area under the curve for the postprandial measurements from 0 to 6 h. The authors concluded that DHA supplementation may further improve cardiovascular health by lowering the small, dense LDL particles in addition to the triglyceride-lowering in both the fasted and postprandial state.

#### 4. Effect of algal DHA supplementation on other CVD risk factors

In addition to hypertension being long-recognized as an important risk factor for CVD, stroke, and related mortality [36], the resting heart rate is well recognized as an independent risk factor for CVD and has been suggested for inclusion in major risk factor assessment [37]. There is considerable evidence that elevated heart rates (particularly above 80 beats/min) are directly associated with the risk of developing both hypertension and atherosclerosis. The early studies by Mori et al. [38] using 4 g of purified DHA per day over a 6-week period reported a significant reduction in both daytime (awake) ambulatory blood pressures as well as 24-h measurements. Relative to placebo, 24-h blood pressure fell by 5.8 and 3.3 mm Hg overall for systolic and diastolic pressures, respectively, and daytime blood pressures fell 3.5 and 2.0 mm Hg, respectively. This group also reported that DHA reduced 24-h heart rates by a mean of 3.5 beats per minute and daytime rates by 3.7 and night time rates by 2.8 beats per minute. A clinical trial from our laboratory [29] in post-menopausal women receiving algal DHA or placebo in a randomized, double-blind, placebo-controlled crossover design with a DHA supplemental intake of 2.8 g/day over 6 weeks gave rise to a highly

significant decrease (by 7%) in the resting heart rate in addition to significant triglyceride-lowering, HDL-cholesterol elevation, and marked reductions in the TG:HDL-cholesterol ratio. The effects on resting heart rate are possibly related to the antiarrhythmic effects of n-3 fatty acids [39–41] which are of considerable interest since reductions in heart rate have been associated with decreased all-cause mortality and sudden cardiac death [37]. Very recently, Theobald and colleagues [42] reported that even a moderate increase in the daily intake of DHA (by providing 750 mg DHA/day from a purified algal source) can provide a modest but statistically significant reduction in diastolic blood pressure (by 3.3 mm Hg overall). In the later study, the tendency for a reduction in resting heart rate (by 2.1 beats per minute) after DHA supplementation did not reach statistical significance at the  $P = 0.05$  level. Very recently, algal-derived DHA omega-3 supplementation at 3 g/day for a 90-day period in men with elevated triglyceride levels was found to significantly decrease the number of circulating neutrophils by 11–12% at days 45 and 91. DHA also reduced the concentrations of C-reactive protein (CRP) by 15%, interleukin-6 by 23%, and granulocyte monocyte-colony stimulating factor by 21% at 91 days [43].

#### 5. Effect of DHA on blood levels of DHA and CVD

It is becoming apparent that increased levels of the long-chain omega-3 fatty acids (particularly DHA+EPA) appear to be inversely related to the risk of the progression of coronary atherosclerosis, sudden cardiac death, and coronary heart disease [1–3]. The work of Albert et al. [44] indicated that subjects dying from sudden cardiac death exhibited significantly lower levels ( $P < 0.005$ ) of DHA in total blood lipid as compared to the control group. Using DHA content in serum phospholipid as the biomarker, higher levels of DHA have also been associated with a lower relative risk for CHD [45]. Recently, higher plasma DHA levels in serum phospholipid have been associated with reduced progression of coronary atherosclerosis in women with coronary artery disease [46]. Supplementation studies using different doses of DHA have shown dramatic increases in DHA levels on both circulating plasma phospholipid and non-esterified fatty acid fractions (DHA as free fatty acid) which approach near-maximal levels following 6 weeks of supplementation [33]. Interestingly, 6 weeks of supplementation with placebo, 750 mg of algal DHA, or 1500 mg of algal DHA per day resulted in concentrations of DHA as non-esterified fatty acid with mean values of 1.5, 7.5, and 12.7  $\mu\text{M}$ . These concentrations of circulating DHA as free fatty acid are of interest since antiarrhythmic cardiac effects of DHA have been reported in isolated myocytes at concentrations of only 5–10  $\mu\text{M}$  [39].

#### 6. Recommended intakes

The 1999 Workshop on the Essentiality of and Recommended Intakes for Omega-6 and Omega-3 Fatty Acids held in Bethesda, Maryland [11] recommended an overall intake for the maintenance of optimal health including cardiovascular care of 650 mg DHA+EPA (combined) for adults with the provision that at least one-third of the mixture should be represented by each of the components (i.e., at least 220 mg of DHA and also of EPA). The rationale for such a recommendation which ensured the consumption of both DHA and EPA was based on the large number of population studies indicating the health benefits of consuming fish; all fish contain a mixture of both long-chain omega-3 fatty acids. The predominant source of DHA+EPA in the Western type diet is fish/seafood [7] and the overall dietary ratio of DHA:EPA is approximately 2.3:1 [8]. Almost all subsequent recommendations

directed toward the optimal intakes of long-chain omega-3 fatty acids for cardiovascular health (prevention and/or management) have not been structured so as to ensure an ample consumption of both DHA+EPA. The American Heart Association provided dietary guidelines which advised the consumption of two fatty fish meals per week for the prevention of CVD [47] without a specific reference to fixed intakes of DHA and/or EPA. It has been estimated that approximately 8 ounces (227 g) of cooked fatty fish per week (two servings of four ounces each) would provide the equivalency of approximately 500 mg/day of DHA+EPA [47,48]. For patients with CHD, the American Heart Association has encouraged patients to increase their consumption of DHA+EPA (total) to approximately 900–1000 mg/day which is very close to the daily supplemental dose used in the GISSI-Prevenzione Study [12]. For blood triglyceride lowering, recommended intakes for DHA+EPA of 2–4 g/day have been suggested by the American Heart Association [47]. Recently, the American Dietetic Association in conjunction with the Dietitians of Canada published a position paper [48] wherein two servings of fatty fish per week are advised. Furthermore, 500 mg/day of DHA+EPA was recommended. Kris-Etherton and Hill [49] have published a concise outline on both North American and global recommendations for the intake of long-chain omega-3 fatty acids and fish for cardiovascular care. Very recently, support for a nutritionally-achievable DRI (Dietary Reference Intakes) has been published [50] with a target daily intake of DHA plus EPA between 250 and 500 mg/day based on the strength of the current literature where a clear, inverse relation between DHA plus EPA intakes and the risk of fatal (and possibly nonfatal) coronary heart disease appears to exist.

## 7. Conclusion

Higher intakes of DHA from fish/seafood and the corresponding elevations in blood levels of DHA have been associated with reductions in the progression of coronary atherosclerosis and the risk of CHD and sudden cardiac death. Supplementation with DHA from an algal source at appropriate dosages has been found to have beneficial effects on risk factors for CVD, including reductions in blood triglyceride levels (as found also for fish oils containing DHA and EPA) and in the triglyceride:HDL-cholesterol ratio, as well as shifts towards moderate elevations in HDL-cholesterol levels. Favorable effects on additional risk factors including blood pressure and resting heart rate have been reported in various studies. The benefits recognized for DHA on risk factors for CVD are consistently observed in almost all studies independent of blood cholesterol lowering. While recommendations from various groups for target intakes for DHA+EPA for the prevention and management of CVD have been forthcoming, specific recommendations on DHA and EPA intakes independently have generally been lacking to date for valid reasons unlike specific recommendations from international groups for DHA intakes in pregnancy, lactation, and infancy.

Finally, it is noted that the average composition of fish/seafood as consumed in most countries generally has greater amounts of DHA as compared to EPA. To date, there have been no long-term controlled supplementation trials which have directly compared only EPA versus only DHA at identical intakes versus mixtures thereof wherein 'hard' endpoints such as cardiac-related mortality have been monitored.

## References

[1] D.J. Holub, B.J. Holub, Omega-3 fatty acids from fish oils and cardiovascular disease, *Mol. Cell Biochem.* 263 (1–2) (2004) 217–225.

- [2] W.S. Harris, M. Miller, A.P. Tighe, M.H. Davidson, E.J. Schaefer, Omega-3 fatty acids and coronary heart disease risk: clinical and mechanistic perspectives, *Atherosclerosis* 197 (1) (2008) 12–24.
- [3] E.M. Balk, A.H. Lichtenstein, M. Chung, B. Kupelnick, P. Chew, J. Lau, Effect of omega-3 fatty acids on serum markers of cardiovascular disease risk: a systematic review, *Atherosclerosis* 189 (1) (2006) 19–30.
- [4] B.J. Holub, Clinical nutrition 4: omega-3 fatty acids in cardiovascular care, *CMAJ* 166 (5) (2002) 608–615.
- [5] H. Iso, S. Sato, A.R. Folsom, et al., Serum fatty acids and fish intake in rural Japanese, urban Japanese, Japanese American and Caucasian American men, *Int. J. Epidemiol.* 18 (2) (1989) 374–381.
- [6] M.L. Daviglus, J. Stamler, A.J. Orenca, et al., Fish consumption and the 30-year risk of fatal myocardial infarction, *N. Eng. J. Med.* 336 (1997) 1046–1053.
- [7] N.R. Raper, F.J. Cronin, J. Exler, Omega-3 fatty acid content of the US food supply, *J. Am. Coll. Nutr.* 11 (3) (1992) 304–308.
- [8] J. Denomme, K. Stark, B.J. Holub, Directly quantitated dietary (n-3) fatty acid intakes of pregnant Canadian women are lower than current dietary recommendations, *J. Nutr.* 135 (2005) 206–211.
- [9] T.A. Dolecek, Epidemiological evidence of relationships between dietary polyunsaturated fatty acids and mortality in the Multiple Risk Factor Intervention Trial, *Proc. Soc. Exp. Biol. Med.* (1992) 177–182.
- [10] K. He, Y. Song, M.L. Daviglus, Liu, et al., Accumulated evidence on fish consumption and coronary heart disease mortality: a meta-analysis of cohort studies, *Circulation* 109 (22) (2004) 2705–2711.
- [11] A.P. Simopoulos, A. Leaf, N. Salem, Workshop on the essentiality of and recommended intakes for omega-6 and omega-3 fatty acids, *J. Am. Coll. Nutr.* 18 (5) (1999) 487–489.
- [12] R. Marchioli, Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-Prevenzione trial, *Lancet* 354 (1999) 447–455.
- [13] T.A. Jacobson, Beyond lipids: the role of omega-3 fatty acids from fish oil in the prevention of coronary heart disease, *Curr. Atheroscler. Rep.* 9 (2) (2007) 145–153.
- [14] A.M. Gotto, Triglyceride: the forgotten risk factor, *Circulation* 97 (11) (1998) 1027–1028.
- [15] P. Cullen, Evidence the triglycerides are an independent coronary heart disease risk factor, *Am. J. Cardiol.* 86 (2000) 943–949.
- [16] A. Onat, I. Sari, M. Yazici, G. Can, G. Hergenc, G.S. Avci, Plasma triglycerides, an independent predictor of cardiovascular disease in men: a prospective study based on a population with prevalent metabolic syndrome, *Int. J. Cardiol.* 108 (1) (2006) 89–95.
- [17] J.M. Gaziano, C.M. Hennekens, C.J. O'Donnell, J.L. Breslow, J.E. Buring, Fasting triglycerides, high-density lipoprotein, and risk of myocardial infarction, *Circulation* 96 (8) (1997) 2520–2525.
- [18] P.O. Szapary, D.J. Rader, The triglyceride-high-density lipoprotein axis: an important target of therapy?, *Am. Heart J.* 148 (2) (2004) 211–221.
- [19] C. Maruyama, K. Imamura, T. Teramoto, Assessment of LDL particle size by triglyceride/HDL-cholesterol ratio in non-diabetic, healthy subjects without prominent hyperlipidemia, *J. Atheroscler. Thromb.* 10 (3) (2003) 186–191.
- [20] W.R. Farwell, H.D. Sesso, J.E. Buring, J.M. Gaziano, Non-high density lipoprotein cholesterol versus low-density lipoprotein cholesterol as a risk factor for a first nonfatal myocardial infarction, *Am. J. Cardiol.* 96 (8) (2005) 1129–1134.
- [21] W.S. Harris, n-3 Fatty acids and serum lipoproteins: human studies, *Am. J. Clin. Nutr.* 65 (s) (1997) 1645s–1654s.
- [22] S. Grimsgaard, K.H. Bonaa, J.B. Hansen, A. Nordoy, Highly purified eicosapentaenoic acid and docosapentaenoic acid in humans have similar triacylglycerol-lowering effects but divergent effects on serum fatty acids, *Am. J. Clin. Nutr.* 66 (3) (1997) 649–659.
- [23] L.J. Schwellenbach, K.L. Olson, K.J. McConnell, R.S. Stolpart, J.D. Nash, J.A. Merenich, The triglyceride-lowering effects of a modest dose of docosahexaenoic acid alone versus in combination with low dose eicosapentaenoic acid in patients with coronary artery disease and elevated triglycerides, *J. Am. Coll. Nutr.* 25 (6) (2006) 480–485.
- [24] J.A. Conquer, B.J. Holub, Supplementation with and algae source of docosahexaenoic acid increases (n-3) fatty acid status and alters selected risk factors for heart disease in vegetarian subjects, *J. Nutr.* 126 (12) (1996) 3032–3039.
- [25] J.A. Conquer, B.J. Holub, Dietary docosahexaenoic acid as a source of eicosapentaenoic acid in vegetarians and omnivores, *Lipids* 32 (3) (1997) 341–345.
- [26] L.M. Arterburn, H.A. Oken, J.P. Hoffman, et al., Bioequivalence of docosahexaenoic acid from different algal oils in capsules and in DHA fortified food, *Lipids* 42 (11) (2007) 1011–1024.
- [27] G.J. Nelson, P.S. Schmidt, G.L. Bartolini, D.S. Kelley, D.J. Kyle, The effect of dietary docosahexaenoic acid on platelet function, platelet fatty acid composition, and blood coagulation in humans, *Lipids* 32 (11) (1997) 1129–1137.
- [28] H.E. Theobald, P.J. Chowienczyk, R. Whittall, S.E. Humphries, T.A. Sanders, LDL cholesterol-raising effect of low-dose docosahexaenoic acid in middle-aged men and women, *Am. J. Clin. Nutr.* 79 (4) (2004) 558–563.
- [29] K.D. Stark, B.J. Holub, Differential eicosapentaenoic acid elevations and altered cardiovascular disease risk factor responses after supplementation with docosahexaenoic acid in postmenopausal women receiving and not receiving hormone replacement therapy, *Am. J. Clin. Nutr.* 78 (5) (2004) 765–773.

- [30] K.C. Maki, M.E. Van Elswyk, D. McCarthy, et al., Lipid responses to a dietary docosahexaenoic acid supplement in men and women with below average levels of high density lipoprotein cholesterol, *J. Am. Coll. Nutr.* 24 (3) (2005) 189–199.
- [31] J. Geppert, V. Kraft, H. Demmelmaier, B. Koletzko, Microalgal docosahexaenoic acid decreases plasma triacylglycerol in normolipidaemic vegetarians: a randomised trial, *Br. J. Nutr.* 95 (4) (2006) 779–786.
- [32] D.S. Kelley, D. Siegel, M. Vemuri, B.E. Mackey, Docosahexaenoic acid supplementation improves fasting and postprandial lipid profiles in hypertriglyceridemic men, *Am. J. Clin. Nutr.* 86 (2) (2007) 324–333.
- [33] J.A. Conquer, B.J. Holub, Effect of supplementation with different doses of DHA on the levels of circulating DHA as non-esterified fatty acid in subjects of Asian Indian background, *J. Lipid Res.* 39 (2) (1998) 286–292.
- [34] F. Karpe, U. de Faire, M. Mercuri, M.G. Bond, M.L. Hellénus, A. Hamsten, Magnitude of alimentary lipemia is related to intima-media thickness of the common carotid artery in middle-aged men, *Atherosclerosis* 141 (2) (1998) 307–314.
- [35] J.J. Agren, O. Hänninen, A. Julkunen, L. Fogelholm, et al., Fish diet, fish oil and docosahexaenoic acid rich oil lower fasting and postprandial plasma lipid levels, *Eur. J. Clin. Nutr.* 50 (11) (1996) 765–771.
- [36] X. Giererd, P. Giral, Risk stratification for the prevention of cardiovascular complications of hypertension, *Curr. Med. Res. Opin.* 20 (7) (2004) 1137–1142.
- [37] P. Palatini, Heart rate as an independent risk factor for cardiovascular disease: current evidence and basic mechanisms, *Drugs* 67 (Suppl 2) (2007) 3–13.
- [38] T.A. Mori, D.Q. Bao, V. Burke, I.B. Puddey, L.J. Beilin, Docosahexaenoic acid but not eicosapentaenoic acid lowers ambulatory blood pressure and heart rate in humans, *Hypertension* 34 (2) (1999) 253–260.
- [39] J.X. Kang, A. Leaf, Antiarrhythmic effects of polyunsaturated fatty acids. Recent studies, *Circulation* 94 (7) (1996) 1774–1880.
- [40] S. Pepe, P.L. McLennan, Dietary fish oil confers direct antiarrhythmic properties on the myocardium of rats, *J. Nutr.* 126 (1) (1996) 34–42.
- [41] S.S. Nair, J.W. Leitch, J. Falconer, M.L. Garg, Prevention of cardiac arrhythmia by dietary (n-3) polyunsaturated fatty acids and their mechanism of action, *J. Nutr.* 127 (3) (1997) 383–393.
- [42] H.E. Theobald, A.H. Goodall, N. Sattar, D.C. Talbot, Cowienzyk, Low-dose docosahexaenoic acid lowers diastolic blood pressure in middle-aged men and women, *J. Nutr.* 137 (4) (2007) 973–978.
- [43] D.S. Kelley, D. Siegel, D.M. Fedor, Y. Adkins, B.E. Mackey, DHA supplementation decreases serum c-reactive protein and other markers of inflammation in hypertriglyceridemic men, *J. Nutr.* 139 (2009) 495–501.
- [44] C.M. Albert, H. Campos, M.J. Stampfer, P.M. Ridker, J.E. Manson, W.C. Willett, J. Ma, Blood levels of long-chain n-3 fatty acids and the risk of sudden death, *N. Engl. J. Med.* 346 (15) (2002) 1113–1118.
- [45] J.A. Simon, M.L. Hodgkins, W.S. Browner, J.M. Neuhaus, J.T. Bernert Jr., S.B. Hulley, Serum fatty acids and the risk of coronary heart disease, *Am. J. Epidemiol.* 142 (5) (1995) 469–476.
- [46] A.T. Erkkilä, N.R. Matthan, D.M. Herrington, A.H. Lichtenstein, Higher plasma docosahexaenoic acid is associated with reduced progression of coronary atherosclerosis in women with CAD, *J. Lipid Res.* 47 (12) (2006) 2814–2819.
- [47] P.M. Kris-Etherton, W.S. Harris, L.J. Appel, AHA Scientific Statement: Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease, *Circulation* 106 (2002) 2747–2757.
- [48] Position of the American Dietetic Association and Dietitians of Canada: Dietary Fatty Acids, *J. Am. Diet. Assoc.* 107 (2007) 1599–1611.
- [49] P.M. Kris-Etherton, A.M. Hill, n-3 Fatty acids: food or supplements, *J. Am. Diet. Assoc.* 108 (7) (2008) 1125–1130.
- [50] W.S. Harris, D. Mozaffarian, M. Lefevre, C.D. Toner, J. Colombo, S.C. Cunnane, J.M. Holden, D.M. Klurfeld, M.C. Morris, J. Whelan, Towards establishing dietary reference intakes for eicosapentaenoic acid and docosahexaenoic acids, *J. Nutr.* 139 (2009) 804S–819S.