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

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Abstract There is evidence to indicate that the high rates of coronary heart disease and myocardial infarction amongst Indians of Asian descent may be partly related to circulating non-esterified fatty acids (NEFA). As docosahexaenoic acid (DHA,22:6n–3) in NEFA form has been found to exhibit anti-platelet aggregatory and anti-arrythmic potential in vitro, the effect of supplementary DHA was examined in healthy subjects of Asian Indian background. Furthermore, time- and dose-dependent changes in absolute levels of DHA as NEFA or phospholipid (PL) were compared. The subjects consumed 8 capsules daily of placebo (DHA-free) or low DHA (0.75 g/day)or high DHA (1.50 g/day) over 6 wks. Fasting blood samples were drawn at days 0, 21, and 42 for analysis of serum lipid/lipoprotein composition. No significant effect of DHA supplementation on the levels of serum lipid/lipoproteins (including Lp[a]) or blood pressure was found. However, the DHA level in serum phospholipid rose by 167% overall with low-dose supplementation (from 2.4–6.4 mol%) but only by an additional 23% upon doubling the dose from 0.75 g to 1.50 g/day. Furthermore, after 6 weeks of supplementation with 0.75 g or 1.5 g DHA/day, absolute concentrations of DHA as PL were not significantly different from the corresponding 3-week values. Interestingly, the absolute concentrations of serum DHA as NEFA showed a marked rise with low-dose supplementation (by 212% overall, from 2.4 to 7.5 μm) and a further 70% rise (to 12.7 μm) upon doubling the supplementation from 0.75 to 1.50 g/day. As well, the 6-week concentrations (DHA-NEFA) were significantly different than the corresponding 3-week values at both dose levels. Elevation of circulating DHA-NEFA levels via DHA supplementation, as shown herein, to concentrations that exhibit anti-thrombotic and anti-arrhythmic potential in vitro needs to be extended to trials where clinical end-points are determined.—**Conquer, J. A., and B. J. Holub.** Effects 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.* 1998. **39:** 286–292.

Supplementary key words docosahexaenoic acid (DHA) • non-esterified fatty acids (NEFA) • cardiovascular disease

Indians of Asian descent are known to exhibit high rates of coronary heart disease and an excess incidence of myocardial infarction, despite their favorable circulating cholesterol concentrations and low dietary intake of saturated fats (1–4). Furthermore, there is evidence to suggest that Asian Indians are predisposed to atherothrombotic risk factors (5). The release of non-esterified fatty acids (NEFA) from intra-abdominal fat cells has been implicated in the unfavorable metabolic profile among Asian Indians (2). There is increased evidence for plasma NEFA playing a central role in coronary heart disease (6).

Particular plasma NEFA may exhibit differing effects on both thrombosis and myocardial functioning (6). In this regard, certain saturated NEFA have been implicated in having both pro-thrombotic and arrythmogenic actions (6, 7) whereas opposite effects (anti-platelet aggregatory and anti-arrhythmic) have been indicated in vitro for docosahexaenoic acid (DHA;22:6n–3) in its NEFA form (8–10). It is noteworthy that Asian Indians (both native and immigrants)have been found to exhibit a very low n–3 fatty acid status, particularly DHA, in their plasma phospholipid (1, 3, 11) which is associated with their higher incidence of coronary heart disease.

The purpose of the present study was to evaluate the potential for a novel vegetarian source of supplementary DHA to increase concentrations of DHA–NEFA in the circulation of Asian Indian subjects to levels that have been found to exhibit anti-platelet aggregatory and anti-arrhythmic effects in vitro. The influence of

Abbreviations: AA, arachidonic acid; BMI, body mass index; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; HDL-C, high density lipoprotein cholesterol; Lp[a], lipoprotein[a]; LDL-C, low density lipoprotein cholesterol; NEFA, non-esterified fatty acids; TC, total cholesterol; TG, triglyceride; VLDL-C, very low density lipoprotein cholesterol.

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DHA supplementation on DHA–NEFA levels was compared with the influence of DHA supplementation on subjects' n–3 status (based on the fatty acid composition of serum phospholipid) and selected risk factors for cardiovascular disease were measured.

SUBJECTS, MATERIALS AND METHODS

Subjects and experimental design

The subjects were 22 healthy persons (14 male and 8 female of Asian Indian background) selected from the Guelph community. Approval for this study was granted by the Human Ethics Committee of the University of Guelph and written informed consent was obtained from each subject. All groups (0, 0.75, and 1.50 g DHA/day) consumed eight capsules per day. The fatty acid composition of the DHA supplement and the placebo supplement is given in **Table 1**. Each group consumed the capsules for a period of 42 d beginning on day 0. Subjects were weighed on each visit (days 0, 21, 42) and height was measured at entry; there were no significant differences among the groups. Subject characteristics at entry are given in **Table 2**. The weight of the subjects was not affected throughout the supplementation period in either group. Results are for 19 subjects as the remaining subjects were not included in the analysis because they dropped out during the course of the study for personal reasons or they did not comply with the required dosages. Compliance was monitored by a capsule count at the end of the study as well as by determining the fatty acid composition of serum phospholipid (PL) after 6 wks.

TABLE 1. Fatty acid composition of DHASCO™ and placebo supplements

Fatty Acids	Supplements	
	DHASCO™	Placebo
	wt % of total fatty acids	
10:0	1.53	n.d.
12:0	8.50	0.02
14:0	21.0	0.04
14:1	0.21	n.d.
16:0	15.8	10.6
16:1	1.63	0.08
18:0	0.44	3.0
18:1	11.2	24.1
18:2n–6	0.74	57.3
18:3n–6	n.d.	n.d.
18:3n–3	n.d.	3.7
20:5n–3 (EPA)	n.d.	n.d.
22:5n–3	0.27	n.d.
22:6n–3 (DHA)	38.6	n.d.
Total saturated	47.3	13.7
Monounsaturated	13.1	24.2
n–6 Polyunsaturated	0.74	57.3
n–3 Polyunsaturated	39.6	3.7
n–6/n–3 Ratio	0.02	15.5

Fatty acid composition of DHASCO™ and placebo capsules were analyzed as described in Subjects, Materials and Methods; n.d., not detected.

Blood collection

The blood from day 0 (presupplementation) and days 21 and 42 (supplementation) was collected after an overnight fast by antecubital venipuncture into siliconized tubes. Whole blood was centrifuged at 1250 *g* for 15 min to obtain serum. Serum was used for measurement of serum total phospholipid fatty acid content, NEFA analysis, total cholesterol (TC), high density lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C), triglyceride (TG), and Lp[a]. Serum was stored at −70°C until all samples were collected and thawed just before analysis of lipids.

Total lipid analysis

The absolute amounts of DHA and other fatty acids in the DHASCO™ (plant/algal source as triglyceride oil provided by Martek Biosciences Corp.) and placebo capsules (corn oil also provided by Martek Biosciences Corp.) were determined after lipid extraction and gas–liquid chromatography in the presence of a known amount of 17:0 as internal standard (12). The composition (amount and fatty acid profile) of total phospholipid from serum was determined after lipid extraction, thin-layer chromatography, and gas–liquid chromatography by a procedure similar to those previously described (12). The composition and quantity of NEFA from serum was determined as follows. Briefly, lipid extraction was performed as described with the addition of a known amount of heptadecanoic acid (17:0) as internal standard (12). The extract was spotted on silica gel G plates and developed in heptane–isopropyl ether–acetic acid 50:50:3. The NEFA band was removed and methylated with 2.0 ml of 14% boron trichloride in methanol.

Cholesterol and triglyceride measurement

TC was measured enzymatically with a diagnostic test (Sigma Diagnostics Procedure No. 352, St. Louis, MO). HDL-C was isolated using a dextran sulfate and Mg ion solution to precipitate the very low density lipoprotein-cholesterol (VLDL-C) and LDL-C from the serum sample. The HDL-C fraction was then assayed by an enzymatic assay (Sigma Diagnostics Procedure No. 352-3, St. Louis, MO). TG was measured enzymatically with a di-

TABLE 2. Subject characteristics at study commencement

Parameter	Supplementation Group: Control (0 g DHA/d) (n = 6)	Low DHA (0.75 g DHA/d) (n = 6)	High DHA (1.50 g DHA/d) (n = 7)
Height (m)	1.66 ± 0.02	1.68 ± 0.02	1.69 ± 0.04
Weight (kg)	65.2 ± 3.2	71.8 ± 4.0	63.9 ± 4.9
BMI (kg/m^2)	22.6 ± 0.7	25.6 ± 1.3	22.2 ± 0.9
Age (y)	30.0 ± 3.5	30.1 ± 3.2	34.1 ± 6.0

Values are reported as means ± SEM. No significant differences among the three groups were found ($P > 0.05$). There were no significant changes in weight or BMI in any of the three groups throughout the duration of the study.

agnostic test (Sigma Diagnostics Procedure No. 339, St. Louis, MO). LDL-C was calculated using the formula of Friedewald, Levy, and Fredrickson (13). Lipoprotein[a] (Lp[a]) was analyzed using the Apo-Tek Lp[a]™ ELISA test system (PerImmune, Inc, Rockville, MD). NEFA were measured colorimetrically using the Wako NEFA C test method (Wako Chemicals USA, Inc., Richmond, VA).

Statistical analysis

All data are reported as mean ± SEM. Data (phospholipid and NEFA analysis) that were not normally distributed were transformed before analysis in order to reach normality. When observations were missing, least-squared means were calculated so that means could be compared. Split-plot design, including time and treatment as factors, was used in the analyses. Statistical analyses were done using the SAS system (SAS Institute, Inc., Cary, NC). Comparison between subject characteristics and cardiovascular risk factors was analyzed by *t*-test (14).

RESULTS

Table 3 gives the values for fasting serum lipids/lipoproteins at entry and after 3 and 6 wks of supplementation in three groups (0 g DHA/day, 0.75 g DHA/day, and 1.5 g DHA/day). DHA, at both supplementation levels, had no significant effect on any of the parameters measured. The mean serum Lp[a] level for all subjects at entry was 16.5 ± 4.7 mg/dL (mean ± SEM, n = 17); no significant effect due to DHA supplementation was seen at 6 wks ($P > 0.05$). Also, the resting heart rate, systolic blood pressure, and diastolic blood pressure for all subjects, which were 73.3 ± 2.8 bpm, 115.4 ± 2.5 mm Hg and 75.0 ± 1.8 mm Hg, respectively, at entry (mean ± SEM, n = 19), were not significantly altered by any supplementation at 3 or 6 wks ($P > 0.05$).

The serum phospholipid concentration at entry (all subjects) was 200.4 ± 7.0 mg/100 ml serum (mean ± SEM); no significant effect of supplementation was found across any of the groups on these levels. **Table 4** shows the levels of fatty acids (mol%) in the total phospholipid of human serum before and after supplementation with DHA. The fatty acid profiles of total serum phospholipid at entry, including eicosapentaenoic acid (EPA; 20:5n–3) and DHA levels, were similar among the three groups. Changes were seen in various fatty acids after 3 wks of DHA supplementation but the data presented here are as % change at wk 6 over control (wk 0) of the corresponding group. Rises in the DHA content (167% in the 0.75 g DHA group and 193% in the 1.50 g DHA group) occurred with capsule supplementation. There was a non-significant rise in EPA levels by 57% in the 0.75 g DHA group and 36% in the 1.50 g DHA group. This was coupled with a rise in the DHA/

TABLE 3. Effect of DHA supplementation on serum lipid and lipoprotein levels

	Supplementation Group: Control (0 g DHA/d) (n = 6)			Low DHA (0.75 g DHA/d) (n = 6)			High DHA (1.50 g DHA/d) (n = 7)		
	Week 0	Week 3	Week 6	Week 0	Week 3	Week 6	Week 0	Week 3	Week 6
Total cholesterol (mmol/l)	4.34 ± 0.28	4.28 ± 0.34	4.24 ± 0.20	4.42 ± 0.50	4.25 ± 0.48	4.81 ± 0.76	4.44 ± 0.34	4.51 ± 0.38	4.41 ± 0.45
HDL-cholesterol (mmol/l)	1.17 ± 0.06	1.16 ± 0.10	1.19 ± 0.83	1.11 ± 0.11	1.10 ± 0.10	1.17 ± 0.13	1.32 ± 0.13	1.37 ± 0.13	1.26 ± 0.17
Total cholesterol: HDL-cholesterol ratio	3.76 ± 0.29	3.83 ± 0.50	3.68 ± 0.43	4.23 ± 0.63	4.12 ± 0.62	3.68 ± 0.58	3.76 ± 0.47	3.51 ± 0.36	3.72 ± 0.47
LDL-cholesterol (mmol/l)	2.71 ± 0.21	2.57 ± 0.26	2.47 ± 0.16	2.66 ± 0.40	2.60 ± 0.41	2.42 ± 0.83	2.61 ± 0.33	2.60 ± 0.37	2.70 ± 0.45
LDL-cholesterol: HDL-cholesterol ratio	2.35 ± 0.21	2.28 ± 0.29	2.13 ± 0.23	2.56 ± 0.47	2.52 ± 0.47	2.19 ± 0.34	2.31 ± 0.39	2.13 ± 0.32	2.31 ± 0.42
Triglyceride (mmol/l)	1.02 ± 0.18	1.20 ± 0.38	1.25 ± 0.35	1.41 ± 0.35	1.20 ± 0.34	1.29 ± 0.44	1.04 ± 0.30	0.91 ± 0.19	0.98 ± 0.33

Values are reported as mean ± SEM. TC, HDL-C, and TG were analyzed as by Sigma Diagnostics Procedures (#'s 352,352-3, and 339, respectively). LDL-C was determined by the formula developed by Friedewald et al. (13).

TABLE 4. Fatty acid composition of serum phospholipid (as mol% of total fatty acids) before and after supplementation

	Supplementation Group								
	Control (0 g DHA/d) (n = 6)			Low DHA (0.75 g DHA/d) (n = 6)			High DHA (1.50 g DHA/d) (n = 7)		
	Week			Week			Week		
Fatty Acid	0	3	6	0	3	6	0	3	6
16:0	29.9 ± 0.5	30.1 ± 0.3	29.5 ± 0.4	29.7 ± 0.6	30.1 ± 0.3	30.3 ± 0.4	29.6 ± 0.5	30.8 ± 0.6	30.0 ± 0.4
18:0	13.6 ± 0.6^{a}	13.6 ± 0.7^{a}	12.8 ± 0.6^{b}	13.7 ± 0.4^{a}	13.7 ± 0.2^{a}	11.7 ± 0.4^{c}	13.3 ± 0.5^{a,b}	12.9 ± 0.7^{a,b}	11.3 ± 0.4^{c}
18:1	10.9 ± 0.4	10.7 ± 0.2	11.3 ± 0.3	11.9 ± 0.8	11.7 ± 0.1	10.2 ± 0.4	11.7 ± 0.9	11.1 ± 0.4	10.5 ± 0.6
18:2n–6	20.9 ± 0.4	20.5 ± 0.6	20.8 ± 0.9	21.1 ± 1.4	19.2 ± 0.4	20.8 ± 1.8	21.2 ± 0.8	20.9 ± 1.9	21.1 ± 0.9
18:3n–3	0.31 ± 0.05	0.28 ± 0.07	0.39 ± 0.06	0.31 ± 0.06	0.29 ± 0.02	0.31 ± 0.02	0.27 ± 0.03	0.24 ± 0.00	0.30 ± 0.06
20:3n–6	2.6 ± 0.2^{a}	2.6 ± 0.2^{a}	2.8 ± 0.2^{a}	2.9 ± 0.3^{a}	2.3 ± 0.1^{b}	2.6 ± 0.2^{a}	2.7 ± 0.2^{a}	1.9 ± 0.1^{c}	2.0 ± 0.1^{c}
20:4n–6 (AA)	8.9 ± 0.6^{a}	9.4 ± 0.5^{a}	9.0 ± 0.6^{a}	8.9 ± 0.8^{a}	8.2 ± 0.6^{a}	8.9 ± 0.7^{a}	8.7 ± 0.2^{a}	6.9 ± 0.7^{b}	7.3 ± 0.6^{b}
20:5n–3 (EPA)	1.0 ± 0.3	1.1 ± 0.3	1.0 ± 0.2	0.7 ± 0.1	0.9 ± 0.1	1.1 ± 0.1	1.1 ± 0.3	1.0 ± 0.2	1.5 ± 0.1
22:4n–6	0.81 ± 0.02^{a}	0.85 ± 0.05^{a}	0.71 ± 0.02^{b}	0.81 ± 0.04^{a}	0.70 ± 0.03^{b}	0.58 ± 0.03^{d}	0.83 ± 0.04^{a}	0.69 ± 0.06^{b}	0.62 ± 0.03^{c}
22:5n–3	0.82 ± 0.12^{a}	0.82 ± 0.12^{a}	0.85 ± 0.09^{a}	0.81 ± 0.10^{a}	0.51 ± 0.04^{b}	0.55 ± 0.07^{b}	0.86 ± 0.11^{a}	0.40 ± 0.06^{b}	0.45 ± 0.04^{b}
22:6n–3 (DHA)	3.0 ± 0.4^{a}	3.2 ± 0.4^{a}	2.8 ± 0.2^{a}	2.4 ± 0.2^{a}	5.4 ± 0.2^{b}	6.4 ± 0.3^{b}	2.7 ± 0.5^{a}	6.3 ± 0.7^{b}	7.9 ± 0.4^{c}
n–6/n–3 ratio	6.7 ± 0.7^{a}	5.3 ± 0.7^{a}	5.6 ± 0.4^{a}	8.1 ± 0.9^{a}	4.3 ± 0.2^{b}	3.3 ± 0.2^{b}	9.1 ± 1.2^{a}	3.6 ± 0.4^{b,c}	3.0 ± 0.1^{c}
EPA/AA ratio	0.12 ± 0.03^{a,b}	0.10 ± 0.03^{a,b}	0.12 ± 0.03^{a,b}	0.08 ± 0.01^{a}	0.11 ± 0.01^{b}	0.10 ± 0.01^{b}	0.11 ± 0.03^{a,b}	0.14 ± 0.04^{b}	0.22 ± 0.03^{c}
DHA/AA ratio	0.34 ± 0.05^{a}	0.29 ± 0.05^{a}	0.32 ± 0.03^{a}	0.28 ± 0.03^{a}	0.68 ± 0.08^{b}	0.61 ± 0.07^{b}	0.35 ± 0.06^{a}	0.78 ± 0.11^{b,c}	1.1 ± 0.11^{c}

Values are reported as mean ± SEM. Differing superscripts across individual rows indicate statistically significant differences ($P < 0.05$). Phospholipids were analyzed using methods similar to those reported (12). Other minor fatty acids (e.g., 14:0, 14:1, 18:3n–6) have been omitted from the table but were included in any calculations.

arachidonic acid (AA; 20:4n–6) ratio (118% in the 0.75 g DHA group and 214% in the 1.50 g DHA group) and the EPA/AA ratio (25% in the 0.75 g DHA group and 100% in the 1.50 g DHA group). AA(−16% in the 1.50 g DHA group only) and the n–6/n–3 ratio (−60% in the 0.75 g DHA group and −67% in the 1.50 g DHA group) decreased. In contrast to the other n–3 fatty acids, the levels of 22:5n–3 were also lowered (by −25% and −44% in the 0.75 g DHA groups and 1.50 g DHA groups, respectively).

The mean total NEFA concentrations (±SEM) for all subjects at entry, as determined by gas–liquid chromatography or colorimetrically, were 447.4 ± 48.9 μm and 352.4 ± 41.0 μm, respectively. At wk 6, NEFA levels (average 651.3 ± 87.9 μm) were not significantly different among the three supplementation groups. The fatty acid composition of serum NEFA (as mol % of total NEFA, **Table 5**) indicates that the only fatty acid to show a statistically significant change with supplementation DHA (in the two DHA supplementation groups).

In terms of absolute concentrations (μm), **Fig. 1** shows a time-dependent rise in serum concentrations

TABLE 5. Fatty acid composition of serum NEFA (as mol% of total fatty acids) before and after supplementation

	Supplementation Group								
	Control (0 g DHA/d) (n = 6)			Low DHA (0.75 g DHA/d) (n = 6)			High DHA (1.50 g DHA/d) (n = 7)		
	Week			Week			Week		
Fatty Acids	0	3	6	0	3	6	0	3	6
14:0	3.4 ± 0.1	3.1 ± 0.1	5.3 ± 0.5	3.1 ± 0.1	3.8 ± 0.4	4.7 ± 0.4	3.4 ± 0.3	4.2 ± 0.5	6.5 ± 1.0
14:1	0.54 ± 0.11	0.63 ± 0.10	1.1 ± 0.2	0.73 ± 0.19	0.72 ± 0.10	0.52 ± 0.15	0.74 ± 0.12	1.0 ± 0.2	0.74 ± 0.20
16:0	26.6 ± 0.5	26.1 ± 0.5	25.7 ± 1.6	27.7 ± 0.6	28.1 ± 1.4	26.9 ± 1.7	26.4 ± 0.7	26.5 ± 0.7	24.7 ± 1.4
16:1	2.3 ± 0.2	2.5 ± 0.2	2.7 ± 0.6	2.7 ± 0.3	3.2 ± 0.5	3.6 ± 0.5	2.4 ± 0.4	2.2 ± 0.7	2.7 ± 0.8
18:0	9.7 ± 0.6	8.4 ± 0.4	6.2 ± 0.6	8.8 ± 0.8	9.2 ± 0.9	6.5 ± 0.7	9.0 ± 0.6	9.1 ± 1.2	6.0 ± 0.3
18:1	36.2 ± 1.0	36.5 ± 0.8	32.4 ± 1.3	35.1 ± 1.1	36.6 ± 1.6	30.8 ± 0.7	36.7 ± 1.4	35.2 ± 2.6	32.5 ± 1.7
18:2n–6	15.3 ± 0.9	16.6 ± 0.3	13.6 ± 0.6	15.7 ± 1.0	12.7 ± 0.6	14.4 ± 1.2	15.7 ± 1.3	13.4 ± 1.8	14.9 ± 1.1
18:3n–3	2.3 ± 0.3	2.2 ± 0.3	2.3 ± 0.2	1.6 ± 0.2	1.7 ± 0.1	2.1 ± 0.2	1.9 ± 0.2	1.6 ± 0.2	2.5 ± 0.4
20:4n–6 (AA)	1.0 ± 0.1	1.2 ± 0.3	0.57 ± 0.11	1.4 ± 0.1	0.78 ± 0.16	0.85 ± 0.28	1.1 ± 0.3	1.2 ± 0.4	0.87 ± 0.24
20:5n–3 (EPA)	0.03 ± 0.01	0.00 ± 0.00	0.05 ± 0.03	0.12 ± 0.06	0.06 ± 0.03	0.16 ± 0.10	0.10 ± 0.08	0.05 ± 0.05	0.10 ± 0.06
22:5n–6	0.03 ± 0.02	0.02 ± 0.01	0.02 ± 0.02	0.02 ± 0.01	0.04 ± 0.02	0.00 ± 0.00	0.04 ± 0.01	0.02 ± 0.02	0.00 ± 0.00
22:5n–3	0.09 ± 0.04	0.07 ± 0.04	0.03 ± 0.03	0.01 ± 0.01	0.04 ± 0.02	nd	0.03 ± 0.02	0.10 ± 0.09	nd
22:6n–3 (DHA)	0.55 ± 0.2^{a}	0.57 ± 0.12^{a}	0.25 ± 0.19^{a}	0.38 ± 0.05^{a}	0.74 ± 0.15^{a,b}	1.3 ± 0.2^{b,c}	0.29 ± 0.08^{a}	1.7 ± 0.8^{b,c}	2.2 ± 0.4^{c}

Values are reported as mean ± SEM. Differing superscripts across individual rows indicate statistically significant differences ($P < 0.05$). Serum NEFA were analyzed as described in Subjects, Materials and Methods. Other minor fatty acids (e.g., 18:3n–6, 18:4n–3) have been omitted from the table but were included in any calculations.

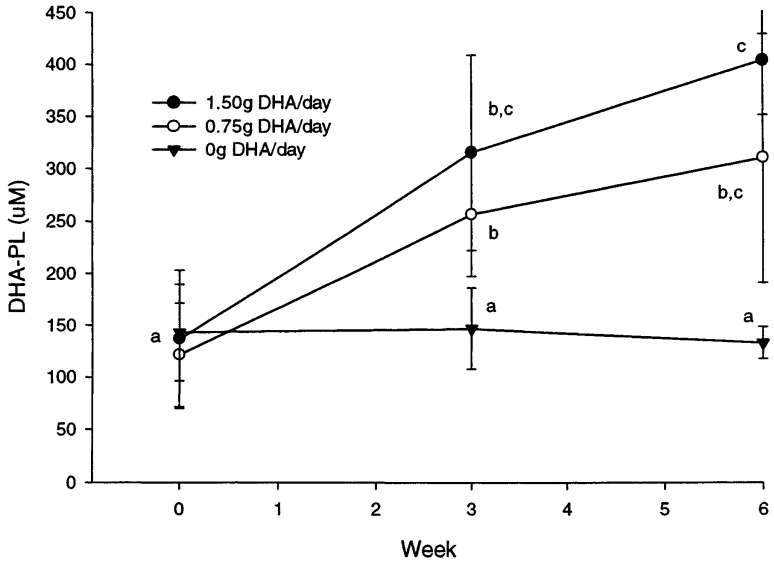

Fig. 1. Concentration of DHA in PL form before and after supplementation. Serum PL were analyzed as described in Subjects, Materials and Methods. Data are represented as mean ± SEM. Differing superscripts indicate statistically significant differences ($P < 0.05$).

of DHA-PL with dietary DHA supplementation. At 6 wks, the concentrations of DHA-PL (mean ± SEM) were 132.8 ± 15.6 μm, 309.1 ± 118.6 μm, and 402.5 ± 52.2 μm for the control (placebo), low DHA, and high DHA groups, respectively. **Figure 2** shows both a time- and dose-dependent rise in serum concentrations of DHA-NEFA with dietary DHA supplementation. At 6 wks, the concentrations of DHA-NEFA (mean ± SEM) were 1.5 ± 1.0 μm, 7.5 ± 0.9 μm, and 12.7 ± 1.1 μm for the control (placebo), low DHA, and high DHA groups, respectively. The very low level of circulating AA-NEFA observed herein (approximately 1 mol% of total NEFA) is similar to very recently reported values using similar methodology (15).

DISCUSSION

The use of two different dose levels of supplementary DHA (0.75 and 1.5 g/day, respectively), both well above estimated North American intakes of <100 mg/day (16), provided information on the apparent quantitative relationship between dietary DHA levels and the corresponding physiological enrichment of serum phospholipid in DHA. The level of DHA in serum phospholipid is regarded as a useful biochemical index for DHA status in the body and a marker for fish intake of EPA/DHA (17–19). Interestingly, the 6-wk data (Table 4) indicates that the DHA level in serum phospholipid rose by 167% overall (2.4 to 6.4 mol %) with 0.75 g of

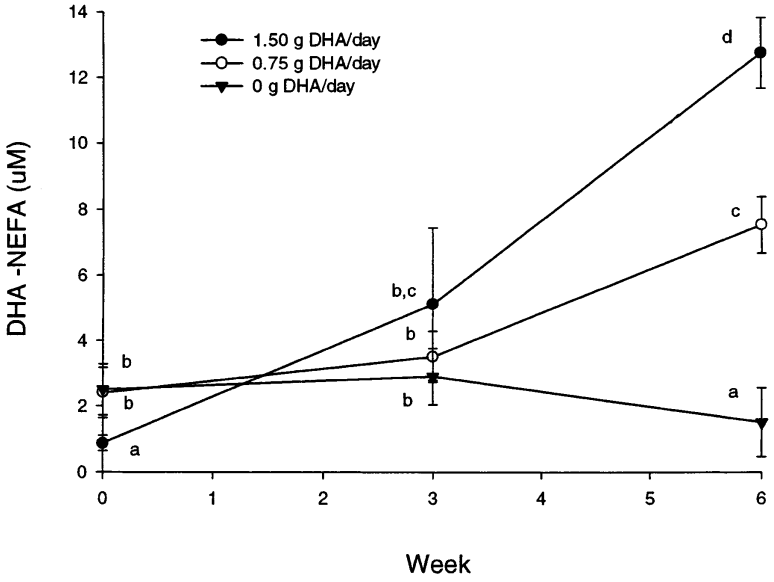

Fig. 2. Concentration of DHA in NEFA form before and after supplementation. Serum NEFA were analyzed as described in Subjects, Materials and Methods. Data are represented as mean ± SEM. Differing superscripts indicate statistically significant differences ($P < 0.05$).

dietary DHA/d. However, an additional 0.75 g rise/d (of 100%) in dietary DHA supplementation (from 0.75 g to 1.5 g) only provided an additional 23% rise in DHA above that seen at 0.75 g DHA/day. Absolute levels of DHA-PL were not significantly different between the two groups supplemented with DHA for 6 weeks. Furthermore, absolute concentrations of DHA as PL, after 6 weeks of supplementation, were not significantly different from the corresponding 3-week values. These findings suggest a plateauing of the dietary DHA–serum phospholipid DHA relationship at the higher levels of dietary intake. Intakes of DHA >0.75 g/day are above that which would generally be consumed in three fish servings per week.

In our previous work (20, 21), we estimated the apparent in vivo retroconversion of dietary DHA to EPA to be 9.4% to 11.3% based on the net mol % rise in EPA (with DHA supplementation) in serum phospholipid as the percentage of the net corresponding mol % rise of DHA plus EPA. Applying this approach to the data (at 6 wks) from subjects on the low- and high-dose regimens of EPA-free DHA yields an apparent mean calculated retroconversion of 9.1% and 7.1%, respectively.

It has been estimated that the mean dietary intake of DHA in North America is 78 mg/day (16). Thus, the 'low' and 'high' supplementation levels used in our trial (0.75 g and 1.5 g, respectively) are 9- and 18-times higher than typical North American dietary intakes. These levels of supplementation did not exhibit any significant effect on the serum lipid/lipoprotein parameters measured (Table 3) or on blood pressure. Previous studies showing significant effects of fish oil concentrates, with respect to a lowering of serum triglyceride, Lp[a], NEFA, and blood pressure, along with a moderate rise in HDL, have occurred with a natural mixture of EPA plus DHA at higher levels of n–3 intake than used herein (reviewed in 22–25). Also, recent work indicates that EPA is primarily responsible for the hypotriglyceridemic effect of fish oil in humans (26) and rats (27), as well as the hypo-NEFA effect (25). Previous work (20) from our lab, which indicated a small but statistically significant decrease in TG, TC/HDL-C ratio, and LDL-C/HDL-C ratios, with DHA supplementation (1.8 g/day over 6 wks), was not observed in the present trial (0.75 or 1.50 g DHA/day over 6 wks) or by Hamazaki et al. (28) using 1.5–1.8 g DHA/day over 13 wks.

The potential for dietary supplementation with DHA to influence the absolute concentration of serum DHA as NEFA was of particular interest as circulating NEFA have been implicated in the unfavorable metabolic profile amongst Asian Indians (2) and in coronary heart disease (6). Interestingly, DHA supplementation provided a dose-dependent rise in the serum DHA-NEFA concentrations, attaining mean levels of 7.5 and 12.7 μm at 6 wks at the low and high doses, respectively. In contrast to the serum phospholipid DHA data (Table 4 and Fig. 1), no plateauing effect in going to the higher level of supplementation was obvious (Fig. 2). Low-dose supplementation (0.75 g/day) increased serum DHA as NEFA by 212% overall (from 2.4 to 7.5 μm) with a further 70% rise (to 12.7 μm) upon doubling the supplementation from 0.75 to 1.5 g/day. Furthermore, both low and high dose supplementation with DHA for 6 weeks resulted in increases in DHA as NEFA compared with the corresponding 3-week values. The near absence of EPA-NEFA in the circulation of subjects given the DHA supplements over 6 wks, in contrast to the serum phospholipid, may reflect the much greater enrichment of adipose triglyceride (from which much of the serum NEFA is derived) in DHA rather than EPA as found in subjects consuming marine sources of EPA plus DHA (29). It is of interest to note that concentrations of DHA (as NEFA) in the 5–10 μm range have been found to exhibit anti-arrhythmic effects in cardiac myocytes (10). Furthermore, in rats, DHA has been shown to be the principal active component of n–3 fatty acids conferring cardiovascular protection (30). Also, concentrations of DHA-NEFA of 10 μm and somewhat lower have been found to markedly inhibit platelet activity in vitro (9). Thus, DHA supplementation at levels approximating <2% of the daily fat intake can provide a strategy for increasing circulating DHA-NEFA to levels (7.5–12.7 μm as found herein and potentially higher) which may offer anti-platelet aggregatory benefits as well as anti-arrythmic effects under physiological conditions. Any potential clinical benefits of DHA supplementation to both Asian Indians and others remains to be evaluated in future more lengthy trials.

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