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Effects of Short Term Supplementation of Fish Oil Capsules on the Blood Fatty Acid Profile of Vegetarians: A Pilot Study

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ABSTRACT

Background and Aims: *Omega-3 fatty acids are essential for cardio vascular as well as overall health. Therefore, fish oil which is a rich source of omega-3 fatty acids namely EPA and DHA is recommended. We supplemented vegetarian volunteers, not consuming fish, with fish oil capsules to observe the changes in lipo-protein metabolism.* ***Methods and Results****: Commercially available fish oil capsules were given to normal healthy volunteers for two weeks and lipid profiles analyzed. Lipid profile normalizing effect of triglycerides and cholesterol was observed in the consuming subjects. Concentration of omega-3 fatty acids increased in the plasma and RBC of all volunteers.* ***Conclusion****: Consumption of omega-3 fish oil capsules is beneficial and heart healthy.*

Keywords: Omega-3 fatty acids, fish oil, heart healthy, cardiovascular disease

INTRODUCTION

During the past several decades, reduction in fat intake has been the main focus of dietary recommendations all over, to decrease the risk of cardiovascular disease (CVD). Results from epidemiological studies and controlled clinical trials have indicated that replacing saturated fat with unsaturated fat is more effective in lowering the risk of cardiovascular disease than simply reducing total fat consumption [1,2]. The adverse effects of the mercury content in fishes (at times) when compared to the appropriate dosage and reduced side effects, makes capsules a safer and better choice for the consumer wishing to increase omega-3 consumption [3]. Consumption of fish oil capsules could therefore benefit vegetarians who otherwise do not eat fish. Fish oil with higher omega3 fat, is known to have favourable effects on both cholesterol and triglyceride levels, and the risk of coronary heart disease [2,4]. The evidence that long chain omega-3 fatty acids can reduce the risk of CVD being sufficiently strong, the American Heart Association and the European Cardiology Society, either recommend increased intake of fish or fish oil [5,6]. Omega-3 fatty acids have been known to decrease blood pressure and improve endothelial functions thereby reducing the risk for CVD [7]. Inflammatory responses resulting from the oxidation of lipids and their invasion into blood vessels is counteracted by fish oils [8]. The two omega-3s eicosa pentaenoic acid (EPA) and docosa hexaenoic acid (DHA) present in fish oil inhibit the production of pro-inflammatory prostaglandin PGE2 and enhance the production of anti-inflammatory prostaglandin PGE3 [9].

A mere concentration of 5% omega-3 of the total fatty acid in the RBC membrane has been associated with a 70% decrease in risk for cardiac attack [10]. Recommendations of 1gram of combined EPA DHA for individuals with existing cardiovascular risk factors may not be realized with manipulations in the diet alone. Fish oil supplements are therefore recommended [11,12]. However, concerns about the possibility of heavy metal contamination in fishes (a rich source of omega-3 fatty acid) and long term ingestion of fish oil have raised apprehensions. Fish consumption may lead to risk of exposure to environmental toxins like mercury and hyper vitaminosis which is markedly reduced through the selectively purification processes developed in recent times. Therefore, clinicians need to approve of supplements which have been passed through the FDA regulatory authorities' thus ensuring safety [13]. The levels of mercury analysed in the fish oil supplements have been found to be in the range of 0.013 to 2.03 ng/g, which is safe for consumption [14]. Other studies also corroborate this finding and consider supplements by popular brands comprising mercury in undetectable limits [15].

A rationale therefore exists for prescribing these supplements to patients with risk for CVD for effective management

[16]. Omega-3 fatty acids therefore seem to exert pleiotropic, and cardio-protective effects and a per day consumption of up to 1 g of omega-3 fatty acids per day does not increase bleeding risk and is also well tolerated except for certain gastric upset [17]. A recent study on myocardial infarction patients who consumed fish oil supplement within a month of being discharged were found to improve considerably when compared to those who did not consume the supplement [18].

Hence a pilot study on a small number of subjects was undertaken to get the leads to corroborate the effects of fish oil capsules on the lipid profile of healthy volunteers not taking any medications.

METHODS

Subjects

A total of 18 subjects who did not have any apparent ailment, and willingly participated in the study were enrolled as seen in Table 1. Based on their acceptability to consume fish oil capsules (mostly vegetarians), the volunteers were enrolled. Blood samples were collected from all the subjects prior to the dietary intervention.

Table 1 Demographic data of volunteers

Sr. No.	Parameter	Fish Oil
1	No. of volunteers	18
2	Mean age	31.6
3	Male	4
4	Female	14
5	Vegetarian	18
6	Non-vegetarian	-
7	Triglyceride <150	17
	>150	1
8	Cholesterol <200	11
	>200	7
9	Age < 30	13 Chol. high-4 TG high-1
	> 30	5 Chol. high-2 TG high-0

Study design

A randomized control trial in which consumption of 4 x 2 fish oil capsules (Softsule Pvt. Ltd.) per week for two weeks was observed.

Ethical consideration

The study was approved by the institutional ethical committee and informed consents obtained from all the volunteers.

Blood collection

A total of 10 ml of blood sample was collected from the volunteers before and after the supplementation. Five millilitre of blood was collected in EDTA bulbs and components separated. RBC, and plasma fractions were taken for analysis of fatty acids; 2 ml of blood was taken for estimating haemoglobin and WBC levels; and remaining 3 ml was collected in heparin bulbs for analysis of serum cholesterol and triglycerides.

Fatty acid analysis

Methyl esters of the plasma and RBC fraction were prepared using the method of Manku, et al. [19]. The methylated fatty acids were analysed by gas chromatography as described and compared with standards [20].

Estimation of haemoglobin, cholesterol and triglyceride level in blood

Estimation of haemoglobin, was carried out by a fully automated cell counter hematology Sysmex KX-21 (Sysmex Corporation, Kobe Hyogo, and Japan).

Estimation of triglyceride was carried out by Enzokit Triglyceride kit (RFCL Limited, Haridwar, India). Estimation of cholesterol, LDL-C, HDL-C and VLDL-C was carried out by Atozyme cholesterol Enzymatic kit (Accurex biomedical PVT. LTD. Mumbai, India).

Statistical analysis

Categorical variables were expressed as means ± standard deviations. Statistical analysis was done by using T-test. $P<0.05$ was considered statistically significant.

RESULTS

Consumer response sensory studies showed that consumption of fish oil was accompanied by a fishy odour and belching.

Haemoglobin levels: There was an apparent increase, in the haemoglobin levels of volunteers as shown in Table 2. Experiments on long term are necessary to ascertain this.

Table 2 Hb concentrations pre and post supplementation

n=18	Hb pre	Hb post
Mean ± SEM	12.155 ± 0.337	12.733 ± 0.356
P value	0.0126	-

Fatty acid profile in RBC and plasma

Mean values of total omega-3 fatty acids in the plasma and RBC of the volunteers at the beginning and at the end of study are presented in Table 3.

Table 3 Fatty acid profile in RBC and plasma of volunteers

Fatty acids (%)	Fish oil plasma-pre	Fish oil plasma-post	P value	Fish oil RBC pre	Fish oil RBC post	P value
SATD	29.247 ± 4.173	28.527 ± 1.330	0.427	35.668 ± 2.174	35.112 ± 2.352	0.328
MUFA	11.538 ± 1.337	12.696 ± 1.877	0.015	8.734 ± 0.705	8.887 ± 1.160	0.486
PUFA	49.665 ± 3.174	48.286 ± 5.472	0.33	29.038 ± 1.366	29.666 ± 1.568	0.094
LA	41.357 ± 2.653	39.796 ± 4.623	0.181	12.517 ± 1.264	12.651 ± 1.298	0.709
GLA	0.378 ± 0.127	0.381 ± 0.071	0.932	0.0576 ± 0.054	0.067 ± 0.062	0.647
AA	6.874 ± 1.151	6.636 ± 1.241	0.569	14.394 ± 1.114	14.459 ± 1.122	0.805
n-6	48.611 ± 2.980	46.814 ± 5.092	0.178	26.969 ± 1.258	27.178 ± 1.827	0.623
ALA	0.331 ± 0.258	0.251 ± 0.184	0.112	0.066 ± 0.049	0.091 ± 0.077	0.226
EPA	0.087 ± 0.051	0.175 ± 0.099	0.0002	0.092 ± 0.056	0.132 ± 0.082	0.052
DHA	0.635 ± 0.245	1.044 ± 0.673	0.0009	1.898 ± 0.796	2.263 ± 0.865	0.00002
n-3	1.054 ± 0.335	1.471 ± 0.732	0.027	2.057 ± 0.826	2.488 ± 0.937	0.0002
n-6:n3	49.985 ± 13.440	39.966 ± 21.943	0.098	14.835 ± 4.845	12.324 ± 4.218	0.001

Though PUFA levels did not change appreciably, significant increases were noticed in EPA, and DHA as seen in Table 3. Also, total omega-3 fatty acid concentrations significantly increased with a concomitant decrease in omega-6/omega-3 ratio.

Cholesterol and triglyceride levels

The data presented in Table 4 shows a decline though not significant in both triglyceride and cholesterol levels of the subjects.

Table 4 Triglyceride and Cholesterol levels of all the enrolled subjects

Fish oil supplementation	Triglyceride (Pre)	Triglyceride (Post)	Cholesterol (Pre)	Cholesterol (Post)
Mean	94.888 ± 5.204	92.166 ± 8.283	187.944 ± 7.732	197.333 ± 5.238
p-value	0.253	-	0.748	-

The subjects were further sub divided into two groups A and B for the purpose of analysis A- those having initially triglyceride levels lower than the normal values of 150 mg/dl, and those having initially higher than the normal triglyceride values of 150 mg/dl. Similarly volunteers having initial blood cholesterol levels below 200 mg/dl were included in A group and above 200 mg/dl were included in the B group. Only the cholesterol group A showed a significant change (p=0.008) as seen in Table 5. Group B volunteers showed decreases in their cholesterol as well as triglyceride levels.

Table 5 Cholesterol and triglyceride levels in the serum of fish oil A and B group volunteers

fish oil group	Triglyceride				Cholesterol			
	Group A (n=17) (<150)		Group B (n=1) (>150)		Group A (n=12) (<200)		Group B (n=6) (>200)	
	Before	After	Before	After	Before	After	Before	After
Mean	89.117 ± 3.731	90.294 ± 8.263	150	94 (37.33% reduction)	168.833 ± 4.267	193.75 ± 5.324	226.166 ± 9.556	204.5 ± 11.819
p-value	0.889334		-		0.00811		0.0809	

HDL, LDL, VLDL levels-Consumption of fish oil capsule showed a significant increase only in the HDL values as observed in Table 6.

Table 6 HDL, LDL, VLDL levels in the serum of volunteers supplemented with fish oil

	HDL (N=18)		LDL (N=18)		LDL/HDL (N=18)		VLDL (N=18)		Total Chol/HD (N=18)	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Mean	47.777 ± 0.424	49.888 ± 0.816	121.622 ± 7.622	129.322 ± 5.689	2.537 ± 0.149	2.6±0.121	18.544 ± 1.007	18.12 ± 1.559	3.926 ± 0.145	3.97 ± 0.12
p-value	0.022		0.362		0.708		0.806		0.781	

DISCUSSION

The data analysed brings out the following facts. Consumer response sensory studies showed fish oil odour accompanied belching complaints in about 40 % of the volunteers. There was a slight but significant increase in haemoglobin (Hb) levels in all the subjects (Table 2). It would be of interest to study the RBC count and Hb levels after 110 days of supplementation of omega-3 supplement to derive at the exact picture.

One of the major findings of the study is the normalizing effect of both triglyceride and cholesterol on consumption of omega-3 supplement fish oil (Tables 4 and 5). On the other hand when the cholesterol and triglyceride levels are on the lower side of the normal, they either get slightly elevated or remain steady, yet remaining within normal limits. In our study, we find an appreciable 33% decrease in the triglyceride level of the sole volunteer whose triglyceride concentrations were above 150. The cholesterol levels of volunteers having lower than 200 mg/l concentration had increased significantly but within normal limits emphasizing the requirements of cholesterol by the body. It is necessary to undertake detailed well-structured studies on larger samples to understand and interpret this interesting finding.

Fish oil capsule consumption shows a significant increase in the healthy HDL levels (Table 6). The volunteers possibly being mostly vegetarians had lower initial levels of total cholesterol and triglycerides. The improvement in lipid profile is significant in just a two week period in all the subjects. Slight but not significant increases in LDL could probably be due to certain other dietary inclusions which have not been monitored using food diaries. Along with significant increase in the cardio-protective HDL cholesterol, a slight decrease in VLDL is observed which is beneficial for atherosclerosis patients.

In case of fish oil supplementation, DHA is directly available to the body. Also, to be noted is the fact the given fish oil capsule contains 90 mg EPA which also would be converted to DHA and be available to the body. A significant increase is seen in the plasma, as well as RBC levels of EPA, DHA, and total omega-3 levels (Table 3). In RBC, a favourable shift of the omega-6: omega-3 ratio is also observed with a significant reduction. MUFA levels also increased favourably in the plasma. Though the study was conducted for a very small period, a positive shift in the lipid profile was observed.

Earlier studies show that *in vitro* both EPA and DHA inhibit triglycerides synthesis and their secretion. In humans, omega-3 fatty acids have been shown to reduce triglycerides with more variable effects on total cholesterol, LDL, and HDL. Our results are in accordance with the above work. Earlier studies also show that dietary omega-3 fatty acids appear to be of value in the secondary prevention of coronary artery disease [21].

For a better understanding; larger studies with omega-3 in different ailments, and different age groups are called for. This pilot study gives us leads for understanding the benefits of short term supplementation of fish oil.

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