EFFECT OF n-3 FATTY ACID SUPPLEMENTATION ON BLOOD GLUCOSE, LIPID PROFILE AND CYTOKINES IN HUMANS: A PILOT STUDY

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ABSTRACT

This study was undertaken to evaluate the effects of n-3 fatty acid supplementation on blood glucose, lipid profile and cytokines in humans. Twenty adult healthy subjects were supplemented with 1g/day fish oil concentrate capsules for 2 weeks. Fasting blood samples were taken at baseline and again after 2 week intervention. Fish oil supplementation significantly lowered fasting serum concentrations of total cholesterol, triacylglycerol, very low density lipoprotein and low density lipoprotein ($P < 0.01$). Also high density lipoprotein increased significantly compared with baseline value ($P < 0.01$). Fish oil did not significantly alter the fasting blood glucose concentration but significantly reduced the serum cytokines ($P < 0.001$). This study demonstrated that eicosapentanoic acid (EPA) and docosahexaenoic acid (DHA) supplementation altered serum lipids, lipoprotein and cytokine concentrations in normal human subjects even at the small dose of 660 mg of EPA plus DHA per day. It appears that EPA plus DHA can be a safe and perhaps beneficial supplement to inflammatory diseases.

KEY WORDS

n-3 fatty acid, Blood Glucose, Lipid profile, Cytokines.

INTRODUCTION

The omega-3 fatty acids (n-3) are essential fatty acids. Alpha – linolenic acid is the most abundant omega-3 fatty acid, occurring naturally in algae and plants. Long chain fatty acids (LCFAs), eicosapentanoic acid (EPA) and docosahexaenoic acid (DHA), are found in high quantities in some types of fish, which enzymatically convert ALA to these longer chain forms. EPA and DHA are the bioactive forms of n-3 fatty acids in humans and they are the preferred dietary form since they do not require any conversion.

Over the past 20 years many studies and clinical investigations have been carried out on the metabolism of polyunsaturated fatty acids (PUFAs) in general and on n-3 fatty acids in particular. Today we know that n-3 fatty acids are essential for normal growth and development and may play an important

role in the prevention and treatment of coronary artery disease, hypertension, diabetes, arthritis, other inflammatory and autoimmune disorders and cancer (1-5). Fish oil supplementation has been shown to lower plasma triacylglycerols (6) and may therefore constitute a potentially effective treatment for obesity-related dyslipidemia. Interleukins (also called lymphokines) are a sub-group of small soluble proteins called cytokines which function as chemical messengers between cells. There are at least 18 known interleukins (IL – 1 to IL – 18) largely secreted by white blood cells (leukocytes) and the role of interleukins is to mediate and control the immunologic and inflammatory response. Vascular endothelial growth factor (VEGF) is an important signaling protein involved in both vasculogenesis (the *de novo* formation of the embryonic circulatory system) and angiogenesis (the growth of blood vessels from pre-existing vasculature). Tumor necrosis factor (TNF) is released by white blood cells, involved in systemic inflammation and the acute phase response generally together with interleukins 1 and 6. Interferons (IFNs) are a class of natural proteins produced by the cells of the immune system of most animals in response to challenges by foreign agents such as viruses, bacteria, parasites and tumor cells. Platelet-derived growth factor

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(PDGF) and Epidermal growth factor (EGF) are proteins that regulate cell growth, cell proliferation and differentiation. PDGF has also been linked to several diseases such as atherosclerosis, fibrosis and malignant diseases. Monocyte chemotactic protein (MCP), a member of the small inducible gene (SIG) family, plays a role in the recruitment of monocytes to sites of injury and infection. Animal and human studies have shown that production of cytokines can be reduced by n-3 fatty acids (7,8,9). This in turn results in reduction of the severity of certain autoimmune, inflammatory and atherosclerotic diseases and reduces cytokine-induced anorexia. Research has been done in animal models, tissue cultures and human beings. However, not many reports have documented the effects of EPA and DHA on blood cytokine levels in healthy humans.

Therefore this study was undertaken to evaluate the effects of n-3 fatty acid supplementation on blood glucose, lipid profile and cytokines in Indian subjects.

MATERIALS AND METHODS

Twenty non-smoking adult male subjects aged between 20-40 years were studied and none of the subjects consumed fish during the experimental period. Also none of them reported a history of cardiovascular disease, arthritis and inflammatory disease or was taking any medication known to affect lipid metabolism during the experimental period.

Supplements were given in the form of Fish oil capsules (Walgreen Co., Deerfield, IL, USA) contained 1000 mg of a fish oil (EPA+DHA) concentrate for two weeks. The fish oil concentrate contained a total of 70% n-3 fatty acids (35% EPA and 25% DHA), thus each capsule contained 660 mg EPA+DHA. This dose of EPA+DHA was used previously to yield a substantial reduction in circulating triacylglycerol concentrations (10).

The study design is paired with comparison of pre and post values. The sample size required to detect at least 1.0 difference between the pre and post intervention values for

IL1- α with 95% confidence level and 90% power is 19 individuals. Hence the sample size was fixed as 20. Fasting blood samples of each subject were collected at the beginning on day 1 before supplementation and again at the end of two weeks (14th day) after supplementation of fish oil.

Blood was collected by venipuncture into evacuated tubes that were free of anticoagulant. After time was allowed for the blood in the tubes without anticoagulant to clot, samples were centrifuged. Serum was used to analyze Glucose, total cholesterol, HDL-cholesterol, and triglyceride concentrations were quantified enzymatically with an autoanalyzer (Maxmat PL analyzer, France). LDL-cholesterol were calculated by using the Friedewald equation (11). VLDL-cholesterol was calculated from measured triglycerides divided by 5. Serum cytokines: Interleukin-1 α (IL1 α), Interleukin-1 β (IL1 β), Interleukin-2 (IL2), Interleukin-4 (IL4), Interleukin-6 (IL6), Interleukin-8 (IL8), Interleukin-10 (IL10), Vascular Endothelial Growth Factor (VEGF), Tumor Necrosis Factor (TNF), Interferon (IFN), Platelet Derived Growth Factor (PDGF), Epidermal Growth Factor (EGF), Monocyte Chemotactic Protein (MCP) were analyzed using Evidence (biochip array technology), Randox Laboratories Ltd., UK.

The results are expressed as Mean $\pm$ SD for all the parameters and changes as percentage to the baseline values. The paired t-test was applied to test the difference in mean changes before and after supplementation of fish oil. The significance level was taken at 5%. All analysis were carried out using SPSS-version 13.

RESULTS AND DISCUSSION

At baseline and at day 14, we evaluated serum glucose, lipids, lipoproteins and cytokines. The serum glucose, lipid and lipoprotein concentrations before and after 2-wk supplementation with fish oil are shown in Table 1. There was no significant change in blood sugar concentration with fish oil supplementation ($P > 0.05$). Fish oil supplementation significantly reduced fasting serum concentrations of total cholesterol (10%), triacylglycerols (25%), VLDL cholesterol

Table 1 : Effect of fish oil supplementation on serum glucose and lipid profile (Values are Mean $\pm$ SD)

TEST	n	Glucose	Total Cholesterol	Triglycerides	HDL	LDL	VLDL
Before supplementation	20	80.60 $\pm$ 6.36	191.40 $\pm$ 38.20	185.10 $\pm$ 69.62	43.30 $\pm$ 4.54	111.30 $\pm$ 30.84	36.80 $\pm$ 13.94
After supplementation	20	79.10 $\pm$ 6.50	173.50 $\pm$ 14.20	139.10 $\pm$ 35.25	49.30 $\pm$ 3.43	96.90 $\pm$ 10.39	27.30 $\pm$ 3.89
p-value		NS *	< 0.01	< 0.01	0.02	< 0.01	< 0.01

* Not significant

Table 2: Effect of fish oil supplementation on serum cytokines (Values are Mean $\pm$ SD)

Cytokine concentration (pg/ml)													
TEST	n	IL1 α	IL1 β	IL2	IL4	IL6	IL8	IL10	VEGF	TNF	IFN	EGF	MCP
Baseline	20	3.35 $\pm$ 1.19	19.92 $\pm$ 6.35	22.67 $\pm$ 5.98	12.88 $\pm$ 3.08	20.51 $\pm$ 7.12	398.20 $\pm$ 114.38	12.09 $\pm$ 2.82	156.60 $\pm$ 47.27	12.20 $\pm$ 6.94	13.18 $\pm$ 4.36	108.30 $\pm$ 31.71	221.40 $\pm$ 56.27
Post-supplementation	20	1.67 $\pm$ 0.32	7.19 $\pm$ 2.71	11.10 $\pm$ 3.68	4.65 $\pm$ 2.78	12.13 $\pm$ 3.29	218.80 $\pm$ 84.23	5.57 $\pm$ 2.95	127.00 $\pm$ 35.32	5.72 $\pm$ 2.22	6.58 $\pm$ 2.74	43.32 $\pm$ 28.37	84.18 $\pm$ 22.37
P-value		< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

(25%) and LDL cholesterol (13%) compared with baseline value ($P < 0.01$). Simultaneously there was an increase in HDL cholesterol concentration (14%, $P < 0.01$).

The role of elevated blood lipids in the etiology of atherosclerosis and cardiovascular disease (CVD) is well established. Moderately elevated triacylglycerol concentrations are recognized as a particularly important risk factor for CVD and myocardial infarction (MI) in human (12,13). In many studies, dietary supplementation with fish oils enriched in n-3 fatty acids reduced triacylglycerol concentrations, usually increased HDL-cholesterol concentrations, but had mixed effects on LDL-cholesterol concentrations (14,15,16).

Similarly, in the present study fish oil supplementation significantly reduced the risk factors for CVD and MI in human.

The serum cytokine concentrations before and after 2-wk supplementation with fish oil are shown in Table 2. Fish oil supplementation significantly reduced serum concentrations of IL1 α (50%), IL1 β (36%), IL2 (49%), IL4 (36%), IL6 (59%), IL8 (55%), IL10 (46%), VEGF (19%), TNF (47%), IFN (50%), EGF (40%) and MCP (38%) ($P < 0.001$).

Inflammation is part of the normal host response to infection and injury. However, excessive or inappropriate inflammation contributes to a range of acute and chronic human diseases and is characterized by the production of inflammatory cytokines, arachidonic acid-derived eicosanoids (prostaglandins, thromboxanes, leukotrienes, and other oxidized derivatives), other inflammatory agents (e.g., reactive oxygen species) and adhesion molecules. Long-chain n-3 PUFAs, as found in oily fish and fish oils, decrease the production of inflammatory eicosanoids, cytokines, and reactive oxygen species and the expression of adhesion molecules. Long-chain n-3 PUFAs act both directly (e.g. by replacing arachidonic acid as an eicosanoid substrate and inhibiting arachidonic acid metabolism) and indirectly (e.g. by altering the expression of inflammatory genes through effects on transcription factor activation). Long-chain n-3 PUFAs also give rise to a family of anti-inflammatory mediators termed

resolvins. Thus, n-3 PUFAs are potentially potent anti-inflammatory agents. As such they may be of therapeutic use in a variety of acute and chronic inflammatory settings (17-20).

In the present study decrease in cytokine concentrations after fish oil supplementation for two weeks support the idea that the n-3 PUFA in fish oil are anti-inflammatory. Therefore, the use of n-3 fatty acids can be recommended to the general healthy population not only to prevent atherosclerosis but also to reduce the risk of inflammatory diseases.

We conclude that the clinical benefits of dietary supplementation with omega-3 fatty acids and possible hazards in critically ill patients remain to be established.

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