Physiological Effects of Omega3 Unsaturated Fatty Acids in Healthy Subjects

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Abstract: Omega-3 polyunsaturated fatty acids (n-3PUFAs), have been known for their potential in the prevention of cardiovascular diseases, however, the physiological impact of their oral supplementation has not been clarified. The main objective of the present study was to assess the influence of (n-3PUFAs) supplementation on the lipid pattern, some hematological and inflammatory parameters in apparently healthy individuals. Thirty four healthy participants, 16 men and 18 women, ranged in age from 25-49 years old were recruited for this study. Subjects were received a daily dose of 1000mg of omega-3 for 12 weeks. Fasting blood samples were withdrawn from each volunteer before and after omega-3 supplementation to evaluate: Serum lipid profile, complete blood picture (CBC) and the inflammatory markers including, C reactive protein (CRP), Interleukin-1(IL-1) and Tumor necrosis factor (TNF-α). Results obtained from this study showed that omega-3 had a hypolipidemic effect upon oral administration for 12 weeks. It also favored cholesterol fraction distribution opposite to the atherogenic process. Moreover, a significant decrease in the monocytic count and platelets count was observed in response to omega-3 intake. In addition, inflammatory markers assayed in sera of the participants experienced a significant decrease after omega-3 administration. On the basis of data obtained from the present study, it may be speculated that long chain omega-3 fatty acids, counteract the atherogenic mechanism even within the physiological limits.
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1. Introduction

The modern concept of the increased prevalence of cardiovascular diseases (CVD) emphasizes the contribution of an inflammatory mechanism to the traditional risk factors for atherosclerosis[1] Traditional risk factor for CVD basically involved altered atherogenic dyslipidemia that involved elevation of plasma cholesterol, triglycerides and/or the disruption of the low to high density lipoprotein cholesterol proportions. Lipoprotein cholesterol fractions maintain the natural balance of the in and out transport of cholesterol in relation to blood vessel[2].

Active systemic inflammation has recently been shown to share in the atherogenic process by altering the histological nature of blood vessels. These changes have been monitored by assaying the acute phase reactants together with the circulating inflammatory monokines such as IL-1 and TNF-α. Damaged blood vessels, altered blood glucose metabolism and creation of a hypercoagulable state due to platelet activation may dramatize the pathologic process leading to arterial occlusion rather than narrowing with a possible fatal outcome[3].

In the last decades, there has been a remarkable increase of public interest in n-3 and n-6 polyunsaturated fatty acids (PUFAs). They are not synthesized in the human body and had to be taken with food where fish have been considered their main prime source[4,5]. Dietary omega-3 polyunsaturated fatty acid have been shown to play an important role as a cardioprotective agent. This latter quality has been attributed to its multifactorial influence on the atherogenic process including lipids metabolism, blood pressure, vascular function, cardiac rhythms, platelet function, and inflammatory responses[6].

Despite the growing body of evidence that oral supplementation of omega-3 can be used as modality to reduce the chance of CHD in high risk individuals, little was done to explore their effects within the physiological limits of lipid pattern i.e in individuals with average risk for CHD. The main purpose of the present study is to assess the impact of long-chain (n-3PUFAs) supplementation on the lipid profile, CBC and some inflammatory markers known to contribute to the atherogenic process in apparently healthy individuals.

2. Subjects and methods

From a pool of apparently healthy individuals 16 men and 18 non pregnant women fulfilled the criteria of inclusion in the present investigation. These criteria included passing complete medical examination, abstinence of drug administration over the last three months, negative family history of coronary heart diseases, within normal reference serum levels of lipid and inflammatory markers including CRP, IL-1 and TNF-α.. Volunteers signed an informed consent before

sharing in this study and they were instructed to not to take fish oil or flaxseed supplements during the experimentation period. Fasting blood samples were withdrawn before omega-3 supplementation and after the completion of the trial. Subjects were supplemented daily with capsules containing 600 mg eicosapentaenoic acid (EPA) and 400 mg docosahexanoic acid (DHA) (Technopharma, Egypt) to supply a total of 1000 mg/day as shown by **Burr *et al.* (1989)**[7] for 12 weeks.

EDTA blood samples were examined for CBC parameters using Sysmex (KX-21) cell counter, with a kit manufactured by (Diamond, Philadelphia, USA). Separated sera were tested for serum total cholesterol, high density lipoprotein (HDL) and triglyceride levels were measured with enzymatic colorimetric methods on Hitachi 902 biochemical analyzer using Roche commercial kits, while serum low density lipoprotein (LDL) levels were calculated according to **Friedewald *et al.* (1972)**[8]**.** Sera were also assayed for C- reactive protein (CRP) by Immunoturbidimetry Assay using kit purchased from (Roche Diagnostics GmbH, Mannheim, Germany) while serum Interleukin -1(IL-1) and Tumor necrosis factor (TNF-α) were determined using the enzyme-linked immunosorbent assay (ELISA) according to **Reale *et al.* (2004)**[9] **& Bonavida (1991)**[10] respectively.

Statistical analysis

Data were represented as mean ± standard error of the mean values. The mathematical differences between parameters assayed at the beginning and at the end of the experiment were assessed by paired t student test according to **Steel and Torrie (1980)**[11]**.**

3. Results

The lipid pattern of sera from volunteers participating in the present study showed significantly decrease in total cholesterol and triglyceride level upon supplementation of omega 3 for 12 weeks. The mean HDL & LDL levels seemed to be reluctant to the effect of omega 3 supplementation, however, LDL HDL risk ratio has been significantly affected by omega3 (table 1).

Table (1): Lipid profile before and after omega-3 supplementation.

Groups / Parameters	Sex M=16 F=18	Before omega-3 supplementation	After omega-3 supplementation
TC (mg/dl)	M	176.62± 5.09	160.93 ±4.73*
	F	185.16±3.06	171.1 ± 3.1**
HDL (mg/dl)	M	47.22 ±1.46	48.62 ±1.49
	F	48.33 ± 1.54	49.83 ±1.51
LDL (mg/dl)	M	105.93 ± 5.33	93.18±5.07
	F	112.5±3.56	103.27 ±3.5
LDL-C/HDL-C	M	2.3 ±0.11	2.0 ± 0.1*
	F	2.4 ±0.1	2.1 ±0.1*
TG (mg/dl)	M	116.62±4.54	93.56 ±4.21**
	F	121.55±4.123	90.11 ± 3.4**

M=Males, F=Females. Data are expressed as mean ± SE; significance at * ($p < 0.05$) - * * ($p < 0.01$).

Table (2) showed the effect of omega-3 intake on the CBC parameters of male and female volunteers. Data from this table showed that the platelets count exceptionally responded to omega-3 supplementation by around 10% decline from their original value.

Table (2): Erythrocytic counts, hemoglobin level, Hematocrit and platelets in healthy individuals before and after omega-3 supplementation

Groups / Parameters	Sex	Before omega-3 supplementation	After omega-3 supplementation
RBCs (n x 10^6µL)	M	5.02 x10^6± 0.04	5.14 x10^6±0.05
	F	4.55x 10^6±0.06	4.74 x10^6± 0.06
HB (g/dl)	M	14.38 ± 0.1	14.61±0.09
	F	13.67±0.16	13.9 ±0.16
Hematocrit (%)	M	43.14 ±0.3	43.83±0.29
	F	41.01± 0.5	41.7± 0.49
Platelets (n x 10^3µL)	M	242 x10^3±6.18	213 x10^3±6.07**
	F	239 x10^3±5.66	208 x10^3±5.15**

M=Males, F=Females. Data are expressed as mean ± SE; significance at * ($p < 0.05$) - * * ($p < 0.01$).

Data from table (3) showed that the absolute monocytic count experienced a significant decrease in response to omega-3 supplementation in both male and female participants. However, this decrease did not go beyond the normal reference value.

Table (3): The Total and differential count of leucocytes before and after omega-3 supplementation.

Parameters \ Groups	Sex	Before omega-3 supplementation	After omega-3 supplementation
Total leucocytes (WBCs x10^3 µL)	M	7.76 x10^3±0.22	7.52 x10^3±0.26
	F	7.05 x10^3±0.27	6.72 x10^3± 0.25
Neutrophil (Absolute x10^3)	M	5.02± 0.14	4.58±0.17
	F	4.43± 0.21	3.98± 0.16
Lymphocytes	M	2.09±0.10	2.37± 0.13
	F	1.99±0.09	2.21±0.12
Monocytes	M	0.47±0.021	0.36± 0.015**
	F	0.49±0.018	0.38 ±0.016**
Eosinophil	M	0.18± 0.03	0.21± 0.02
	F	0.14± 0.02	0.15 ± 0.02
Basophil	M	0.00	0.00
	F	0.00	0.00

A significant decrease in all inflammatory markers (CRP, IL-1&TNF-α) after 12 weeks of omega-3 intake was markedly demonstrated in table (4).

Table (4): The inflammatory markers before and after omega-3 supplementation.

Parameters \ Groups	Sex	Before omega-3 supplementation	After omega-3 supplementation
C RP (mg/dl)	M	0.40±0.02	0.32± 0.01**
	F	0.42 ± 0.02	0.33 ± 0.01**
IL-1(pg/ml)	M	2.72 ±0.07	1.91 ± 0.08**
	F	2.35 ±0.14	1.74±0.11**
TNF- α (pg/ml)	M	12.55±0.19	11.26 ±0.3**
	F	12.43±0.19	11.16 ±0.28**

4. Discussion

The pathogenesis of the atherogenic process is the outcome of an intricate interaction of serologial and cellular mechanisms. Disruption of serum lipid pattern has been known for its potential in the contribution of the pathogenic process[12]. Atherogenesis is initiated by accumulation of cholesterol-rich lipid strikes in the arterial wall favored by lipoprotein oxidation[13,14] ollowed by aggregation and retention[15,16,17] endothelial alteration, monocyte recruitment, and foam cell formation. Subsequent self-perpetuating chronic inflammatory response leads to further immune reactions and lipid deposition, which lead to atherosclerosis[18]. One possible mechanism to avoid atherosclerosis is the prevention of cholesterol deposition on the vessel intima via reverse cholesterol transport.

Data from the present study showed that, the administration of omega-3 for 12 weeks resulted in a significant decrease in the levels of the total cholesterol and triglycerides as compared with their levels in the same subjects before omega-3 intake. Similar findings have been reported by **Simopoulos (1991)[19]** who found that (n-3PUFAs) reduced plasma cholesterol and triglyceride concentrations by inhibiting the biosynthesis of very-low-density lipoproteins (VLDL) and triglycerides in the liver. Other studies had shown that n-3PUFAs supplementation decreased serum lipid concentrations and inflammatory markers. Thus, DHA supplementation reduced serum triglycerides by more than 25% in hypertriglyceridemic men. The hypolipidemic effect of omega-3 has been attributed to the inhibition of hepatic very low-density lipoprotein (VLDL)-TG synthesis and secretion that resulted in a secondary decrease in TG synthesis. This decrease in VLDL-TG secretion has been assumed to be due to the decrease in the expression of hepatic gene transcription factor, SREBP-1c, which is the key switch in controlling lipogenesis[20]. Other suggested triglyceride lowering mechanism by n-3PUFAs included the decreased activity of the key enzymes involved in TG biosynthesis, such as phosphatidic acid phosphohydrolase or diacylglycerol

(DG) acetyltransferase that catalyzes phosphatidate to DG and DG to TG, respectively [21]

The present study also attracted the attention to the possible role of omega-3 supplementation in modulating the in and out cholesterol transport across the blood vessel wall. This was most pronounced from the decrease in the LDL to HDL ratio caused by omega-3 administration.

Platelets aggregation is a major factor in the thrombotic process. The inflammatory events such as those observed in the early stages of atherosclerosis and triggered by the reduction in the mechanisms implicated in maintaining endothelial antithrombotic properties are very important for the disease pathogenesis. These include the activity of reactive oxygen species (ROS) generated by atherosclerotic risk factors[22], and an increased prothrombotic and pro-inflammatory mediators in the circulation[23]**.** Platelet aggregation is very much enhanced by platelet count increase even if the latter was within the normal range[24]**.** In view of data of the present study, where the circulating platelets count were significantly decreased after supplementation of omega-3, it is likely that this was also associated with a concomitant decrease in their adhesive properties. Consistent with this assumption **Hendra & Betteridge (1989)**[25] have reported that dietary omega-3 fatty acids inhibited platelet aggregation and exerted a favorable effect on coagulation profile in high and low doses. However the ability of omega-3 to reduce platelets number and their correlation with modulation of their aggregation potential needs further exploration.

The cellular mechanism of atherosclerosis emphasizes the role of monocytes/macrophage series in the contribution of this process as previously demonstrated. Through their migration to the subintimal layer, they start the release of inflammatory cytokines which leads the recruitment of other inflammatory cells and aggravation of the inflammatory process. Omega-3 supplementation to healthy volunteers suppressed the serum interleukin-1 (IL-1) and tumor necrosis factor (TNF-α) levels in the present investigation which was consistent with data reported by **Weber and Leaf (1991)**[26]**.**The decrease in the number the circulating monocyte count in response to omega-3 supplementation (table 3) may herald an explanation for the reduced IL-1 and TNF-α serum level. The exact mechanism of the decline in the monocytic count has not been resolved in the present study. It is most likely that omega3 intake had modulated the signals required to promote monocyte differentiation and/ or release from bone marrow. Despite the assumption that omega-3 –induced decline in IL-1 and TNF-α due to the decrease in the circulating monocyte number other mechanisms such as alteration in the cell membrane phospholipid fatty acid composition, disruption of lipid rafts, inhibition of activation of the pro-inflammatory transcription nuclear factor kappa B involved in the expression of inflammatory genes should not be ignored as demonstrated by **Calder (2003)**[27]**.**

The most extensively studied biomarker of inflammation in CVD is C-reactive protein (CRP) produced by the liver [28]. Among healthy populations, the (CRP) is also a powerful predictor of cardiovascular risk [29,30]. A cross-sectional study of healthy Australian adults showed an inverse association of CRP with n-3intake [31,32]. This was in agreement with the results in the present study, which showed a significant decreased in the concentration of CRP after omega-3 intake.

In conclusion, data from the present study emphasize the role of omega-3 fatty acids supplementation in apparently normal individuals from both sexes in modulating the lipid pattern, platelet count and inflammatory markers known to contribute to the atherogenic process in clinically predisposed individuals.

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