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Dietary Changes with Omega-3 Fatty Acids Improves the Blood Lipid Profile of Wistar Albino Rats with Hypercholesterolaemia

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ABSTRACT

Background: *Lipid profile is a reasonably reliable parameter for the assessment of cardiovascular risk, besides the anthropometric measurements. Serum lipid dysfunctions in the HDL and LDL components are commonly observed in cardiac patients. Omega-3 fatty acids exhibit a hypolipidemic potential which could be exploited in preventing the onset of this alarmingly increasing problem globally.* ***Aims:*** *To evaluate and compare the effects of different sources of omega-3 fatty acids, on the lipid profile parameters in rats induced with hyperlipidaemia.* ***Methods and material:*** *In our present study, we supplemented omega-3 oils from the plant source as well as the fish source to hypocholesteraemia induced Wistar albino rats for a period of three months. Wistar albino rats were fed normal chow along with 1% cholesterol for a period of three months to induce hypocholesteraemia. To this 1% flax oil and 0.1% fish oil were mixed separately and fed to two groups of rats for another period of three months to check for hypolipidemic effects if any.* ***Results and conclusions:*** *A significant reduction in total cholesterol, LDL, and glucose levels with increases in HDL levels in the flax oil as well as fish oil groups is observed. Also, a noticeable change though not significant was observed in the plasma triglyceride concentrations after the supplementation period. This significant hypolipemic effect by omega-3 fatty acids from both the sources, demonstrates their possible therapeutic use in patients with cardiac risk.*

Keywords: Hyperlipidaemia, omega-3 fatty acid, cardiovascular risk, fish oil, flax oil

Abbreviations: PUFA: Polyunsaturated Fatty Acid; EPA: Eicosapentaenoic Acid; DHA: Docosahexaenoic Acid; HDL: High Density Lipoprotein; LDL: Low Density Lipoprotein

INTRODUCTION

Arterial disease is not only one of the primary reasons of deaths globally, but also a major challenge affecting the health care economy. Nevertheless, many researchers suggest suitable lifestyle alterations to prevent major health detriments [1,2]. Several researches have demonstrated the association between cardiovascular risk and hypercholesterolemia [3]. Primarily this abnormality in lipid metabolism has origins either of a familial or genetic type. Secondarily it could have its origins in hepatic, renal or endocrine diseases [4]. Concomitant increases of the levels of triglyceride-rich VLDL and cholesterol-rich LDL is recognized as being associated with an increased risk of premature coronary artery disease [5]. Atherosclerosis is majorly understood to be a dysfunction of lipid metabolism. Increases in the concentrations of low density lipoproteins and decreases in high density lipoprotein concentrations are known alterations in the lipid transport system [6]. Atherosclerosis is also characterized by inflammation and dysfunction of the arterial lining apart from the build-up of cholesterol and lipids, which results in plaque formation, obstruction in the flow of blood along the vessels, and a diminished oxygen supply to the target organs [7]. LDL retention in the arterial wall [8] and its subsequent oxidation by free radicals triggers a series of biochemical reactions enhancing the inflammatory process [9,10]. The oxidized cholesterol products thus produced in arterial plaques and blood, alter the structure of the plasma membrane, rate of cell growth and apoptosis, and accelerate atherosclerosis [11].

Earlier studies show a strong correlation between high plasma cholesterol levels and increased cardiovascular and mortality risk. Hence the reduction of cholesterol is one of the major factors in improving the condition. This has paved the way to many researches in controlling hyperlipidaemia through dietary and lifestyle modifications. Dietary intake of omega-3 poly unsaturated fatty acids has been associated with decreased incidence of thrombosis as well as

atherosclerosis. Decrease in levels of plasma cholesterol and triglycerides, has been shown to reduce the aggregation of platelets, lower the risk of thrombosis, and prevent heart attacks and strokes. It has also been suggested that abnormal blood glucose concentration in normal as well as dyslipidaemia subjects forecasts the occurrence of cardiovascular problems later in life [12].

Omega-3 polyunsaturated fatty acids (PUFAs) supplements are usually consumed in the form of marine oils from fish or flaxseed oil from plants. While fish oil contains both docosahexaenoic acid (DHA, C22:6 w-3) and eicosapentaenoic acid (EPA, C20: 5 w-3), flaxseed oil contains only ALA, the parent omega-3 compound. Human trials indicate that w-3 fatty acids from either fish or fish oil may considerably reduce risk for cardiovascular problems, and untoward events too. Despite the supporting data, dietary awareness and endorsement has not been widespread [13]. Ample scientific evidence shows that EPA and DHA omega-3 fatty acids found in fish oil may help prevent the development of blood clots and plaque thereby helping treat atherosclerosis. Omega-3 fatty acids have also been shown to help decrease blood pressure, level of blood triglycerides and prevent heart disease. One preliminary study found that fish oil consumption by people with high cholesterol levels lowered their blood cholesterol about as much as people who took statins. Patients with a risk of cardiovascular problems or those who need to lower the blood triglycerides level may therefore benefit from consumption of fish oil supplements [14], especially those exhibiting hyperlipidaemia [15]. The bioavailability of the omega-3 fatty acid ALA from plant source is greater in oil than in milled or the whole flaxseed [16]. The present study was designed to evaluate and compare the effects of different sources of omega-3 fatty acids, on the lipid profile parameters in rats that were induced with hyperlipidaemia.

METHODS

The experiments were conducted according to international protocols for the use of animals in experimental studies and the study was approved by the King Fahd Medical Research Centre Animal Ethics Committee. During the entire treatment period, animals were kept at room temperature of 22°C ± 2°C with 12 h light/dark cycles. Male Wistar rats weighing between 150 g to 200 g aged 8 weeks were maintained at the animal house of the King Fahd Medical Research Centre for this study. Forty-Eight Male Wistar albino rats were randomly assigned to four cages of twelve animals each. The negative control Group I contained rats that were fed with standard rat chow and water ad libitum. Group II serving as positive control received standard rat chow and cholesterol for a period of 3 months to induce hypercholesterolemia [17]. High cholesterol diet was made weekly by adding 2% cholesterol to their standard chow for 12 weeks. The supplemented test Group III and Group IV were fed with standard rat chow, cholesterol, combined with flax oil (Group III) and fish oil (Group IV) respectively for the next 3 months. The flax oil dose of 1% and fish oil dose of 0.1% (containing 40% DHA) were added to their high cholesterol diet respectively.

All feed was administered orally. All rats were weighed every two weeks from the beginning till the end of the study. Blood collection was done at the end of the 12-week period.

Group allocation

Forty-Eight male rats were used in this study and they allocated randomly into the following groups:

Group I: Normal Control group rats (n=12).

Group II: Hyperlipidaemic group rats (n=12).

Group III: Hyperlipidaemic, flax oil treated group rats (n=12).

Group IV: Hyperlipidaemic, fish oil treated group rats (n=12).

Collection and preparation of blood samples

Blood sample collection was done using the orbital venous plexus bleeding technique. Serum was separated and used for the measurement of total Cholesterol, HDL-Cholesterol, LDL-Cholesterol, and triglycerides. Tail blood was taken and blood glucose estimated using a glucometer.

Analysis of serum cholesterol and plasma glucose

Total cholesterol, triglycerides and HDL-cholesterol were analyzed enzymatically using kits obtained from Randox Laboratories Limited, Crumlin, United Kingdom (UK). Plasma LDL-cholesterol was determined from the values of

total cholesterol and HDL-cholesterol using the Friedewald formula, LDL-c=TC-(HDL-c) [18]. Blood glucose levels was analyzed using the *in vitro* diagnostic test systems Brussels [19].

Statistical analysis

The data gathered were subjected to analyses of variances (ANOVA). The analyses were performed using the SPSS statistical software for Windows Version 20.0 (SPSS Inc., Chicago, IL, 2011). A value of $p<0.05$ was regarded as statistically significant. The data is presented as the mean ± standard deviation for the individual groups.

RESULTS

The body weight gain of the rats at the initial and at the end of the three-month period of the study is presented in Table 1. A significant body weight gain was observed in rats fed a high cholesterol diet as compared to the control group with p=0.000. Weight gain when compared to the hypercholesterolemia rats was significant in Group III rats fed flax oil with p=0.024 and not as appreciable in Group IV rats fed fish oil with p=0.44 as seen in Table 2. Feed consumption data shows a decrease in the feed consumption during weeks 4-8 in the cholesterol fed group (p=0.839), and the flax oil Group III (p=0.997) when compared to the control. Though a modest decrease in feed consumption was observed in the fish oil group (p=0.999) as compared with cholesterol group in week 6, the rats regained consumption soon after (Table 3 and Table 4).

Table 1 Weekly changes in rat weights after supplementation of flax oil and fish oil

Groups n=10	Control	Cholesterol fed group	Chol+Flax oil group	Chol+Fish oil group
Initial weight (g)	187.16	174.72	168.93	158.25
Week 2	198.18	221.94	209.12	204.07
Week 4	230.1	260.5	271.2	271.2
Week 6	257.31	291.6	296.03	276.13
Week 8	280.29	294.26	302.41	280.29
Week 10	288.08	333.87	318	288.08
Week 12	302.41	354.72	325.54	294.26
Weight gain	115.25	180	156.61	136.01
Compared to control group	-	64.75	41.36	20.76
Compared to cholesterol group	-64.75	-	-23.39	-43.99
Compared to control group	-	*0.000	*0.024	0.44

Table 2 Total weight gain in rats after supplementation of flax oil and fish oil

Parameter	N	Control Group I ± SD	Hypercholesterolemia Group II	Probability
Total Weight gain	10	115.3 ± 23.4	*180 ± 36.3	*0.000
Parameter	N	Control Group I ± SD	Hypercholesterolemia with Flax Oil Group III ± SD	-
Total Weight gain	10	115.3 ± 23.4	*156.6 ± 27.5	*0.024
Parameter	N	Control Group I ± SD	Hypercholesterolemia with Fish Oil Group IV ± SD	-
Total Weight gain	10	115.3 ± 23.4	136.01 ± 34.1	0.44

Table 3 Weekly feed consumption by each group

Groups n=10	Control	Cholesterol fed group	Chol+Flax oil group	Chol+Fish oil group
Week 2	27.83	26.86	26.73	27.06
Week 4	29	27.16	29.06	28.73
Week 6	29.93	27.23	29.43	28.96
Week 8	30.1	27.63	30.63	31.96
Week 10	30.3	28.8	33.9	33.7
Week 12	34.83	31.13	35.53	34.03
Total feed consumed	181.99	168.81	185.28	184.44
Compared to control	-	-13.18	3.29	2.45

Table 4 Total feed consumption by each group

Parameter	N	Control Group I ± SD	Hypercholesterolemia Group II ± SD	Probability
Total Feed consumption	10	182 ± 12.67	168.8 ± 25.29	0.839

Parameter	N	Control Group I ± SD	Hypercholesterolemia with Flax Oil Group III ± SD	-
Total Feed consumption	10	182 ± 12.67	185.3 ± 15.34	0.997
Parameter	N	Control Group I ± SD	Hypercholesterolemia with Fish Oil Group IV ± SD	-
Total Feed consumption	10	182 ± 12.67	184.46 ± 21.8	0.999

Table 5 Lipid profile levels in normal and hypercholesteraemic rats

Parameter	N	Control Group I ± SD	Hypercholesterolemia Group II ± SD	Probability
Total Cholesterol	10	1.355 ± 0.26	*1.633 ± 0.24	*0.007
HDL	10	1.181 ± 0.24	1.066 ± 0.123	0.427
LDL	10	0.335 ± 0.11	**0.534 ± 0.14	**0.000
Triglyceride	10	0.929 ± 0.34	1.097 ± 0.396	0.681
*p<0.05 denotes significant and; **p<0.001 denotes highly significant values. Significant changes in the total cholesterol, LDL, and blood glucose was observed in rats fed cholesterol.				

Table 6 Lipid profile in hypercholesteraemic and flax oil fed rats

Parameter	N	Hypercholesterolemia group 2 ± SD	Flax oil fed group 3 ± SD	Probability
Total Cholesterol	10	1.633 ± 0.24	*1.348 ± 0.14	*0.006
HDL	10	1.066 ± 0.123	*1.342 ± 0.8	*0.004
LDL	10	0.534 ± 0.14	*0.361 ± 0.06	*0.002
Triglyceride	10	1.097 ± 0.396	0.984 ± 0.24	0.875
*p<0.05 denotes significant and; **p<0.001 denoting highly significant values.				

Table 7 Lipid profile levels in hypercholesteraemic and fish oil fed rats

Parameter	N	Hypercholesterolemia group 2 ± SD	Fish oil fed group 4 ± SD	Probability
Total Cholesterol	10	1.633 ± 0.24	*1.328 ± 0.13	*0.003
HDL	10	1.066 ± 0.123	1.258 ± 0.19	0.067
LDL	10	0.534 ± 0.14	*0.408 ± 0.07	*0.036
Triglyceride	10	1.097 ± 0.396	0.841 ± 0.34	0.336
*p<0.05 denotes significant and; **p<0.001 denotes highly significant values.				

Total cholesterol and LDL levels reduced significantly, with appreciable changes in the HDL, and a little lesser in the triglyceride levels in the fish oil group.

Table 8 Effect of omega-3 supplementation on blood glucose

S. No	Groups	N	Blood glucose	p-value
1	Control group	10	6.8 ± 0.42	-
2	Hypercholesterolemia group	10	*8.26 ± 1.43	*0.025
3	Flax oil fed group	10	**6.1 ± 0.964	**0.000
4	Fish oil fed group	10	*6.87± 1.28	*0.036
*p<0.05 denotes significant and; **p<0.001 denoting highly significant values.				

A significant increase in blood glucose was observed in the hypercholesteraemic group while the flax oil and fish oil group displayed significant decreases.

Results for the serum lipid profile are presented in Tables 5-7. There were significant differences in the total cholesterol (p=0.007) and LDL levels (p=0.000) in the cholesterol fed Group II as compared to the control. But HDL and triglyceride levels did not show any significant changes when compared to the control group. The experimental Group III and Group IV exhibited positive changes in the lipid profile. The total cholesterol, and LDL levels of the flax oil group decreased significantly with p values 0.006 and 0.002 respectively and HDL levels increased significantly (p=0.004) when compared to the hypercholesteraemic group. The triglyceride concentrations of flax oil group decreased, though not to a significant level (p=0.875) as compared to the hypercholesteraemic group. The total cholesterol, and LDL values of the fish oil group significantly decreased with p=0.003, and 0.036 respectively when compared to hypercholesteraemic group. The HDL levels showed an increase though not significant (p=0.067) in the fish oil group as compared to the hypercholesteraemic group (Table 5). Triglyceride levels of fish oil group also decreased though not significantly (p=0.336) as compared to the hypercholesteraemic group. Blood glucose increased in Group II rats fed cholesterol as compared to the control with p=0.025. Also, both groups fed omega-3 fatty acids

showed significant decreases in blood glucose levels with p=0.000 for the flax oil group and p=0.036 for the fish oil group respectively as seen in Table 8.

DISCUSSION

Omega-3 fatty acids have emerged as pleiotropic molecules exhibiting varied biological actions that are beneficial in the management of inflammatory disorders like hypertension, cardiovascular disease, diabetes etc. These actions are modulated by cytokines and eicosanoids causing activation of the peroxisome proliferator activated receptor (PPAR) which in turn regulates gene expression of some key components of the fatty acid metabolism. Past researches show that elevated levels of the anti-inflammatory resolvins (produced from omega-3 fatty acids), activation of PPAR-α target genes of the lipid metabolism and up-regulation of nuclear factor (NRF2) mediated antioxidant enzymes, may be responsible for the beneficial effects noted [20]. The resultant increase in lipolytic activity thus favourably alters the lipid profile parameters. There are speculations of increase in LDL particle size to thereby reduce the atherogenic potential [21,22].

In the present study, we used the rodent model to test the effects of supplementation of low doses of omega-3 fatty acids from plant and fish sources on the lipid profile and glucose levels of hypercholesteraemic rats. There were significant differences between the experimental and control groups for most of the measured indices even on supplementation with low dosages.

There was a considerable though not significant reduction of serum triglycerides in rats treated with either the flax oil or fish oil after 12 weeks of treatment. A larger time and large sample size may show significant effects in the triglyceride levels too. This lowering of triglycerides in hyperlipidaemic rats after omega-3 supplementation has also been observed by earlier researchers [23,24].

In our study, we observed a considerable decrease in the serum concentrations of LDL and total cholesterol with a concomitant small increase in HDL concentrations which was consistent in both the flax oil as well as fish oil group. This finding is in agreement with Kobatake, et al. [24] who observed that omega-3 significantly reduced serum total cholesterol after 20 days of therapy in hyperlipidaemic subjects. It has been suggested that reduction of serum cholesterol may be due to inhibition of HMG-CoA reductase. This enzyme catalyzes the conversion of HMG-CoA to mevalonate which in turn decreases the cholesterol synthesis [25,26].

Our experiments showed a notable increase though not significant in the levels of HDL after 12 weeks of fish oil treatment which is quite in accordance with a previous study [27]. In our study, we have used lower dosage of fish oil (0.1%) containing the potent omega-3 fatty acids eicosapentaenoic acid and docosahexaenoic acid which also proved effective. HDL increase could possibly be influenced by significant reduction in total cholesterol levels in hyperlipidaemia by omega-3 fatty acids [28]. The mechanism underlying the increase in HDL-c levels observed during omega-3 therapy though is poorly understood. It might possibly be the decrease in VLDL-cholesterol production, and an increased removal of the same, or both processes occurring together [29]. There is also evidence that increases in HDL-c due to supplementation of omega-3 fatty acids may be related to the decrease in the activity of cholesteryl ester transfer protein. This decrease in transfer protein activity could be due to depletion of levels of very low-density lipoprotein and LDL particles as is observed in statin therapy [30].

Researches have shown that omega-3 fatty acid supplementation may cause a reduction of weight gain in the obese. A dose-dependent reduction in visceral fat associated with a decrease in adipocyte size was observed when supplementation of omega-3 fatty acids was done in rats on high fat diets. High omega-6 fatty acid intake leads to decreased insulin sensitivity in the muscle and promotes accumulation of fat in the adipose tissue. Nutritional approaches with dietary omega-3 fatty acids may therefore control body fat, improve insulin sensitivity, and reverse the dysregulated system [31]. Our results too show reduction in weights and an improved insulin sensitivity on supplementation with omega-3 fatty acids.

Earlier experiments show that rats fed a lard-fish oil diet exhibited lesser subcutaneous and visceral adipose tissue, suggesting that the omega-3 fatty acids present in fish oil may be protective against body fat accumulation This could possibly be due to the modulation of satiety in the obese and overweight subjects during weight loss. Most likely there is an altered gene expression favouring an increase in lipid oxidation in adipose, as well as other tissues and a reduced deposition of fat in the adipose tissue. Though preliminary evidence suggests an attenuation of postprandial

satiety, rat experiments report an improved body composition, and no reductions in food intake [31]. In our study, too, though food intake was not changed in the fish oil group and to a lesser extent in the flax oil group but rat weights were yet considerably lesser than in the hyperlipidaemic group fed only cholesterol. This may be suggestive that omega-3 fatty acids promote enhancement in the concentrations of lean tissue, thereby increasing metabolic rate and body fat reduction [31]. Earlier experiments on goslings too show that diets containing lower ratios of omega-6 to omega-3 fatty acids could inhibit fat synthesis by decreasing fat deposition.

The observed results indicate that the supplementation of omega-3 fatty acids from plant as well as fish source may induce substantial favourable alterations in the serum lipids profile of rats, within the physiological limits. The mechanisms for the effects of these improvements on lipid metabolism may be associated with the anti-inflammatory action of omega-3 fatty acids. Blood glucose concentrations also appear to be favourably improved due to omega-3 fatty acid consumption exhibiting an improved insulin sensitivity. Omega-3 fatty acids could therefore have a great therapeutic potential in averting risk for cardiovascular disease.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this paper.

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