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Hypocholesterolemic Effects of Polyunsaturated Fatty Acid Concentrate Prepared from Fish Oil

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Hypocholesterolemic effects of a concentrate of polyunsaturated fatty acids (PUFA) prepared from commercially available fish oil were evaluated in albino rats. PUFA concentrate containing about 76% of total polyunsaturated acids (36% of docosahexaenoic acid, (DHA) and 23% of eicosapentaenoic acid, (EPA) was incorporated in a hypercholesterolemic diet at 1% level and fed to rats for two different periods, viz., one month and three months. The results showed that one month was too short a period for the hypocholesterolemic effects to be manifested. Continuation of the same diet for three months resulted in lowering of serum cholesterol level to about 36% of that in the control group. Cholesterol content of the liver of the animals fed on PUFA-supplemented diet was more than two times that of the control group (2.71 and 1.17 g/100g, respectively). Total lipid content of the liver of the animals also showed a similar trend, suggesting that n–3 PUFA probably would lower serum cholesterol levels by effective redistribution of total cholesterol between serum and tissues.

Keywords: PUFA, Fish oil, DHA, EPA, Hypocholesterolemic effect.

Interest in the nutritional and pharmacological advantages of marine oils *vis–a–vis* atherosclerotic heart diseases has been on the rise ever since the epidemiological studies of Bang et al (1971) and Kromhout et al (1985) established the highly significant low incidence of these diseases among Greenland Eskimos. It was hypothesised that the biological activity of long chain n–3 polyunsaturated fatty acids (n–3 PUFA), mainly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) present in substantial quantities in Eskimo diets were responsible for the beneficial effects (Dyerberg 1986; Kagawa et al. 1982). Investigations have been conducted in animals and human volunteers on different aspects of the biological activity of long chain PUFA of marine origin. Earlier studies have shown that fish oil in the diet at 10% level, when substituted for coconut oil or groundnut oil, significantly lowered serum cholesterol levels in albino rats (Viswanathan Nair and Gopakumar 1981). However, divergent views exist on the role of fish oils and n–3 PUFA in lipid metabolism and their hypolipidemic properties. Spady et al (1995) have reported that n–3 PUFA lower total cholesterol in plasma of rats, fed diets enriched with cholesterol and saturated fatty acids but had no effect on liver cholesterol. It has been demonstrated (Ide et al. 1996) that fish oil containing very long chain n–3 PUFA such as EPA and DHA reduce serum lipid concentrations in animals and humans. Ikeda et al (1994) observed that in rats, DHA at a dose of 1g/100g diet significantly reduced plasma cholesterol concentrations compared with an α linoleic acid-rich diet, whereas the effects of an EPA -rich diet were between those of DHA and α linoleic acid diets. Kobatake et al (1984) demonstrated that serum cholesterol concentrations of young rats fed a hypercholesterolemic diet were significantly decreased by DHA and that triglyceride (TG) concentration was decreased by both EPA and DHA. DHA was found to be effective in lowering total serum cholesterol concentrations in hyperlipidemic patients after 8 weeks of administration. DHA–rich fish oil supplement consumed for 6 weeks by healthy male volunteers resulted in lower levels of plasma triglycerides and VLDL cholesterol and higher levels of HDL, HDL_2 –cholesterol and apolipoprotein B (Sanders and Hinds 1992). In an exhaustive review on the effects of fish oil on plasma and lipoprotein metabolism in humans, Harris (1989) has concluded that dietary n–3 PUFA lower plasma triglyceride levels virtually in all patients and total and LDL cholesterol are affected only in comparison with a diet richer in saturated acids.

The interest in fish oil and n–3 PUFA concentrate as hypolipidemic and antithrombotic agents is high and a new and rapidly developing field of fish oil concentrate is fast emerging. A basic limitation in the preparation of PUFA concentrate from fish oil is that the maximum EPA+DHA concentration that can be achieved for fish oil triglyceride is about 40% (Ackman 1992). Most of the fish oil concentrates available in the market have about 30% EPA+DHA, EPA being the major constituent. Higher concentrations of these acids are possible by hydrolysing the glycerides and

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concentrating the acids thus obtained. DHA is the major PUFA in most of the fishes from Indian waters and PUFA concentrates prepared from these fish oils have this acid as the major constituent. Stability and bioavailability of PUFA in the free state as against PUFA – enriched triglycerides is a factor, which may have strong influence on its hypolipidemic properties (Ackman 1992). Reconversion of separated and enriched PUFA to triglycerides may not be a viable idea, as it will involve considerable losses and other chemical changes in the PUFA. The influence of duration of administration of PUFA on lipid profile also merits serious study. Lu et al (1996) have reported that in a study lasting for 4 weeks only, n–3 PUFA actually raised VLDL and LDL levels in hamsters fed on diets supplemented with cholesterol at 5g/kg level. Our earlier studies on the influence of n–3 PUFA on lipid profile in albino rats were for a period of three months. The present study was, therefore, undertaken to investigate the hypocholesterolemic effects of free n–3 PUFA and the influence of the duration of administration of the acids in detail.

Materials and Methods

Six weeks old male albino rats (Wistar strain), bred in the Institute's animal house and each weighing around 100 g were used for the study. Twenty rats were divided into two groups of ten rats each. The rats were housed individually in polypropylene cages. About 10 kg of the basal diet containing casein (15%), salt mixture (USP) (4%), vitamin mixture (Chapman et al. 1959) (1%), cellulose (5%), cholesterol (1%) and methionine (0.1%) and corn starch to make it up to 90% was prepared. According to the daily requirement, an amount of this basal diet was mixed with coconut oil at 10% level for the control diet and coconut oil 9% + PUFA concentrate 1% for the test diet before feeding. The rats were fed on this diet. Feed and water were given *ad libitum*. PUFA concentrate used in this study was prepared in our laboratory from commercially available fish oil by removing the major part of saturated and monounsaturated fractions as urea inclusion compounds (Ackman et al. 1988). The concentrate was stored under nitrogen at -20°C, and only the required amount was taken for daily use.

After one month, (short term feeding) five rats from each group were sacrificed and blood, liver and heart were removed. Serum separated was analyzed for total and HDL cholesterol using Sigma diagnostic kit. Total cholesterol was estimated according to Sigma procedure No. 352 and quantitative isolation of HDL fraction of serum was done according to procedure No. 352–3.

Heart and liver were weighed and their sizes calculated as percentage of the body weight (Song and Wander 1991). Lipid from the tissue was extracted with chloroform–methanol mixture of 2:1 ratio (Folch et al. 1957). Fat was saponified and NS matter extracted according to AOAC (1990). Total cholesterol in the NS matter was estimated using ferric chloride (Rudel and Morris 1973).

The remaining five rats from each group were fed the same diet for 2 more months (long term, total 3 months) and then sacrificed. The analysis of serum, heart and liver was carried out as given above for the 1 month-fed rats. The data were analyzed statistically using ANOVA (Snedecor and Cochran 1973).

Results and Discussion

The animals in all the groups were in good health till the end of the experiment. Weight gain of the two groups is given in Table 1. Difference in weight gain between the experimental (PUFA supplemented) and control (unsupplemented) groups at the end of three months was not significant, though it was highly significant at the end of one month. Feed consumption was lower for the experimental group and this may be the reason for the lower weight gain for this group. The experimental feed was found to increase the liver lipid contents of the animals and the differences between the groups were highly significant at the end of three months. Lipid content of the liver of experimental group was 13.80%, while that of the control group was 7.18%. Weight of liver as a percent of body weight was somewhat similar for both the groups. Huang et al (1986) observed that lipid content of liver increased substantially in cholesterol–fed rats. But in the present study, it was not the effect of cholesterol alone, but appeared to be due to the influence of the n–3 PUFA in the diet. The difference in the lipid content of the liver was not observed in the short term feeding trial, i.e., at the end of 30 days. Dietary supplementation of long chain n–3 PUFA has been found to affect liver, adipose tissue etc. Parish et al (1991) reported that the mass of liver and spleen increased as a result of n–3 PUFA supplementation, whereas mass of adipose tissue decreased significantly. Rustan et al (1993) have observed decrease in epidermal tissue together with a decreased content of liver

TABLE 1. WEIGHT GAIN AND LIPID CONTENT OF HEART AND LIVER OF RATS FED PUFA SUPPLEMENTED DIET

	1 Month			3 Months		
	Control	PUFA fed	P value	Control	PUFA fed	P value
Weight gain, g	158.1 ± 10.29	112.4 ± 12.90	0.00026**	292.0 ± 19.3	217.7 ± 12.9	0.846#
Feed consumption, g	435.8 ± 37.70	361.3 ± 24.80	0.00612**	1402 ± 42.0	1201 ± 40.0	5.6 E-05**
Liver weight, g	8.6 ± 1.12	7.0 ± 0.93	0.0389*	13.85 ± 1.13	11.29 ± 0.52	0.0017**
Heart weight, g	0.8 ± 0.09	0.68 ± 0.50	0.0117*	1.09 ± 0.07	1.09 ± 0.02	0.9088#
Liver lipids, %	8.0 ± 1.20	8.7 ± 0.37	0.4795#	7.18 ± 0.52	13.82 ± 1.44	1.1 E-05**
Heart lipids %	3.6	2.75		3.1	3.65	

** Highly significant, P<0.01 * Significant, P<0.05

Not significant @ Statistical analysis not done

and muscle glycogen after 7–8 weeks of feeding long chain n–3 PUFA. Liver plays a vital role in regulating lipid metabolism and it seems n–3 PUFA exerts wide ranging influence in this control mechanism. The mechanisms involved and the biological significance of accumulation of lipid in the liver of PUFA fed rats are not well understood.

Cholesterol contents of serum, liver and heart of the rats are given in Table 2. The data clearly show that differences in serum total cholesterol and HDL cholesterol between the groups are highly significant. Total serum cholesterol in the experimental group was lowered to about 36% of that in the control group. The proportion of serum HDL cholesterol increased by about 30% in the PUFA-fed animals and the atherogenic index (Haglund et al. 1991) was lowered by about 46%. These results clearly show the very strong hypocholesterolemic effects of free n–3 PUFA at a level as low as 1% of the diet, when administered for a reasonably long period.

Several reports have appeared in literature, contradicting the hypocholesterolemic properties of n–3 PUFA. Lu et al (1996) have found that n–3 PUFA raised VLDL and LDL cholesterol concentrations in hamsters, when 5g/kg of cholesterol was supplemented in the diet. Field et al (1987) observed significant increases in total and HDL cholesterol in rabbits fed a menhaden oil diet. Demke et al (1988) and Harris et al (1988) have also reported similar findings. According to Suzukawa et al (1995), dietary supplementation of n–3 PUFA increases the oxidizability of LDL, which may counteract some of the beneficial effects of fish oil and therefore adequate amounts of antioxidants such as vitamin E should be incorporated in the diet. In the present study, no antioxidant was added to the diet, except traces of BHA/BHT, which was added at the time of preparation of PUFA concentrate, that might have remained in the PUFA preparation. Thus, the oxidizability of the LDL fraction due to presence of n–3 PUFA does not appear to be the sole reason for the contradictory observations.

The present data show that duration of feeding has a strong influence on the manifestation of the hypochloresterolemic effects of n–3 PUFA. Cholesterol concentrations in the rats-fed PUFA -supplemented

TABLE 2. CHOLESTEROL CONTENTS OF SERUM, LIVER AND HEART LIPIDS IN CONTROL AND PUFA SUPPLEMENTED RATS

	1 Month			3 Months		
	Control	PUFA fed	P value	Control	PUFA fed	P value
Cholesterol, total-liver, g/100g	1.407 ± 0.289	1.573 ± 0.08	0.168#	1.174 ± 0.17	2.71 ± 0.26	0.00011**
Cholesterol total-heart, mg/100g @	138.5	117.4	-	143.0	169.0	-
Cholesterol, total-serum, mg/100ml	72.6 ± 7.75	70.9 ± 4.00	0.6743#	135.9 ± 17.90	48.9 ± 7.30	8.4 E^{06} **
Cholesterol HDL, mg/100ml	-	-	-	34.4 ± 1.70	18.9 ± 2.90	8.3 E^{06} **
HDL cholesterol as % of total cholesterol @	-	-	-	25.3	38.5	-
Atherogenic index	1.16 ± 0.12	1.46 ± 0.099	0.1149#	2.95 ± 0.43	1.59 ± 0.64	0.01194*
Cholesterol consumed by each rat, g @	4.36	3.61	-	14.02	12.01	-

** Highly significant, P < 0.01 * Significant, P <0.05

Not significant @ Statistical analysis not done

diet for one month did not show any significant difference, when compared with the control group, whereas after three months, the effects were highly significant. In the light of this observation, the results of some of the short duration studies have to be re-examined.

When fish oil is used in the diet as a source of n-3 PUFA, a fairly high proportion of the oil (up to 20%) (Ackman 1992) is to be incorporated in the diet for obtaining a significant degree of hypocholesterolemic effects. Moreover, this proportion may vary depending upon the fatty acid composition of the oil. In the present study, a concentrate containing about 76% PUFA in the free acid form was used and the results confirmed that in rats, this concentrate was a powerful hypocholesterolemic agent at 1% level in the diet. The biological availability of n-3 PUFA from fish oils depends on the positional distribution of the fatty acids in the glyceride molecule. When free acids or methyl esters are included in the diet, they are available to the organ sites or even the walls of the vascular system in a format different from those originating from fish oils (Ackman 1992).

Another important observation was the high concentration of cholesterol in the liver of the animals-fed PUFA-supplemented diet. In this group, cholesterol level was about two times higher than that in the control group at the end of three months. There was no significant difference between the two groups after 30 days. The accumulation of cholesterol in the liver is coinciding with the depletion of serum cholesterol and therefore, the two phenomena seem to be inter related. A mechanism suggested for lowering of serum cholesterol levels by PUFA was the redistribution of cholesterol from serum to organs (Crocker et al. 1979). The shifts in serum lipid levels have been attributed to changes in hepatic secretion of very low density lipoprotein (VLDL) in receptor- mediated clearance of LDL by liver (Woollet et al. 1992, Wong et al. 1984). Influence of dietary n-3 PUFA on hepatic cholesterol balance is complex. They decrease cholesterol synthesis (Ventura et al. 1989) and secretion of VLDL (Benner et al. 1990; Nestel et al. 1984) and increase LDL receptor activity in rat liver.

Comparative hypocholesterolemic efficiencies of the two major n-3 PUFA (EPA and DHA) are not established beyond doubt. Some studies (Childs et al. 1988, Kobatake et al. 1984) suggest that the two acids have different effects on plasma cholesterol and triglyceride levels. But Spady (1993) had found that both EPA and DHA were effective in reducing the rate of LDL formation and stimulating LDL receptor activity. The PUFA concentrate used to supplement the feed in the present study had DHA as the major component (35.8%) and a lesser proportion of EPA (23.0%). Oils of most species of fishes of this region have DHA as the major polyunsaturated fatty acid and concentrates prepared from these oils, therefore, have DHA as the major PUFA. This study has shown that PUFA concentrate with a high proportion of DHA is a strong hypocholesterolemic agent.

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