

Effect of Fish Protein on Isoprenaline-Induced Alterations in Lipid and Antioxidant Status in Adipose Tissue of Rats

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The present study was designed to examine the protective effect of 20% fish protein in adipose tissue of isoprenaline-treated male albino rats. The protective effect was determined based on the following criteria: total body and adipose tissue weight, protein concentration in adipose tissue, total lipid in adipose tissue, concentrations of total cholesterol, triglycerides, free fatty acids and phospholipids of serum and adipose tissue and anti-peroxidative enzymes in adipose tissue. Fish protein feeding brought about a significant decrease in the total body weight and adipose tissue weight and in levels of adipose tissue total lipid, cholesterol, triglycerides and free fatty acids. Fish protein fed rats showed significant rise in adipose tissue protein content. It also prevented a rise in antiperoxidative enzymes catalase and superoxide dismutase and elevated the levels of reduced glutathione which indicates that fish protein protects against lipid peroxidation. Fish protein feeding has also led to lowering of serum cholesterol, triglycerides and free fatty acids and increased the proportion of HDL cholesterol. The protective effect of fish protein is believed to be due to its unique amino acids content namely, the reduced methionine: glycine and lysine: arginine ratios and increased sulphur containing amino acids.

Key words : Fish protein, adipose tissue, antiperoxidative enzymes, amino acid ratio.

Throughout the world the food and pharmaceutical industries are increasingly getting involved in identifying products that have the potential to prevent or retard the progression of degenerative diseases like cardiovascular disease, hypertension, obesity and cancer. Such degenerative diseases are diet related and have a major contribution to mortality worldwide (Ames *et al.* 1993, Gage, 2005). It is widely accepted that certain foods popularly referred to as functional foods may confer a health benefit protecting against or retarding the progression of degenerative diseases (Ivor, 1998). Dietary proteins are reported to influence lipid peroxidation and activities of antioxidant enzymes (Chiang & Kimura, 1991).

Populations consuming predominantly fish-based diet have always attracted attention due to low incidence of cardiovascular diseases among them. The positive effects of fish and its constituents on lipid parameters, antioxidant status and lipid peroxidation have generally been attributed to the n-3 Poly Unsaturated Fatty Acids (Morris, 1994). Very few studies directly address the role of fish protein in regulating lipid levels and lipid peroxidation. Fish protein may be considered a functional food as several reports substantiate its beneficial effects on human health. In a particular study it was established that feeding fish protein improved anti-oxidative defense status in various tissues like liver and heart

of rats and attenuated the development of hypertension (Dalila *et al.*, 2003). Work carried out earlier in our laboratory had established the lipid lowering effect, especially cholesterol lowering effect of fish protein in serum and heart of albino rats (Ammu *et al.*, 1989). Fish protein strengthens antioxidant system and is hypolipidemic. Myocardial infarction (MI) and other cardiovascular diseases are characterized by increases in lipid content in serum and tissues and lipid peroxidation in tissues including adipose tissue. Therefore fish protein may have beneficial effects in such conditions. Adipose tissue is well appreciated as a tissue important for normal physiological growth and metabolism and not just an energy reservoir for emergency situations (Mohammed-Ali *et al.*, 1989). The alterations induced by MI in heart have been well documented. It has been reported to cause oxidative stress and altered lipid metabolism in the myocardium resulting in infarct like necrosis of the heart muscle (Vijaya & Devi, 2000; Sasikumar & Devi, 2000; Rajdurai & Prince, 2006). Lipid metabolism and antioxidant status in adipose tissue under conditions of MI has not received much scientific attention. Hence the study was designed to investigate the potential of fish protein to protect against isoprenaline-induced alterations in adipose tissue, which has been increasingly recognized as a complex organ that affects an array of metabolic and other functions in the organism. Isoprenaline hydrochloride is 3, 4-Dihydroxy- α -[(isopropylamino) methyl] benzyl alcohol hydrochloride, a synthetic catecholamine well-characterized as lipolytic agent (Sreepriya *et al.* 1998).

Materials and Methods

Fish protein from rohu was prepared in the laboratory by defatting with hexane. All chemicals used were of analytical grade.

Twenty four male albino rats of Wistar strain weighing 100-120 g were obtained from Animal Facility of the institute. The animals were housed in polypropylene cages maintained at controlled temperature ($22 \pm 2^\circ\text{C}$) and 12-hour day and 12-hour night cycle. They were fed on feed formulated in the laboratory. Food and water were provided *ad libitum*. The animals were allowed to acclimatize with the laboratory conditions prior to the study. The animals were maintained for a period of 90 days; their body weights and feed intake per day were recorded. The present study was implemented according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India and authorized by the Animal Ethics Committee of the Institute.

The following experimental design was carried out. Four groups of six rats each were fed with the following diets:

Group I or normal control and group III or treated control were fed formulated diet mixed with casein, the protein source, at 20%. Group II or normal test and group IV or treated test were given commercial feed mixed with defatted-fish powder at 20%. Groups III & IV were given intra-peritoneal injection of isoprenaline at a dosage of 8 mg per kg body weight at the end of experimental period. The animals were sacrificed by anesthetizing with chloroform. Adipose tissue was dissected out, rinsed in 0.1 M phosphate buffered saline (pH 7.4), and stored at -20°C until further analysis.

Adipose tissue was weighed and homogenised in 0.1 M Tris buffer (pH 7.4). Fat was extracted from adipose tissue by the method of Floch *et al.*, 1957. Levels of total cholesterol (Parekh & Jung, 1970),

triglycerides (Rice, 1970), phospholipids (Fiske & Subba Rao, 1925) and free fatty acids (Horn & Menahan, 1981) in adipose tissue and serum were determined. HDL and LDL (Burnstein & Scholnick, 1972) cholesterol in serum were also determined. Antiperoxidative enzymes catalase (CAT) (Takahara *et al.*, 1960), superoxide dismutase (SOD) (Misra & Fridovich, 1972) and glutathione peroxidase (Pagila & Valentine, 1967) (GPX) were estimated in adipose tissue. Thiobarbituric acid reactive substances (TBARS) (Okahawa *et al.*, 1979), reduced glutathione (GSH) (Ellman, 1959) and protein Lowry *et al.*, 1951) content of adipose tissue were also determined. The amino acid composition (Ishida *et al.*, 1981) of proteins of control and experimental diets were analysed.

Values are expressed as mean $\pm$ SD for 6 animals. The differences between the 2 groups were analyzed by t test for comparison of various parameters and *P* value of less than 0.05 was considered significant.

Results and Discussion

Fish protein-fed rats exhibited lower body weight gain, final body weight, absolute and relative adipose tissue weight, but similar food intake compared with casein-fed rats (Table 1). On the other hand, isoprenaline treated control rats showed the highest body weight gain and final body weight, absolute and relative adipose tissue weights among all the groups. Fish protein-fed rats treated with isoprenaline had significantly lower levels of the above parameters, comparable to control rats. Fish protein feeding also led to increase of protein concentration in adipose tissue in Groups II and IV rats compared to Groups I and III where protein concentration was decreased.

Tables 2 and 3 show the lipid profile. Fish protein lowered serum cholesterol, total lipid, cholesterol, triglycerides, phospholipids and free fatty acids increased the fraction of HDL cholesterol and lowered LDL cholesterol compared to casein based diet. Isoprenaline led to increase in the above

Table 1. Body weight gain, final body weight, food intake, absolute and relative adipose tissue weight and adipose tissue protein in normal and experimental groups of rats.

	Group I	Group II	Group III	Group IV
Body weight gain g/day	4.2 $\pm$ 0.55	3.01 $\pm$ 0.28	5.84 $\pm$ 0.81 ^b	4.70 $\pm$ 0.57 ^d
Final Body weight g	329 $\pm$ 9.0	298 $\pm$ 5.0	388 $\pm$ 12 ^a	346 $\pm$ 10 ^c
Food intake g/day	15.68 $\pm$ 0.58	14.62 $\pm$ 0.86	15.9 $\pm$ 1.06	15.1 $\pm$ 1.92
Weight adipose tissue g (absolute)	4.37 $\pm$ 0.64	3.69 $\pm$ 0.22	5.14 $\pm$ 0.30 ^b	4.71 $\pm$ 0.61 ^d
Weight adipose tissue g/100g (Relative)	1.33 $\pm$ 0.2	1.23 $\pm$ 0.09	1.32 $\pm$ 0.05	1.36 $\pm$ 0.1
Adipose tissue protein mg/g	45.3 $\pm$ 2.6	67.5 $\pm$ 3.8	28.1 $\pm$ 6.5 ^a	41.4 $\pm$ 3.5 ^c

Group I, rats fed on regular rodent diet; group II, rats fed on defatted fish powder; group III, rats administered with isoprenaline; group IV, rats on defatted fish powder administered with isoprenaline.

Values expressed as mean $\pm$ SD for 6 animals in each group. ^a: *p*<0.001 and ^b: *p*<0.01 significant as compared to Group I, ^c: *p*<0.001 and ^d: *p*<0.01 significant when compared to Group III.

Table 2. Concentration of lipid fractions in the serum of normal and experimental groups of rats:

	Group I	Group II	Group III	Group IV
Triglycerides	72.4±2.4	68.2±3.6	108.4±11.6 ^a	83.6±6.4 ^c
Cholesterol	93.9±6.2	88.6±7.2	113.6±10.5 ^a	101.5±8.6 ^d
LDL C	53.2±3.6	39.7±3.1	74.3±5.8 ^a	58.1±6.5 ^c
HDL C	28.6±2.2	36.2±3.6	19.5±1.4 ^b	25.4±4.1 ^d
Phospholipids	86.5±5.8	81.1±8.8	106.8±7.2 ^a	98.3±4.9 ^c
FFA	16.6±1.8	10.3±2.2	26.2±3.8 ^a	18.7±1.7 ^c

Group designations are same as in Table 1. Values expressed as mg/dl serum and as mean ± SD for 6 animals in each group. ^a: p<0.001 and ^b: p<0.01 significant as compared to Group I, ^c: p<0.001 and ^d: p<0.01 significant when compared to Group III.

mentioned parameters which were brought towards normal on feeding fish protein in group IV. Isoprenaline administration (Group III) caused significant increase in the levels of these parameters in adipose tissue (p<0.001 & p<0.01) compared with normal Group I rats. In isoprenaline treated rats fed fish protein, the drug-induced changes were reversed, bringing the concentration of the lipid fractions to levels comparable to that of control group.

Table 4 shows the levels of antiperoxidative enzymes, reduced glutathione and TBARS in adipose tissue. Adipose tissue SOD and CAT activities were significantly lower whereas GPx activity was significantly enhanced in the group fed fish protein compared with casein. Isoprenaline treatment resulted in the activities of SOD and CAT to increase significantly (p<0.001)

while the activity of GPx was significantly reduced (p<0.01) compared with casein and fish protein based diets. In the group that were fed fish protein prior to isoprenaline injection, the activities of SOD and CAT were lowered, while the activity of GPx was increased to reach levels comparable to normal rats. Compared with casein, TBARS concentration was markedly decreased in adipose tissue of rats fed fish protein diet. Also feeding fish protein significantly enhanced the content of reduced glutathione compared with casein based diet. Levels of TBARS were significantly increased (p<0.001) and glutathione content reduced (p<0.001) in isoprenaline-intoxicated rats compared to both casein and fish protein diets. Fish protein rendered a protective effect to minimize the isoprenaline-induced changes in the antioxidant status in adipose tissue of Group IV animals by restoring the levels of

Table 3. Concentration of lipid fractions in adipose tissue of normal and experimental groups of rats:

	Group I	Group II	Group III	Group IV
Total lipid	649.4±12.1	587±8.6	684±14.8 ^a	658±11.6 ^c
Triglycerides	552±14.3	501±9.1	597±12.7 ^a	566±9.5 ^c
Cholesterol	38.9±4.2	27.3±2.5	56.8±3.8 ^a	43.9±2.7 ^c
FFA	19.47±1.6	11.6±3.1	24.3±2.4 ^b	21.2±3.3 ^d
Phospholipids	12.74±0.8	7.04±0.9	15.5±2.9 ^b	9.7±1.6 ^d

Group designations are same as in Table 1. Values expressed as mg/dl serum and as mean ± SD for 6 animals in each group. ^a: p<0.001 and ^b: p<0.01 significant as compared to Group I, ^c: p<0.001 and ^d: p<0.01 significant when compared to Group III.

Table 4. Activities of catalase, superoxide dismutase, glutathione peroxidase and concentrations of reduced glutathione and thiobarbituric acid reactive substances in adipose tissue of normal and experimental rats

	Group I	Group II	Group III	Group IV
CAT ¹	0.039±.004	0.028±.005	0.068±.003 ^a	0.045±.004 ^c
SOD ²	5.44±0.39	4.07±0.48	8.53±0.47 ^a	6.10±0.51 ^c
GPX ³	0.037±.005	0.058±.004	0.020±.005 ^b	0.031±.005 ^d
GSH ⁴	7.05±0.35	8.02±0.5	3.58±0.83 ^a	5.97±0.43 ^c
TBARS ⁵	2.24±0.35	1.20±0.23	8.38±0.86 ^a	4.18±0.70 ^c

Group designations are same as in Table 1. Values expressed as mean ± SD for 6 animals in each group. ^a: p<0.001 and ^b: p<0.01 significant as compared to Group I, ^c: p<0.001 and ^d: p< 0.01 significant when compared to Group III. ¹: catalase, umol/min/mg protein; ²: superoxide dismutase, U/mg protein; ³: glutathione peroxidase, U/g protein; ⁴: reduced glutathione, nmol /mg protein, ⁵: thiobarbituric acid reactive substances, nmol/g tissue.

the antiperoxidative enzymes and reduced glutathione and lowering the levels of TBARS.

Table 5 describes the composition of the normal control diet and the experimental diet containing fish protein. Amino acid composition of the proteins of the control and experimental diets, are given in table 6. In the casein-based diet, the ratio of methionine to glycine is 1.05 and lysine to arginine is 2.06 whereas in case of fish protein-based diet the former is 0.20 and the latter is 1.14.

Although food intake was similar, rats fed fish protein diet exhibited significantly lower body and adipose tissue weights

Table 5. Composition of the diets fed to the control and experimental groups of rats

g/Kg	Casein (Control) Diet	Experimental Diet
Casein	200	-
Defatted fish powder	-	200
Methionine	1.0	1.0
Salt mixture	40	32.0
Cellulose	50	50
Vitamin Mixture	10	10
Coconut oil	100	98.8
Corn starch	599	599
Water	-	8.2

compared with those fed on casein diet (table 1) or those treated with isoprenaline. The data suggests the probability of development of visceral obesity that would eventually cause peripheral insulin resistance in isoprenaline fed rats. Isoprenaline administration is known to cause insulin resistance (Kirsch *et al.*, 1983) which may be a direct consequence of gain in body and adipose tissue weight as evidenced by the data in table 1. Isoprenaline causes breakdown of myocardial lipids that spill into the blood and eventually are taken up by the peripheral tissues including the adipose tissue, contributing to the weight gain. Isoprenaline may cause enhanced lipolysis in adipose tissue leading to the increase in lipid parameters. Prior feeding of fish protein reversed these changes in group IV rats and conferred a protective effect. The beneficial effects of fish protein are likely to reflect better insulin sensitivity caused by a lower weight. It has been demonstrated that fish protein improves glucose tolerance and insulin sensitivity compared with casein in rats (Charles *et al.*, 2000). In the study hypertriglyceridemia and elevated levels of free fatty acids are seen in casein fed isoprenaline treated rats and their levels decreased in rats on fish protein based diets. In cases of peripheral insulin resistance, hypertriglyceridemia and elevated levels of

free fatty acids are found to occur, further impairing glucose utilization (Broden, 1999; Grundy, 1999). This is in good agreement with those published in other studies (Hurley *et al.*, 1995; Iritani *et al.*, 1996) showing the hypolipidemic effect of fish protein compared with casein in rats. Fish protein-fed rats showed lower levels of free fatty acids in adipose tissue compared to casein-fed rats which may be due to decreased activity of lipoprotein lipase. According to Beynen & Sugano (1990) increased insulin sensitivity, resulting from feeding fish protein, may decrease tissue fatty acid mobilization and, in turn, decrease synthesis and secretion of VLDL triglycerides from the liver, reducing plasma triglyceridemia, thus leading to weight loss. Interestingly, Demonty *et al.* (1998) recently demonstrated that LPL activity was lower in skeletal muscle of rats fed cod and soy proteins than of those fed casein. It is therefore possible that feeding fish protein lowered the activity of LPL activity in adipose tissue that in turn can reduce the supply of lipids reaching the blood stream and peripheral tissues, conferring a lipoprotective effect.

In the present study fish protein feeding lowered the plasma cholesterol levels compared to casein. Isoprenaline injection brought about an increase in plasma cholesterol which was normalized through prior feeding of fish protein in group IV. The low plasma cholesterol is the result of decreased synthesis of cholesterol in liver due to diminished activity of HMG CoA reductase (Zhang & Beynen, 1993). This may be attributed to the increased levels of arginine in fish protein, which is more than 1.5 times higher than casein. Fish protein feeding might enhance the plasma arginine content which in turn elevates glucagon levels (Sugano, *et al.* 1984). Glucagon inhibits HMG CoA reductase activity (Beg & Brewer, 1982). Also studies

describe that fish protein in diet contributes to weight loss by increasing the proportion of HDL cholesterol, and by lowering acyl-CoA: cholesterol acyltransferase activity (Sachs *et al.* 1994). The hypolipidemic effect of defatted fish powder may also be due to its low methionine-glycine and lysine-arginine ratios (table 6) unlike casein in which both the ratios are significantly high. It had been reported by Gudbrandsen *et al.* (2005) that feeding proteins with low methionine-glycine and lysine-arginine ratios, reduced total cholesterol, and cholesteryl esters in plasma and liver, increased fecal cholesterol and bile acid levels and increased the hepatic

Table 6. Amino Acid Composition (g/Kg protein) and ratios of amino acids of Dietary Proteins fed to the control and experimental groups of rats

Amino Acid	Casein	Defatted Fish Powder
Asp	66	80
Thr	46	41
Ser	55	53
Glu	207	130
Pro	116	58
Gly	18	123
Ala	30	69
Cys	7	12
Val	46	41
Met	19	25
Ile	42	28
Leu	92	55
Tyr	58	42
Phe	44	21
His	34	14
Lys	74	70
Arg	36	61
Glycine	18	123
Methionine	19	25
Lysine	74	70
Arginine	36	61
Amino acid ratios		
Methionine / glycine	1.05	0.20
Lysine / arginine	2.06	1.14

gene expression of cholesterol 7 α hydroxylase, suggesting that the mechanism of action of such proteins was via the enterohepatic circulation. Also, the content of sulphur containing amino acids methionine, cysteine and taurine is more in fish protein and there are studies that report that diets supplemented with these amino acids lower plasma cholesterol concentration in rats (Kawasaki *et al.*, 1998). Isoprenaline intoxicated rats fed fish protein thus register a decrease in the lipid parameters through enhanced insulin sensitivity provided by fish protein in addition to directly lowering lipid parameters. It is probable that fish protein, by virtue of its low methionine-glycine, lysine-arginine ratios and high sulphur containing amino acids has the above effects on lipid metabolism which has negated the effects of isoprenaline administration.

Isoprenaline administration causes oxidative stress in the heart (Guo-Zhing *et al.*, 2005; Rathore *et al.*, 1998). In the study increase of antioxidant enzymes SOD, CAT and elevation in TBARS; and decrease in GPx and reduced GSH occurred in adipose tissue of isoprenaline-intoxicated rats suggesting the occurrence of oxidative stress. Source of lipid peroxidation is free radicals and in cases where their generation is more as indicated by elevated TBARS, the activity of scavenging enzymes (SOD and CAT) increases, as seen in this group. A decrease in GPx activity in this group is explained by the fact that isoprenaline causes depletion of reduced GSH which leads to lowering of GPx activity as the later requires GSH for its activity (Stadman, 1980). Fish protein feeding causes significant lowering of the activities of SOD and CAT as expected since the incidence of lipid peroxidation is significantly low. On the other hand, fish protein enhances the antioxidant status of adipose tissue by elevating the levels of reduced GSH which is the cause for elevation in the

activity of GPx in fish protein fed rats. Boukourt *et al.* (2004) reported that fish protein improves total antioxidant status in experimental rats with induced diabetes. In group IV rats, fish protein conferred protective effect against isoprenaline intoxication by strengthening the antioxidant system of the adipose tissue. Fish protein, on the one hand, reinforces the antioxidant defences, and on the other hand, by virtue of presence of certain amino acids, such as arginine and glycine, directly scavenges oxygen free radicals (Fang *et al.*, 2004). Fish protein seems to have a preventive effect against oxidative stress, and this is very promising for application in the treatment of diseases involving free radicals like hypertension.

In conclusion, defatted fish protein exhibits a protective effect in adipose tissue against isoprenaline-induced alterations through its antilipidemic and antioxidant activities. Since a variety of life-style diseases like atherosclerosis, cancer, hypertension, obesity, cancer etc. seem to involve among other factors, discrepancies in lipid metabolism and oxidative stress, it is likely that inclusion of fish in diet may seriously cut down on the risk of acquiring these diseases.

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