Influence of Dietary Proteins on Cholesterol levels in Albino Rats

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Six groups of albino rats were fed identical diets, differing in their protein sources for sixty days. The protein sources used were fat free casein, defatted groundnut cake, and defatted fish powders from three species of fishes, namely, the fresh water fish *Labeo rohita,* the marine fishes, *Rastrelliger kanagurta* and *Otolithus argenteus* and marine prawns, *Parapenaeopsis stylifera.* After sixty days, the levels of cholesterol in the serum, liver and heart of the rats were estimated. The casein group recorded the highest level of cholesterol. Compared to casein, the groundnut protein was distinctly hypocholesterolemic. The fish proteins had a still greater cholesterol lowering ability. Of the proteins used in this study, the proteins of prawns had the maximum hypocholesterolemic effect. An attempt is made to correlate the behaviour of the proteins in this respect, with their respective amino acid compositions.

The influence of diet on the serum cholesterol levels was known since long. Quality and quantity of various dietary components have been found to influence the serum cholesterol levels. It has been established that saturated fats in the diet generally tend to be hypercholesterolemic while fats rich in polyunsaturated fatty acids lower serum cholesterol levels (Kinsell *et al.,* 1952; Kritchevsky *et al.,* 1954; Ahrens, 1957; Peifer *et al.,* 1962; Feigenbaum *et al.,* 1961; Jones, 1974). The importance of the quality of dietary proteins in controlling the levels of serum cholesterol, has also been pointed out (Kritchevsky, 1959; Carroll & Hamilton, 1975; Hamilton & Carroll, 1976; Huff *et al.,* 1977; Huff & Carroll, 1980; Forsythe *et al.,* 1980). It was postulated that vegetable proteins generally lower cholesterol levels in serum, whereas animal proteins tend to elevate the levels. Soyabean protein was the vegetable protein used in most of these studies and casein was the standard protein used for comparison. Very few studies used egg, meat or fish as the protein source.

It was observed that even in the presence of a known cholesterol elevating agent like coconut oil, the fatty fish, *Sardinella longiceps* in the diet could bring down the serum cholesterol level in albino rats (Viswanathan Nair & Gopakumar, 1981). This effect was thought to be due to the high content of polyunsaturated fatty acids in the fish oil. However, subsequent studies revealed that fish protein also has a hypocholesterolemic effect (Viswanathan Nair *et al.,* 1985). But the effect of species difference, if any, was not studied. The present study was undertaken to compare the effect of different dietary proteins on the cholesterol levels in albino rats.

Materials and Methods

Six weeks old male albino rats (Wistar strain), weighing around 100 g were used for the study. They were divided into six groups of five rats each and were housed individually. The diets for the six groups were identical in all respects except the protein source. The fish powder samples used were prepared from edible portions from the fresh fishes, rohu *(Labeo rohita),* mackerel *(Rastrelliger kanagurta),* jew fish *(Otolithus argenteus)* and prawns *(P. stylifera).* The minced fishes were dried in an electrically operated hot air tunnel drier. The dried muscle samples were powdered and defatted thoroughly by repeated extraction with petroleum ether. Solvent was distilled off and last traces of the solvent were removed under vacuum. The groundnut cake used was also defatted in a similar way. Casein used was fat free. Protein content of all these powders were estimated by the Kjeldahl method.

Protein contents of all the diets were adjusted to be 15% by taking the appropriate powder in required quantities. 32% glucose, 5% refined groundnut oil, 4% salt mixture (Hubbel *et al.,* 1937) and 1% vitamin mixture (Chapman *et al.,* 1959) in cellulose and 0.5% cholesterol were added to these proteins and the diets made upto 100% by adding the required amount of corn starch.

Rats were first adapted to the respective diets by feeding for five days and then their initial body weight was noted. After this, they were fed on the respective diets for 60 days. Feed and tap water were supplied *ad libitum.* Weight gain was noted at weekly intervals. At the end of 60 days the rats were starved for 24 hours and sacrificed. Blood, liver and heart from each rat were collected separately and serum was separated by centrifugation of the blood samples. Tissues were kept frozen at –18°C pending analysis.

Total and free cholesterol in serum samples, lipids from liver and heart were determined by the method of Rudel & Morris (1973). Total lipids from the liver and heart tissue samples were extracted by the method of Bligh & Dyer (1959). Total and free cholesterol in these samples were separated by thin layer chromatography on silica gel G using a solvent system of hexane: ethyl ether: glacial acetic acid (80:20:1) The plates were developed by exposure to iodine vapour and the spote scraped and quantitatively extracted with chloroform. Cholesterol in the extracts was then estimated by the above method. Amino acid compositions of the dietary proteins were determined using a Technicon NC–2P amino acid analyser.

Results and Discussion

All groups of rats, except the groundnut protein group, recorded very good weight gain till the endof 4 weeks. After 10 weeks, gain in weight was slower in all cases. Table 1 gives the amino acid composition of the protein sources for the six diets. Compared to the other proteins, groundnut protein is deficient in lysine, leucine and isoleucine. This explains the poor rate of growth

Table 1. *Amino acid composition of the different dietary proteins (g/16 g N)*

Amino acid	Jew fish protein (Diet 1)	Mackerel protein (Diet 2)	Prawn protein (Diet 3)	Rohu protein (Diet 4)	Casein (Diet 5)	Groundnut protein (Diet 6)
Taurine	1.30	1.38	0.38	0.25	—	—
Aspartic acid	10.74	13.00	9.52	8.10	6.80	9.20
Threonine	5.20	6.86	3.75	3.85	4.90	2.50
Serine	4.20	5.00	3.73	3.10	3.06	2.70
Glutamic acid	20.16	20.26	20.16	14.50	22.00	15.30
Glycine	4.99	6.96	5.60	4.25	5.40	4.60
Alanine	6.69	7.50	5.80	5.70	2.45	3.55
Valine	11.14	7.15	4.40	4.90	5.35	3.09
Cystine	0.25	—	0.95	1.20	0.40	2.10
Methionine	1.51	2.56	3.06	3.02	2.05	1.09
Isoleucine	3.97	6.40	3.90	3.60	4.35	2.38
Leucine	9.25	11.48	8.30	6.40	7.42	4.40
Tyrosine	4.57	4.90	2.10	2.03	5.90	4.50
Phenylalanine	3.00	4.03	5.00	2.70	5.50	4.80
Histidine	3.80	11.24	2.30	2.13	3.50	3.24
Lysine	8.29	10.30	9.50	10.20	8.20	3.50
Arginine	3.34	4.25	4.50	4.60	2.80	9.20
Tryptophan	1.15	1.33	—	1.65	1.35	1.40
Proline	3.99	7.30	2.25	2.94	12.82	9.30

observed in this group in the active growth period. But after 4 weeks' feeding, weight gain was found to decrease in the casein fed group also, which probably indicates a change in the amino acid requirements after this stage. It is known that casein is a balanced dietary protein only during the active growth period. The amino acid requirements of the adult rat are better met by fish proteins (Mukundan *et al.,* 1986).

Cholesterol levels in the serum, liver and heart of the different groups of the rats are presented in Table 2. The data clearly shows the influence of dietary protein on serum cholesterol levels. The variations were not as pronounced in heart and liver as in serum though the trend was generally the same. The casein group recorded the highest level of serum cholesterol followed by the groundnut protein group and then by the four fish protein groups. Among the fish based groups, prawn protein group showed the lowest level of serum cholesterol. As the protein sources in all cases were thoroughly defatted and as the diets were identical in all other respects, including fat source, the variations can be attributed to the protein component only. Viswanathan Nair *et al.* (1985) had ruled out any effect due to residual lipids in the protein components, as fatty acid composition of different diets with the different protein sources but having the same fat source did not show any variation. Differences in the total intake of diet between the groups were marginal. In general, the intake was higher in all fish based diets compared to other diets. So intake of dietary cholesterol is also likely to be more in these groups. Inspite of this, these groups recorded lower levels of cholesterol. Variations in any other micronutrient components in these diets were not considered in this study.

Compared to the casein group, the groundnut protein group recorded a decrease in serum cholesterol to the extent of 45%. When casein was substituted by fish proteins in the diet, serum cholesterol levels showed a decrease by nearly 68%. Compared to the groundnut protein group, fish protein group registered a decrease in serum cholesterol to the extent of about 40%, showing the pronounced cholesterol lowering ability of fish proteins. The same trend was seen in the values for esterified cholesterol also. While casein group had a serum esterified cholesterol: serum free cholesterol ratio of 2.28 ± 0.25 the groundnut protein group gave a value of 2.02 ± 0.14, whereas in all the fish based groups, the corresponding value was around 1.7 only. The values followed the same pattern in levels of cholesterol in heart

Table 2. *Cholesterol levels (mean values with standard deviation) in serum, heart and liver of the six groups of rats given different dietary proteins*

	Serum TC mg/100 ml	Serum FC mg/100 ml	Serum EC mg/100 ml	Serum EC/FC	Heart TC g/100g	Heart FC g/100g	Heart EC mg/100 g	Heart EC/FC	Liver TC g/100g
Diet 1	51.35 ± 0.89	18.25 ± 1.56	33.00 ± 0.94	1.81 ± 0.21	0.174 ± 0.034	0.148 ± 0.040	26.00 ± 11.24	0.150 ± 0.010	0.873 ± 0.094
Diet 2	52.50 ± 1.77	19.90 ± 2.45	32.60 ± 2.01	1.67 ± 0.32	0.165 ± 0.020	0.134 ± 0.030	31.00 ± 3.40	0.230 ± 0.012	0.861 ± 0.252
Diet 3	43.15 ± 2.55	15.45 ± 1.55	27.70 ± 1.89	1.78 ± 0.19	0.131 ± 0.019	0.117 ± 0.017	14.00 ± 2.16	0.120 ± 0.010	0.62 ± 0.106
Diet 4	55.00 ± 6.07	20.22 ± 1.70	34.78 ± 4.55	1.71 ± 0.13	0.174 ± 0.017	0.146 ± 0.014	27.00 ± 2.70	0.190 ± 0.010	0.706 ± 0.079
Diet 5	156.20 ± 11.03	48.02 ± 6.98	108.18 ± 4.80	2.28 ± 0.28	0.180 ± 0.022	0.145 ± 0.018	35.00 ± 3.77	0.240 ± 0.014	1.289 ± 0.305
Diet 6	84.80 ± 4.77	28.12 ± 1.80	56.68 ± 3.70	2.02 ± 0.14	0.173 ± 0.017	0.145 ± 0.014	28.00 ± 3.29	0.190 ± 0.011	0.722 ± 0.098

TC = Total Cholesterol; FC = Free Cholesterol; EC = Esterified Cholesterol.

also. However, the variations in cholesterol in the liver did not strictly follow the same pattern.

The results were subjected to analysis of variance to test the statistical significance of the variations (Table 3). Variations in total, free as well as esterified cholesterol in serum were found to be highly significant ($P < 0.001$) when values for all the six groups were compared statistically. However variations between the values for the three fish based groups were not found significant at 1% level. When comparison is made between the four fish based (including prawn) groups, values, again became significant at 1% level, showing the significant cholesterol lowering effect of prawn proteins. The esterified to free cholesterol ratio in the heart was noticeably low in the group fed prawn proteins whereas in casein and mackerel proteins groups this ratio was high.

The lipoprotein receptors play a crucial role in the uptake and degradation of cholesterol carrying lipoproteins and thus regulate the plasma cholesterol level in man (Michael *et al.*, 1981). But certain proteins lower the serum cholesterol level to a greater extent compared to the other proteins. The mechanism involved in this is not clear.

Different groups of workers have put forward divergent theories to explain the hypocholesterolemic effect of dietary proteins (Kritchevsky, 1979; Huffe & Carrol, 1980, Olson *et al.*, 1970). However all seem to agree that the amino acid composition of the dietary protein plays a critical role in the cholesterol metaboilism.

It was suggested that the lysine: arginine ratio in a protein is the critical factor deciding its role in cholesterol metabolism (Nagata *et al.* (1981). The higher the ratio, the more atherogenic the protein is likely to be. It was reported that lysine inhibits liver arginase activity and will thus make more arginine available for incorporation into an arginine rich apolipoprotein which is known to be atherogenic (Goldstein & Brown, 1977). Rajamohan & Kurup (1986) postulated that a lysine to arginine ratio of 1.0 conferred hypocholesterolemic properties to the dietary protein whereas values above or below 1.0 for this ratio lead to noticeable decrease in their hypocholesterolemic properties. The present study does not show any clear relation between the lysine: arginine ratio and the behaviour of the protein with regard to its cholesterol lowering effect (Table 4). Prawn proteins which was most effective in lowering serum cholesterol had almost the same lysine: arginine ratio compared to casein and fish proteins. But groundnut protein having a significantly lower lysine: arginine ratio did not show a corresponding pronounced hypocholesterolemic effect. The ratio $\frac{\text{Ileu, Leu, Thr, Tyr, Ser}}{\text{Arg, Gly, Glu}}$ (Huff & Caroll, 1980) and the ratio glutamic acid to essential amino acids (Olson *et al.*, 1970) were also suggested as the critical factors. Some other reports

Table 3. *Statistical analysis*

Groups	Serum TC	Serum FC	Serum EC	Serum EC/FC	Heart TC	Heart FC	Heart EC	Heart EC/TC	Liver TC
1–6	S	S	S	S	NS	NS	S	S	S
1–4 & 6	S	S	S	NS	—	—	NS	—	NS
1–4	S	S	S	—	—	—	—	—	—
1, 2 & 4	NS	NS	NS	—	—	—	—	—	—
1, 3, 4 & 6	—	—	—	—	—	—	—	—	—

S = Significant at 1% level
NS = Not significant at 1% level
— = Not calculated
Groups : 1 = Jew fish, 2 = Mackerel, 3 = Prawn, 4 = Rohu, 5 = Casein, 6 = groundnut cake

suggested that the actual content of specific amino acids like lysine and arginine (Kritchevsky, 1979) methionine (Hamilton & Carroll, 1976) and glycine (Hermus et al., in Lancet 11, 48 cited by Nagata *et al.*, 1981) in a protein decided its hypocholesterolemic properties, These ratios for the proteins used in this study are presented in Table 4. However none of these could explain the pronounced cholesterol lowering property of fish proteins satisfactorily.

Table 4. *Comparison of the various suggested amino acid ratios for the six dietary proteins tested*

Diet	Lys/ Arg	(*Ileu, Leu, Thr, Yyr, Ser*)/ (Arg, Gly, Glu)	*Glutamic acid* to the essential amino acids	Methionine content	Alanine/ Proline
Diet 1	2.48	0.95	0.398	1.51	1.67
Diet 2	2.40	1.10	0.308	2.56	1.02
Diet 3	2.10	0.72	0.450	3.06	2.58
Diet 4	2.20	0.81	0.330	3.02	1.94
Diet 5	2.90	0.85	0.480	2.05	0.19
Diet 6	0.38	0.57	0.430	1.09	0.38

An examination of the data on the amino acid composition of the proteins tested (Table 1), on the other hand, reveals another feature. In both casein and groundnut protein, content of alanine was significantly low and proline recorded noticeable high values. The four fish proteins resembled each other in their content of these amino acids. But prawn proteins which had the maximum hypocholesterolemic effect had a high alanine and a very low pıoline content. The alanine: proline ratio of a protein seems to have a better correlation to its hypocholesterolemic property. The data shows that the higher this ratio for a protein, the more hypocholesterolemic it will be.

Variations in the total period of feeding may also explain the divergent results reported by different workers. Age is an important factor in determining the requirements and utilization of dietary proteins (Mukundan *et al.*, 1986).

Interactions between the dietary proteins and lipids is another factor to be taken into consideration. Viswanathan Nair *et al.* (1985), suggested that dietary fish protein, in some way, interfered with the incorporation of dietary fatty acids into serum lipids. Casein diets, when given with corn oil, were not found to be hypercholesterolemic; but corn oil did not reduce cholesterol levels to any significant extent in animals fed on soya protein (Carroll & Hamilton, 1975). All these results suggest that the influence of dietary protein and lipids on the serum cholesterol levels, cannot be an isolated effect of any single dietary component.

Minerals like copper, vanadium, chromium etc. and dietary fibre are also reported to influence cholesterol metabilism (Linder, 1985). Jaya & Kurup (1987) found that magnesium in diet is also critical in determining the serum cholesterol levels. Dietary zinc is also known to influence the lipid levels in serum. But in the present comparative study using identical diets differing only in the protein sources, the effect of minerals contributed by the protein source, if any, has not been taken into consideration. It is clear from the results that dietary protein plays a critical role in determining the serum cholesterol levels, and that, in this respect fish proteins are better than even vegetable proteins.

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