Full Length Research Paper

Effect of soy protein on serum lipid profile and some lipid-metabolizing enzymes in cholesterol fed rats

E. C. Onyeneke[1], O. M. Oluba[1], S. I. Ojeaburu[1]*, O. Adeyemi[2], G. E. Eriyamremu[1], K. E. Adebisi[1] and O. Adeyemi[2].

[1]Department of Biochemistry, University of Benin, P. M. B. 1154, Benin-City, Nigeria.
[2]Department of Biochemistry, Adekunle Ajasin University, P.M.B. 1, Akungba Akoko, Ondo, State, Nigeria.

The effect of soy protein on serum lipid profile and some lipid metabolizing enzymes in rats fed with cholesterol diets was examined in this study. Rats were subjected to feeding trial over a period of six weeks on formulated diets containing: 20% soy protein with 0% cholesterol (group A), 20% soy protein with 5% cholesterol (group B), 20% soy protein with 10% cholesterol (group C), 0% soy protein with 20% cholesterol (group D), and 5% soy protein with 20% cholesterol (group E). Serum total, VLDL, LDL, and HDL-cholesterol and triglyceride concentrations were found to be reduced significantly in Groups B and C when compared with Groups D and E ($p < 0.05$). The lipid profile in group C did not, however, differ significantly when compared to the control group ($p > 0.05$). Groups D and E showed significant increased levels ($p < 0.01$) when compared to other groups. Lipoprotein lipase (LPL), lecithin:cholesterol acyl transferase (LCAT), and triglyceride lipase (TGL) activities were found to be significantly reduced ($p < 0.05$) in groups B and C when compared with groups D and E. Though groups B, C, D, and E rats all showed significant elevation ($p < 0.05$) in the enzyme activities when compared to the control.

Key words: Soy protein, cholesterol, lipid metabolizing enzymes.

INTRODUCTION

It is well known that cardiovascular disease (CVD) is the leading cause of death in both men and women in developed countries. Hypercholesterolemia has been established as a primary risk factor in the development of CVD. Studies in this area are mostly preventive and have focused on ways of reducing serum cholesterol by nutritional means. Preventing or reducing the increase in serum cholesterol is associated with reducing risk for CVD. Diet plays an important role in controlling blood lipids. Although elevation of serum or tissue cholesterol after excessive cholesterol intake depends on the animal species, cholesterol administration has been shown to increase total cholesterol in rats (Kummerow, 1982). The hypercholesterolemia that develops when animals are fed on high fat diet is consistently characterized by an increase in the molecular weight of serum low density lipo-

proteins (LDL) (Vegroesen, 1972; Berg et al., 1986). The hypercholesterolemic effect of soy protein has been recognized for more than thirty years (Zhan and Ho, 2005). Animal studies show that substituting soy protein for dietary animal protein reduces serum total and LDL cholesterol concentrations (Colacurci et al., 2005). The magnitude of the cholesterol lowering effect of soy protein is similar to that observed for other plant-based foods such as oat bran, or garlic and has been confirmed to be a major clinical means of treating patients with genetically based hypercholesterolemia (Setchell, 1998). However, the real potential of soy protein appears to be preventing the rise in serum cholesterol which is diet- induced in almost 40% of the adult population and therefore in lowering the overall risk for CVD.

Changes in serum lipids are controlled by those enzymes responsible for lipid metabolism; Lipoprotein lipase (LPL) is the crucial enzyme in the metabolism of triglyceride rich lipoproteins. Elevated triglyceride rich lipoprotein levels not only may promote a more rapid progression of atherosclerosis, but could also lead to myocardial ische-

*Corresponding author. E-mail: samojeaburu@yahoo.com. Tel: +2348035844852.

Table 1. Feeding regimen for each group.

Feed composition	Group				
	A	**B**	**C**	**D**	**E**
Maize Flour	70	65	60	70	65
Fish Meal	10	10	10	10	10
Soy Protein	20	20	20	-	5
Cholesterol	-	5	10	20	20
Total	100	100	100	100	100
Calorie Equivalent	450	475	500	510	530
% Soy Protein incorporated	20	20	20	0	5
% Cholesterol Protein incorporated	0	5	10	20	20

mia particularly in subjects with high triglyceride/low HDL traits which is frequently present in coronary artery disease (CAD) (Nordis and Mack, 1995). Earlier reports have shown that sub endothelially located LPL exhibited proatherogenic effect by increasing oxidative suscep- tibility of LDL facilitating the uptake of triglyceride rich lipoproteins by macrophages. The latter promotes foam cell formation which is the hallmark of atherogenesis (Babaev et al., 1999; Libby et al., 2002; Milosavjevic et al., 2003).

Lecithin:cholesterol acyl transferase (LCAT) is a serum enzyme that esterifies free cholesterol, primarily at the surface of the HDL particle after which the cholesteryl ester molecules migrate to the inner core of this lipo- protein. Through this action, LCAT plays a key role in the maturation of HDL particles. In humans, almost all serum cholesteryl esters are formed by the activity of LCAT and individuals devoid of this enzyme show abnormal plasma lipoproteins (Glomset, 1968; Glomset et al., 1970; Nordby and Norum, 1975). Experimental findings have indicated that modification of serum proteins by LCAT alters rates of cholesterol flux between cells and medium and results in a net loss of cholesterol from the cells accompanied by cellular cholesterol synthesis (Glomset, 1970; Glomset, 1976; Ray et al., 1980; Relimpio and Iriarte, 1981). Studies have also shown that an increased activity of LCAT is proatherogenic (Ononogbu and Okpara, 1986; Onyeneke et al., 1991).

High levels of serum triglyceride has been strongly linked with low serum HDL cholesterol (Richards et al., 1989; Avogaro et al., 1991) and these low HDL Levels may contribute to increased risk for CVD (Assmann et al., 1986). Triglyceride lipase is a major factor in the reduc- tion of serum triglyceride concentration (Kantor et al., 1985).

The enzymes; lipoprotein lipase (LPL), LCAT and triglyceride lipase (TGL) have been hypothesized to participate in the regulation of cholesterol metabolism. This hypothesis coupled with the effect of soy protein on serum lipid profile is explored in this present study.

MATERIALS AND METHODS

Soy protein

Matured soy beans (uncooked) were purchased from Iwaro Market, Oka Akoko, Ondo State, Nigeria and were identified as *Glycine maximus* (soybean) by a taxonomist in the Department of Crop Science, Faculty of Agriculture, University of Benin, Nigeria. This was ground into powder and used in the diet formulation. Some of the soy protein was subsequently deposited at the herbarium of the faculty.

Animals and diets

Thirty five (35) twelve weeks old albino rats (Wister Strain) weighing between 60 – 70 Kg purchased from the animal house of the department of Biochemistry, University of Ilorin, Nigeria were used for the study. The rats were housed in stainless steel cages with raised wire floors at a temperature of 30 degrees centigrade and fed on rat chow and water *ad libitum* for a period of two weeks to acclimatize. The animals were then divided into five groups of seven rats each designated A (control), B, C, D and E (experi- mental) were then placed on five different dietary regimen as shown in Table 1. Before the commencement of the dietary regimen, the animals were fasted overnight but allowed access to water *ad libitum*. One rat from each group was sacrificed on Day 0 and its serum collected to determine the baseline level of the test para- meters studied.

Assays

At weekly intervals, one rat from each group was sacrificed and 2 ml of blood was collected from the animal by cardiac puncture. The blood was allowed to stand at room temperature to clot and centrifuged at 10,000 g for 5 min using Hettich (Universal II) centri- fuge to separate serum from the cells. The supernatant (serum) was carefully decanted and analysis was carried out immediately. Determination of total and free cholesterol in the serum was by the method of Searcy and Bergquist (1960). Serum triglyceride (TG) was determined using the method of Tiez (1990) after enzymatic hydrolysis by lipases LDL and HDL-cholesterol as well as VLDL- cholesterol were determined according to the methods described by Friedwald et al. (1972). Protein content was determined by the Biuret reaction described by Gornall et al. (1949) while lipoprotein lipase and triglyceride lipase activities were determined by the method of Kamimura et al. (1999). LCAT activity was determined

Table 2. Change in mean body weights (g) of rats fed soy protein and cholesterol diet.

Time on diet (weeks)	Mean body weight (g)				
	A	B	C	D	E
0	0	0	0	0	0
1	6.58	4.13	6.90	8.68	9.00
2	8.38	14.57	11.59	13.15	9.06
3	18.77	19.18	22.90	19.78	19.66
4	25.33	20.54	23.82	35.75	30.80
5	19.29	13.89	9.54	5.56	6.19
6	14.58	14.41	12.39	11.90	6.25

by the methods described by Varman and Soloff (1976). Lipoprotein lipase and triglyceride lipase activities were measured in mmol of free fatty acids liberated per minute while LCAT activity was expressed in mg cholesterol ester formed per 100 ml serum.

Statistical analysis

Statistical analysis was done by one way analysis of variance (ANOVA) and Duncan Multiple range test (DMRT). The level of significance was p < 0.05.

RESULTS

The animals consumed their daily rations satisfactorily and showed increase in their body weight. The weekly percentage increase in body weight for the respective groups is presented in Table 2. Table 3 shows the weekly changes in serum total, free, esterified VLDL-, LDL-, and HDL-cholesterol concentration observed for the respective groups over the six weeks treatment period. Results obtained indicated that rats in groups B and C fed 20% soy protein with 5 and 10% cholesterol, respectively, showed decreases in their serum cholesterol levels when compared to the control while groups D and E rats fed 20% cholesterol with 0 and 5% soy protein, respectively, showed significant increases (p < 0.05, p < 0.01) in their serum cholesterol levels when compared to the control and groups B and C. Generally, the reduction in group B rats was statistically significant (p < 0.05) when compared to the control and in all the cholesterol fractions. Group E (5% soy protein + 20% cholesterol) rats, however, had reduced cholesterol fraction levels (p < 0.05) when compared to group D rats (0% soy protein + 20% cholesterol). Table 4 shows the changes in the serum triglyceride concentration for the respective groups studied. Although there was no significant mean difference in group B and C rats (p > 0.05), group D rats fed 20% cholesterol without soy protein showed significantly elevated levels of serum triglyceride (p < 0.05).

Table 5 shows lipoprotein lipase activity (expressed in mmol of free fatty acid hydrolyzed per minute) for the respective groups over the six weeks period. Rats in

groups B, C, D and E showed a significant increase (p < 0.05) in their serum lipoprotein lipase activity when compared to the control. The enzyme activity in group D is about 2-folds that of the control and inclusion of soy protein showed significant reduction (p < 0.05) in the activity of LPL when compared to group D rats. The esterifying activity of serum LCAT expressed as mg cholesterol ester formed per 100 ml serum is as shown in Table 6. The results obtained showed that rats in groups D and E fed 20% cholesterol with 0% soy protein and 5% soy protein, respectively, showed about 3-fold and 2.5-fold increase in activity when compared to the control rat at the end of the feeding trial. Though rats in groups B, C, D, and E showed a significant increase (p < 0.05) in LCAT activity when compared with the control, and higher activity was observed in group D when compared with groups B, C and E. Table 7 shows the results obtained for serum triglyceride lipase (TGL) activity. Rats in group D showed elevated levels in serum triglyceride lipase (TGL) (p < 0.01) when compared to the control. The groups on soy protein supplementation (groups B, C and E) showed significant reduction (p < 0.05) in TGL levels when compared to group D (0% soy protein + 20% cholesterol).

DISCUSSION

Cholesterol rich diet has been demonstrated by several workers to increase serum total VLDL and LDL cholesterol as well as serum triglyceride concentrations (Berg et al., 1986; McGill, 1988; Onyeneke et al., 1991). Contrary to the expected elevation of serum total cholesterol, VLDL cholesterol, LDL cholesterol and triglyceride concentrations, the results obtained from this study show decreased total VLDL and LDL cholesterol as well as triglyceride concentrations. The results obtained for groups B and C rats showed reductions in their serum total VLDL and LDL cholesterol concentrations as well as triglyceride concentrations. This could be attributable to the inclusion of 20% soy protein in the animal diet. This observation is justifiable since group D rats fed diet con-

Table 3. Weekly changes in serum total, free, esterified VLDL-, LDL- and HDL-cholesterol concentrations (mg/dl) of rats fed soy protein and cholesterol diet.

Time on diet (weeks)		Groups				
		A	B	C	D	E
0	TC	197.0 ±2.0 [.a]	193.3 ±3.0 [a]	198.3 ±6.0 [a]	195.3 ±4.0 [a]	197.0 ±4.0 [a]
	VLDL – C	19.7 ±0.8 [a]	19.3 ±0.8 [a]	19.8 ±0.5 [a]	19.5 ±0.6 [a]	19.7 ±0.7 [a]
	LDL - C	128.3 ±3.0 [a]	126.0 ±4.0 [a]	129.0 ±4.0 [a]	127.0 ±2.0 [a]	128.0 ±3.0 [a]
	HDL - C	49.0 ±1.0[a]	48.0 ±2.0 [a]	49.5 ±1.0 [a]	48.8 ±1.0[a]	49.3 ±1.0 [a]
	FC	65.0±1.0[a]	64.3±2.0 [a]	66.3±2.0 [a]	65.3±2.0 [a]	66.0±2.0 [a]
	CE	132.0±3.0 [a]	129.0±2.0 [a]	132.0±3.0 [a]	130.0±2.0 [a]	131.0±1.0 [a]
1	TC	195.5 ± 3.0 [a]	192.2 ±1.0 [a]	199.0 ±3.0 [b]	200.0 ±4.0 [b]	199 .0±3.0 [b]
	VLDL – C	19.5 ±0.9 [a]	19.2 ±0.6 [a]	20.9 ±0.5 [a]	20.0 ±0.4 [a]	19.9 ±0.5 [a]
	LDL - C	127.0 ±3.0 [a]	125.0 ±3.0 [a]	129.9 ±2.0 [a]	130.0 ±3.0 [a]	129.3 ±2.0 [a]
	HDL - C	49.0 ±2.0 [a]	48.0 ±1.0 [a]	47.2 ±3.0 [a]	50.0 ±2.0 [a]	49.8 ±2.0 [a]
	FC	64.5±2.0 [a]	64.2±3.0 [a]	66.0±2.0 [a]	67.0±2.0 [a]	66.0±2.0 [a]
	EC	131.0±2.0 [a]	128.0±3.0 [a]	133.0±2.0 [a]	133.0±2.0 [a]	133.0±3.0 [a]
2	TC	193.3 ±3.0 [a]	190.7 ±2.0 [b]	192.2 ±3.0 [b]	207.5 ±5.0 [c]	204.4 ±3.0 [c]
	VLDL – C	19.3 ±1.2 [a]	19.2 ±0.8 [a]	19.2 ±0.6 [a]	20.7 ±0.7 [a]	20.4 ±0.5 [a]
	LDL - C	126.0 ±1.0 [a]	124.0 ±2.0 [a]	125.0 ±3.0 [a]	135.0 ±2.0 [b]	133.0 ±2.0 [b]
	HDL - C	48.0 ±3.0 [a]	47.5 ±1.0 [a]	48.0 ±2.0 [a b]	51.8 ±1.0 [b]	51.0 ±2.0 [b]
	FC	64.3±3.0 [a]	63.7±3.0 [a]	64.2±1.0 [a]	69.5±3.0 [a]	68.4±2.0 [a]
	EC	129.0±1.0 [a]	127.0±2.0 [a]	128.0±3.0 [a]	138.0±2.0 [a]	136.0±2.0 [a]
3	TC	198.8 ±2.0 [a]	190.9 ±3.0 [b]	193.5 ±3.0 [b]	221.7 ±4.0 [c]	213.9 ±5.0 [d]
	VLDL – C	19.8 ±0.6 [a]	19.1 ±0.6 [a]	19.3 ±0.4 [a]	22.2 ±0.8 [b]	21.0 ±0.8 [b]
	LDL - C	129.0 ±3.0 [a]	124.0 ±1.0 [b]	126.0±1.0[ab]	144.0 ±2.0 [c]	139.0 ±1.0 [d]
	HDL - C	50.0 ±3.0 [a]	47.8 ±0.5 [a]	48.2 ±1.2 [a]	55.5 ±0.5 [b]	53.5 ±1.0 [b c]
	FC	66.8±1.0 [a]	64.9±2.0 [a]	64.5±2.0 [a]	74.7±2.0[b]	71.0±4.0[b]
	EC	132.0±2.0 [a]	127.0±2.0 [b]	129.0±2.0[ab]	148.0±3.0 [c]	143.0±4.0 [c]
4	TC	195.6 ±3.0 [a]	189.1 ±4.0 [b]	195.6 ±2.0 [a]	246 .1±8.0 [c]	229 .2±7.0 [d]
	VLDL – C	19.6 ±0.2 [a]	18.9 ±0.5 [a]	19.6 ±0.6 [a]	24.6 ±0.6 [b]	22.9 ±0.9 [c]
	LDL - C	127.0 ±2.0 [a]	123.0 ±2.0 [b]	127.0 ±1.0 [a]	160.0 ±3.0 [c]	149.0 ±1.0 [d]
	HDL - C	49.0 ±1.0 [a]	47.2 ±2.0 [a]	49.0 ±1.0 [a]	61.5 ±2.0 [b]	57.3 ±2.0 [b]
	FC	65.0±2.0 [a]	63.0±3.0 [a]	65.0±4.0 [a]	82.0±3.0 [b]	76.0±3.0 [b]
	EC	131.0±1.0 [a]	126.0±2.0[b]	131.0±2.0[a]	164.0±4.0 [c]	153.0±3.0[d]
5	TC	198.9 ±4.0 [a]	189.1 ±1.0 [b]	196.9 ±3.0 [a]	253.9 ±9.0 [c]	237.0 ±6.0 [d]
	VLDL – C	19.8 ±0.1 [a]	18.9 ±0.4 [b]	19.7 ±0.3 [a]	25.4 ±0.7 [c]	23.7 ±0.5 [d]
	LDL - C	129.0 ±2.0 [a]	123.0 ±1.0 [b]	128.0 ±2.0 [a]	165.0 ±4.0 [c]	154.0 ±1.0 [d]
	HDL - C	50.0 ±1.0 [a]	47.2 ±2.0 [a]	49.2 ±0.5 [a]	63.5 ±1.0 [b]	59.3 ±1.0 [c]
	FC	66.0±1.0 [a]	63.0±2.0 [b]	66.0±1.0 [a]	85.0±2.0 [c]	79.0±2.0 [d]
	EC	132.0±2.0 [a]	126.0±1.0 [b]	131.0±3.0 [a]	169.0±5.0 [c]	158.0±3.0 [d]
6	TC	198.8 ±3.0 [a]	187.5 ±3.0 [b]	195.6 ±2.0 [a]	267.5 ±4.0 [c]	258.3 ±3.0 [d]
	VLDL – C	19.8 ±0.3 [a]	18.7 ±0.5 [b]	19.6 ±0.4 [a]	26.7 ±0.5 [c]	25.8 ±0.6 [d]
	LDL - C	129.0 ±1.0 [a]	122.0 ±2.0 [b]	127.0 ±3 [a]	174.0 ±1.0 [c]	168.0 ±2.0 [d]
	HDL - C	50.0 ±1.0 [a]	46.8 ±0.5 [b]	49.0 ±1.0 [a]	66.8 ±1.0 [c]	64.5 ±1.0 [c]
	FC	66.0±1.0 [a]	62.0±1.0 [b]	65.0±2.0 [a]	89.0±1.0 [c]	86.0±2.0[d]
	EC	132.0±1.0 [a]	125.0±3.0 [b]	131.0±2.0 [a]	178.0±3.0 [c]	172.0±3.0 [c]

Tabulated results are means of five determinations ± SEM. Values in the same row carrying different superscripts are significantly different (p < 0.05). TC = Total cholesterol, VLDL-C = very low density lipoprotein cholesterol, LDL-C = low density lipoprotein cholesterol, HDL-C = high density lipoprotein cholesterol, FC = free cholesterol, EC = esterefied cholesterol.

Table 4. Serum triglyceride concentrations (mg/dl) of rats fed soy protein and cholesterol diet.

Time on diet (weeks)	Serum triglycerides concentration (mg/dl)				
	A	B	C	D	E
0	134 ±2 [a]	131 ±3 [a]	137 ±5 [a]	136 ±3 [a]	133 ±3 [a]
1	137 ±4 [a]	135 ±2 [a]	138 ±4 [a]	150 ±3 [b]	146 ±2 [b]
2	139 ±3 [a]	137 ±2 [b]	142 ±3 [b]	168 ±5 [c]	160 ±3 [c]
3	138 ±2 [a]	141 ±2 [a]	140 ±3 [a]	189 ±4 [b]	181 ±3 [c]
4	140 ±3 [a]	143 ±2 [a]	143 ±2 [a]	206 ±9 [b]	209 ±7 [b]
5	143 ±4 [a]	146 ±2 [a]	144 ±3 [a]	220 ±6 [b]	222 ±5 [b]
6	144 ±3 [a]	148 ±2 [a]	146 ±2 [a]	233 ±7 [b]	237 ±5 [b]

Tabulated results are means of five determinations ± SEM.
Values in the same row carrying different superscripts are significantly different (p < 0.05).

Table 5. Serum lipoprotein lipase activities (mmol/min ×10^{-2}) of rats fed soy protein and cholesterol diet.

Time on diet (weeks)	Serum lipoprotein lipase activity (mmol/min ×10^{-2})				
	A	B	C	D	E
0	8±0.4 [a]	9±0.8 [a]	8±0.5 [a]	8±0.3 [a]	9±1.0 [a]
1	10±1.0 [a]	12±0.9 [b]	11±0.5 [b]	23±2.0 [c]	22±2.0 [c]
2	11±2.0 [a]	13±1.0 [a]	15±2.0 [a]	25±2.0 [b]	26±2.2 [b]
3	14±1.4 [a]	20±2.0 [b]	21±1.3 [b]	32±2.0 [c]	30±1.4 [c]
4	13±1.6 [a]	25±3.1 [b]	28±2.6 [b]	37±2.3 [c]	33±1.7 [c]
5	11±0.9 [a]	16±0.8 [b]	17±1.4 [b]	22±1.8 [c]	20±2.0 [c]
6	9±0.8 [a]	15±1.5 [b]	16±1.0 [b]	20±2.0 [c]	17±1.3 [c]

Tabulated results are means of five determinations ± SEM. Values in the same row carrying different superscripts are significantly different (p < 0.05).

Table 6. Serum LCAT activities (mg cholesterol ester/100 ml serum) of rats fed soy protein and cholesterol diet.

Time on diet (weeks)	Serum (LCAT) activity (mg cholesterol ester/100 ml serum)				
	A	B	C	D	E
0	58.5±2.0 [a]	58.0±2.0 [a]	58.0±2.0 [a]	58.0±2.0 [a]	58.0±2.0 [a]
1	61.0±3.0 [a]	93.5±3.0 [b]	89.0±4.0 [b]	101.5±6 .0 [c]	115.0±4.0 [d]
2	58.0±6.0 [a]	117.5±7.0 [b]	115.5±5.0 [b]	120.5±3.0 [b]	118.0±2.0 [b]
3	57.0±1.0 [a]	121.0±2.0 [b]	119.0±3.0 [b]	155.0±6.0 [c]	137.0±3.0 [d]
4	58.5±2.0 [a]	132.5±4.0 [b]	130.0±2.0 [b]	160.5±1.0 [c]	152.0±2.0 [d]
5	60.5±1.0 [a]	140.5±3.0 [b]	139.5±2.0 [b]	163.5±0.5 [c]	154.5±1.0 [d]
6	61.5±0.8 [a]	142.5±0.6 [b]	141.0±0.3 [b]	170.5±1.0 [c]	157.5±0.6 [d]

Tabulated results are means of five determinations ± SEM. Values in the same row carrying different superscripts are significantly different (p < 0.05).

taining no soy protein showed significantly (p < 0.05) raised levels serum total cholesterol, VLDL cholesterol, LDL cholesterol and triglyceride concentrations, while group E rats fed diet containing 5% soy protein showed significant decrease (p < 0.05) in their serum total VLDL and LDL cholesterol concentrations as well as triglyceride

Table 7. Serum triglyceride lipase activities (mmol/min x10^{-2}) of rats fed soy protein and cholesterol diet.

Time on diet (weeks)	Serum triglyceride lipase activity (mmol/min x10^{-2})				
	A	**B**	**C**	**D**	**E**
0	9±0.8 [a]	9±1.2 [a]	9±0.5 [a]	9±1.3 [a]	9±0.8 [a]
1	10±1.2 [a]	12±1.6 [a]	11±1.8 [a]	19±1.5 [b]	17±2.0 [b]
2	12±0.6 [a]	17±1.9 [b]	15±2.0 [b]	25±3.1 [c]	21±0.7 [d]
3	14±1.2 [a]	23±2.0 [b]	22±2.2 [b]	33±3.4 [c]	29±1.4 [d]
4	17±2.2 [a]	28±1.2 [b]	25±2.6 [b]	35±1.2 [c]	32±2.0 [c]
5	12±0.8 [a]	17±1.5 [b]	16±1.4 [b]	29±2.1 [c]	27±1.8 [c]
6	11±1.2 [a]	13±2.0 [b]	13±1.6 [b]	24±2.2 [c]	22±1.3 [c]

Tabulated results are means of five determinations ± SEM.
Values in the same row carrying different superscripts are significantly different ($p < 0.05$).

concentrations when compared to group D rats. This results further strengthens the earlier reports on the hypocholesterolemic effect of soy protein (Zhan and Ho, 2005; Colacurci et al., 2005). Comparative clinical trials by Anderson et al. (1995) showed that consumption of diets rich in soy protein as opposed to those high in animal protein significantly lowers blood total cholesterol level, LDL cholesterol and triglycerides without lowering helpful HDL cholesterol. Kirk et al. (1998) have also demonstrated that diet containing intact or unextracted soy protein significantly reduces lesion areas in wild type mice when compared to those fed phytooestrogen extracted soy protein. They equally observed that there was no difference in the extent of atherosclerosis in the LDL receptor deficient mice fed intact soy protein and those fed phytoestrogen extracted soy protein. This suggests that cellular LDL receptors play a significant role in soy protein hypocholesterolemic effect (Baum et al., 1998). The activities of lipid metabolizing enzymes especially lipoprotein lipase, LCAT and triglyceride lipase have been reported to be responsive to nutritional factors (Richards et al., 1989; Fielding and Fielding, 1980). Exogenous cholesterol does contribute significantly in the regulation of the activities of these enzymes. Hypertrigly-ceridemia, a major risk factor for CVD has been demonstrated to be associated with increased LPL and TGL activity (Kantor et al., 1985; Richards et al., 1989; Patsch and Gotlo, 1995). Similarly, variation in the serum level of HDL cholesterol has been reportedly linked to the activity of LPL and TGL (Richards et al., 1989; Avogaro et al., 1991; Patsch and Gotlo, 1995). Low levels of HDL cholesterol observed in patients at high risk of CVD have been associated with increased LPL and TGL activities. The result of our present study shows that consumption of soy protein reduces to baseline levels, serum LPL and TGL activity. Earlier reports by Onyeneke et al. (1991) and Ononugbu et al. (1986) have demonstrated that

increased LCAT activity is proatherogenic. The results obtained from this study supports this claim. This is justified since group D rats fed diets containing 0% soy protein with 20% cholesterol showed significantly higher LCAT activity when compared with group B and C rats fed 20% soy protein. The action of soy protein on the activity of these lipid metabolizing enzymes could be attributable to its hypocholesterolemic effect. Thus, reduction in serum levels of cholesterol and triglycerides may lead to a corresponding decrease in the activity of these enzymes. It is therefore suggested that the inclu-sion of soy protein in the diet improves serum lipid profile as well as control the activities of the lipid metabolizing enzymes. Hence, soy protein could be beneficial in reducing the increased risk to and consequent death (resulting from intake of high fat diet) due to atherosclerosis.

The action of soy protein over the activities of these lipid-metabolizing enzymes could be ascribed to its hypo-cholesterolemic attributes; thus reductions in serum levels of cholesterol and triglycerides may lead to a corresponding decrease in the activities of these enzymes

REFERENCES

Anderson JW, Johnstone BM, Cook-Newell ME (1995). Meta-analysis of the effects of soy protein intake on serum lipids. N. Engl. J. Med. Aug. 3(5): 276-282

Assman G, Schulte H, Oberwittler W, Hause WH (1986). New aspects in the prediction of coronary artery disease: the prospective cardio-vascular monster study. In: Fidge NH, Nestel PJ, eds. Atheros-clerosis VII. Amsterdam, Netherlands: Elsevier Science Publishers. pp. 19-24.

Avogaro P, Ghiselli G, Soldan S, Bittolo Bon G (1991). Relationship of triglycerides and HDL cholesterol in hypertriglceridemia. Athero-sclerosis. 92: 79-86

Babaev VR, Fazio S, Gleaves LA, Carter KJ, Semenkovich CF, Linton MF (1999). Macrophage lipoprotein lipase promotes foam cell forma-

tion and atherosclerosis *in vivo.* J. Clin Invest. 103(12): 697-705.

Baum JA, Teng H, Erdman JW, Weigel RM, Klein BP, Persky VW, Freels S, Surya P, Bakhit RM, Ramos E, Shay NF, Potter SM (1998). Long term intake of soy protein improves blood lipid profiles and increases mononuclear cell low density lipoprotein receptor messenger RNA in hypercholesterolemic, post menopausal women. Am. J. Clin. Nutr. 68(3): 545-51.

Berg KA, Borrensen H, Dahlen G (1986). Serum hydro-density lipoproteins and atherosclerotic heart diseases. Lancet. 1: 499-501.

Colacurci N, Chiantera A, Fornaro F, de Novellis V, Manzella D, Arciello A, Chiatere V, Improta L, Paolisso G (2005). Effects of soy isoflavones on endothelial function in healthy postmenopausal women. Menopause. 12: 299-307.

Fielding CJ, Fielding PE (1980). Characteristic of triacylglycerol and partial acylglycerol hydrolysis by human plasma lipoprotein lipase. Biochim Biophys. Acta. 620: 440-446.

Friedwald WT, Levy RT, Fredickson DS (1972). Estimation of the concentration of low density lipoprotein cholesterol in plasma, without use of preparative ultracentrifuge. Clin. Chem. 18: 499-502.

Glomset JA (1968). The Plasma Lecithin: Cholesterol acyl transferase reaction. J. Lipid Res. 9: 155-167

Glomset JA, Norum KR, King W (1970). Plasma lipoproteins in familial lecithin: cholesterol acyl transferase deficiency: lipid composition and reactivity *in vitro*. J. Clin Invest. 49: 1827-1937.

Glomset JA (1976). Lecithin: cholesterol acyl transferase in lipoprotein metabolism,ed. By Greton, H; Springer-Verlag, Berlin, New York, pp. 28-41.

Gornall AG, Bardawill CT, David MM (1949). Determination of serum protein by means of biuret reaction. J. Biol. Chem. 177: 751-766.

Kamimura ES, Mendieta O, Sato HH, Pastore G, Maugeri F (1999). Production of lipase from *Geotricum sp* and adsorption studies on affinity resin. Braz. J. Chem. Eng, 16(2): 103-112.

Kirk EA, Sutherland P, Wang SA, Chait A, LeBaeuf RC (1998). Dietary isoflavones reduce plasma cholesterol and atherogenesis in C57BL/6 mice but not LDL receptor-deficient mice. J. Nutr. 128(6): 954-959.

Kantor MA, Bianchini A, Bernier D, Sady SP, Thompson PD (1985). Androgens reduce HDL_2-cholesterol and increase hepatic trigly-ceride lipase activity. Med. Sci. Sports Exerc. 17:462-465.

Kummerow FA (1982). The possible involvement of dietary fats in atherosclerosis. Prog. Lipid Res. 21: 743-746

Libby P, Ridker PM, Maseri A (2002). Inflammation and atherosclerosis. Circulation. 105 (9): 1135.

McGill HC (1988). The pathogenesis of atherosclerosis. Clin Chem: 34 (8B): B33- B39.

Milosavjevic D, Kontush A, Griglio S, Le Naour G, Thrillet J, Chapman MJ (2003). VLDL-induced triglyceride accumulation in human macrophages is mediated by modulation of LPL lipolytic activity in the absence of change in LPL mass. Biochem. Biophys. Acta. 1631(1): 51-60.

Nordby G, Norum KR (1975). Substrate specificity of lecithin:cholesterol acyl transferase, esterification of demosterol, β-sitosterol and cholecalciferol in human plasma. Scand. J. Clin. Lab. Invest. 35: 677-682

Nordis HN, Mack WJ (1995). Triglyceride-rich lipoproteins and pro-gression of coronary artery disease. Curr Opin Lipidol. 6: 209-214.

Ononogbu IC, Okpara GC (1986). Effect of garri diet on lecithin: cholesterol acyl transferase. Nutr. Rep. Int. 33: 19-87.

Onyeneke EC, Alumanah EO, Ononogbu IC (1991). Changes in plasma lecithin: cholesterol acyl transferase activity in rats fed on cholesterol diet. J. Clin. Biochem Nutr. 10: 27-34.

Patsch W, Gotto AM (1995). High density lipoprotein cholesterol, plasma triglyceride and coronary heart disease. Pathophysiology and management. Adv. Pharmacol. 32: 375-426.

Ray E, Bellini F, Stoudt G, Hemperly S, Rothblat G (1980). Influence of lecithin: cholesterol acyl transferase on cholesterol metabolism in hepatoma cells and hepatocytes. Biochim. Biophys. Acta. 617: 318-334.

Relimpio A, Iriarte AJ (1981). Re-evaluation of cholesterol homeostasis and atherosclerosis. J. Biol. Chem. 256: 4478-4488.

Richards EG, Grundy SM, Cooper K (1989). Influence of plasma triglycerides on lipoprotein patterns in normal subjects and in patients with coronary artery disease. Am. J. Cardiol. 63: 1214-1220.

Searcy RL, Berquist LM (1960). A new colour reaction for the quantification of serum cholesterol. Clin. Chim. Acta. 5: 192-199.

Setchell KD (1998). Phytoestrogens: the biochemistry, physiology and implications for human health of soy isoflavones. Am. J. Clin. Nutr. 68(6): 1333-1346.

Tiez NW (1990). Clinical guide to laboratory tests. 2nd ed. W.B. Saunders Company, Philadelphia, U.S.A. p. 554.

Varman KG, Soloff LA (1976). A method for the purification of milligram quantities of stable human phosphatidyl choline-cholesterol acyl transferase. Biochem. J. 155: 583-599.

Vegroesen AJ (1972). Dietary fat and cardiovascular disease: Possible modes of action of linoleic acid. Proc. Nutr. Soc. 31: 323-329.

Zhan S, Ho SC (2005). Meta-analysis of the effects of soy protein containing isoflavones on the lipid profile. Am. J. Clin. Nutr. 81: 397-408.