International Journal of Medicine and Medical Sciences Vol. 1 (9), pp. 400-406, September, 2009
Available online at http://www.academicjournals.org/IJMMS

Full Length Research Paper

Effects of soy protein on selected enzymes in tissues of rats fed a cholesterol diet

Olarewaju M. Oluba[1*], E. Chukwu Onyeneke[1], Godwin C. Ojieh[2] and George O. Eidangbe[2]

[1]Department of Biochemistry, University of Benin, P. M. B. 1154, Benin-City, Nigeria.
[2]Department of Medical Biochemistry, College of Medicine, Ambrose Alli University, Ekpoma, Nigeria.

Accepted 14 August, 2009

Soy protein, an important component of soybeans is unique among plant-based protein because of its associated isoflavones. Isoflavones are a group of compounds with variety of biological properties that may potentially benefit human health. Their actions in various tissues have motivated researchers to access the possible related mechanisms and functions. This study is aimed at determining the effect of soy protein on selected tissue enzymes (used as aids in early diagnosis of cardiovascular disease) in rats fed a cholesterol diet. 24 male Wistar albino rats were assigned randomly into three groups. The first group serving as control was placed on normal diet while groups two and three were fed diet containing 5% cholesterol and 5% cholesterol plus 5% soy protein, respectively. The animals were placed on their respective diet for 7 weeks and at weekly interval LDH, ALT, AST and γ-GT activities in the liver, kidney and heart were monitored. At the end of the study period, LDH, ALT, AST and γ-GT activities were substantially reduced in the liver, kidney and heart of rats fed soy protein plus cholesterol diet compared with those fed cholesterol diet without soy protein. These results indicate that soy protein reduces the accumulation of excess fat in the liver, kidney and heart and thus prevent cell death due to lipotoxicity.

Key words: Soy protein, cholesterol, diet, enzyme, lipotoxicity, cardiovascular disease.

INTRODUCTION

Soy protein is an important component of soybeans and provides an abundant source of dietary protein. Among the dietary proteins, soy protein is considered a complete protein in that it contains ample amounts of all essential amino acids plus several other macronutrients with a nutritional value roughly equivalent to that of animal protein of high biological values (Young, 1991). Soy protein is unique among the plant-based protein because it is associated with isoflavones, a group of compounds with a variety of biological properties that may potentially benefit human health. An increasing body of literature suggests that soy protein and its isoflavones may have beneficial role in coronary heart disease (CHD) (Potter, 1995; Anderson et al., 1999). A large number of nutritional intervention studies in animals and humans indicate that consumption of soy protein reduces fat mass in addition to lowering serum total cholesterol, low-density lipoprotein cholesterol (LDL-C), and triglycerides as well as hepatic cholesterol and triglycerides (Zhan and Ho, 2005; Weggemans and Trautwein, 2003). Studies in animals showed that soy protein intake exhibits its hypolipidemic effect by reducing intestinal cholesterol absorption and increasing faecal bile acid excretion, thereby reducing hepatic cholesterol content and enhancing removal of LDL-C (Greaves et al., 2000). Studies by Lovati et al. (1987) as well as Kirk et al. (1998) have also shown that dietary soy protein could exert a direct effect on hepatic cholesterol metabolism and LDL receptor activity.

Lipid abnormalities associated with CHD consist of increased overall production of lipids with elevated levels of fatty acids, triglycerides, and LDL-C, as well as very-low-density lipoprotein (VLDL) cholesterol. Excess fat intake especially in the form of saturated fatty acids and cholesterol leads to the formation and deposition of lipids in various fatty tissues. Elevated serum concentrations of cholesterol in the total, LDL-C and VLDL-cholesterol as well as free fatty acids (FFA) have been shown to play a key role in contributing to the development of CHD (Bell, 2000). In addition, evidence exists that suggests that

*Corresponding author. E-mail: olubamike2000@yahoo.co.uk. Tel: 2347030496639, 2348070613653.

Table 1. Diet composition (% by weight).

Feed composition	Control	CO	SPC
Maize flour	70	65	60
Fish meal	10	10	10
Groundnut cake	20	20	20
Cholesterol	-	5	5
Soy protein	-	-	5
Total	100	100	100

Note: CO, Cholesterol only; SPC, Soy protein plus cholesterol

accumulation of excess fat and FFA in non-adipose tissues, such as the liver, kidneys, skeletal muscle, and blood vessels may impair their functions and contribute to cell dysfunction or cell death, a phenomenon known as lipotoxicity (Schaffer,2003; Montani et al., 2004).

Preventive or therapeutic strategies to control CHD should target these abnormalities. Various dietary modifications designed to control excess serum cholesterol and dyslipidemia have focused on the manipulation of the amounts and nature of dietary fat intakes. In recent years, increased attention has focused on the role of dietary protein with particular interest on soy protein in the management of CHD. Studies directly related to the effect of soy protein on tissues enzymes such as lactate dehydrogenase (LDH), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma-glutamyl transpeptidase (γ-GT) which are considered as markers of tissue injury in rats are scanty. Moreover, these enzymes have been reported to be useful as diagnostic aids in predicting risk to cardiovascular disease (Oluba et al., 2008a; Oluba et al., 2008b). The present study is therefore focused on the effect of soy protein on the activities of these enzymes (LDH, ALT, AST and γ-GT) in rats fed a cholesterol-based diet.

MATERIALS AND METHODS

Chemicals

All chemicals used were of analytical grade and were products of BDH Chemicals Ltd, Poole, England.

Soy protein

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

Animals and diets

Twenty-four (24) twelve week old male albino rats (Wistar strain) mean weight 85±2 g obtained from the Animal Laboratory of the Department of Biochemistry, University of Ilorin (Nigeria) were used for the study. Rats were housed individually in stainless steel cages with raised wire flour in an environment of 28 - 30^{O}C, 50 - 60% relative humidity and a 12 h light-dark cycle. The animals were fed commercial rat pellets (Guinea feeds, Nigeria) and tap water *ad libitum*. Maintenance and treatment of animals were in accordance with the principles of the "Guide for care and use of laboratory animals in research and teaching" prepared by the National Academy of Sciences and published by the National Institutes of Health (NIH) publication 86 - 23 revised in 1985. Rats were acclimatized to the facility for two weeks before the start of the experiments. Rats were then assigned to one of three groups (n = 8) designated: Control, cholesterol only (CO) and soy protein plus cholesterol (SPC) and placed on their respective diet for a period of 7 weeks. Composition of each diet is presented in Table 1. Prior to the commencement of the feeding experiments, the animals were fasted overnight but allowed access to water *ad libitum*.

Tissue preparation

At weekly interval one rat from each group was anaesthetized (in chloroform saturated chamber) and while still under anesthesia, the liver, kidney and heart samples were quickly excised and placed on ice and subsequently weighed. Portions of each tissue were homogenized separately to give a 20% homogenate and centrifuged at 10,000 g for 15 min as described by Aksnes and Njaa (1981) to obtain clear supernatant as the source of LDH, ALT, AST and γ-GT as well as total cholesterol (TC) and triacylglycerol (TAG).

Assays

Tissue total cholesterol (TC) and triacylglycerol (TAG) were determined using commercial kits (Randox Lab. Ltd., Crumin, UK). LDH activity was assayed by the method of Kubowitz and Otti (1946). ALT and AST activities were determined using the method described by Reitman and Frankel (1957). γ-GT activity was determined according to the methods of Szaz (1976). Each test for each group was done in triplicates.

Statistical analysis

The data are presented as mean ± SEM of triplicate determinations. Effect of experimental diets were examined using one way analysis of variance (ANOVA) and Duncan multiple range test (DMRT) using SPSS 11.0. p values ≤ 0.05 were considered statistically significant.

RESULTS

The animals consumed their daily rations satisfactorily and showed increase in body weight. The increase in

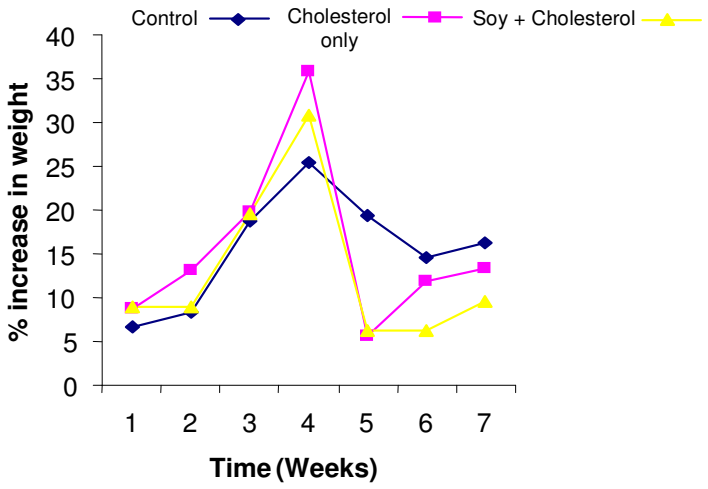

Figure 1. Percentage increase in body weight during the experiment.

Table 2. Mean weekly changes in organ weight (g/100 g body weight).

Time	Liver			Kidney			Heart		
(weeks)	Control	CO	SPC	Control	CO	SPC	Control	CO	SPC
0	3.8±0.3	3.6±0.5	3.8±0.2	1.3±0.3	1.3±0.3	1.3±0.3	1.6±0.4	1.6±0.3	1.5±0.2
1	3.9±0.2	3.9±0.2	3.8±0.3	1.5±0.2	1.4±0.3	1.4±0.1	1.7±0.1	1.8±0.3	1.6±0.1
2	4.2±0.1	4.3±0.2	4.0±0.2	1.8±0.2	1.7±0.2	1.7±0.1	1.8±0.3	2.1±0.1	1.8±0.1
3	4.3±0.2	4.5 0.3	4.3±0.3	1.8±0.3	2.0±0.3	1.8±0.2	2.0±0.3	2.5±0.2*	2.1±0.1
4	4.5±0.2	5.1±0.1*	4.6±0.3	1.9±0.1	2.3±0.1	2.0±0.3	2.0±0.3	2.6±0.3*	2.2±0.3
5	4.5±0.1	5.4±0.2*	4.7±0.2	1.9±0.2	2.5±0.1*	2.0±0.3	2.1±0.1	2.8±0.3*	2.3±0.2
6	4.6±0.3	5.6±0.5*	4.8±0.3	2.0±0.2	2.7±0.3*	2.3±0.1	2.2±0.3	2.9±0.3*	2.4±0.2
7	4.6±0.3	5.8±1.0*	5.0±0.7*	2.1±0.3	3.0±0.5*	2.4±0.2	2.3±0.3	3.2±0.3*	2.6±0.2

CO and SPC represent the groups fed cholesterol diet only and soy protein plus cholesterol diet defined in Materials and Methods. Values are mean ± SEM of triplicate determinations. * $p < 0.05$ vs. corresponding control value.

Table 3. Changes in liver, kidney and heart triacylglycerol (TAG) concentration (mg/g wet tissue).

Time	Liver			Kidney			Heart		
(weeks)	Control	CO	SPC	Control	CO	SPC	Control	CO	SPC
0	0.2±0.1	0.2±0.1	0.2±0.1	0.1±0.1	0.1±0.1	0.1±0.1	0.1±0.1	0.1±0.1	0.1±0.1
1	0.2±0.1	0.3±0.1	0.2±0.1	0.1±0.1	0.1±0.1	0.1±0.1	0.1±0.1	0.3±0.1	0.1±0.1
2	0.2±0.1	0.3±0.1	0.3±0.1	0.1±0.1	0.2±0.1	0.2±0.1	0.1±0.1	0.4±0.1	0.2±0.1
3	0.3±0.1	0.6±0.2	0.3±0.1	0.2±0.1	0.3±0.1	0.2±0.1	0.1±0.1	0.5±0.2	0.2±0.1
4	0.3±0.1	0.8±0.2*	0.4±0.2	0.2±0.1	0.4±0.1	0.3±0.1	0.2±0.1	0.5±0.1	0.3±0.1
5	0.4±0.1	1.0±0.4*	0.5±0.2	0.2±0.1	0.6±0.3*	0.3±0.1	0.2±0.1	0.6±0.3*	0.4±0.1
6	0.4±0.1	1.2±0.3*	0.6±0.2	0.2±0.1	0.8±0.2*	0.4±0.1	0.2±0.1	0.8±0.2*	0.4±0.1
7	0.5±0.3	1.7±0.2*	0.8±0.2	0.3±0.1	1.3±0.3*	0.6±0.2	0.4±0.1	1.1±0.2*	0.5±0.2

CO and SPC represent the groups fed cholesterol diet only and soy protein plus cholesterol diet defined in Materials and Methods. Values are mean ± SEM of triplicate determinations. * $p < 0.05$ vs. corresponding control value.

body weight was in order of control > SPC > CO (Figure 1). As indicated in Table 2, weights of the three organs were similar at the start of the experiment, however, at the end of the study, the sizes of the organs were significantly higher ($p < 0.05$) in the group fed cholesterol diet (CO group) compared with the control as well as the soy protein fed (SPC) groups. The weights of the three organs were not significantly different in the control and SPC groups.

Tables 3 and 4 show changes in liver, kidney and heart

Table 4. Changes in liver, kidney and heart cholesterol concentration (mg/g wet tissue).

Time (weeks)	Liver			Kidney			Heart		
	Control	CO	SPC	Control	CO	SPC	Control	CO	SPC
0	2.0±0.1	2.0±0.3	2.1±0.2	1.0±0.	1.1±0.2	1.0±0.2	1.0±0.2	1.0±0.3	1.0±0.2
1	2.5±0.1	2.6±0.3	2.6±0.3	1.1±0.1	1.4±0.2	1.3±0.1	1.3±0.1	1.5±0.5	1.3±0.3
2	3.2±0.2	4.5±0.4	4.2±0.2	1.3±0.1	1.5±0.3	1.6±0.1	1.4±0.3	2.9±0.3*	1.8±0.1
3	3.7±0.1	7.8±2.3**	4.9±0.2*	1.3±0.2	2.3±0.6*	2.2±0.3*	1.7±0.3	3.3±0.3**	2.4±0.2*
4	4.2±0.3	11.2±5.5**	5.3±0.2*	1.6±0.1	5.0±1.2**	2.8±0.3*	1.8±0.1	3.7±0.7**	2.8±0.2*
5	4.3±0.2	19.6±4.1**	7.7±1.4*	1.7±0.2	7.8±1.0**	3.1±0.3*	2.0±0.1	4.5±0.5**	3.2±0.3*
6	4.5±0.3	25.0±6.0**	12.5±5.6*	1.7±0.2	10.2±1.3**	4.4±0.3*	2.3±0.3	6.1±1.5**	4.7±0.5*
7	4.5±0.5	33.8±9.3**	17.4±1.8*	1.8±0.5	16.1±4.5**	4.5±0.6*	2.3±0.2	10.7±1.2**	5.3±1.0*

CO and SPC represent the groups fed cholesterol diet only and soy protein plus cholesterol diet defined in Materials and Methods. Values are mean ± SEM of triplicate determinations. * $p < 0.05$ and ** $p < 0.01$ vs. corresponding control value.

Table 5. Serum LDH, ALT, AST and GGT activities (U/L).

Time (weeks)	LDH			ALT			AST			γ–GT		
	Control	CO	SPO	Control	CO	SPO	Control	CO	SPO	Control	CO	SPC
0	217±5	220±4	219±4[a]	3.2±0.6	3.8±0.6	4.4 ±1.0	17±1.0	20±2.1	18±3.8	17 ± 1.0	20 ± 2.1	18 ± 3.8
1	250±3	300±4*	237 ±4*	3.9±0.2	5.4±0.2*	5.1 ±0.6*	19±2.1	23±3.1*	22±2.1*	19.± 2.1	23 ± 3.1*	22 ± 2.1*
2	268±	382±9*	262±13	4.7±0.4	7.3±0.1*	6.0 ± 0.4*	18±0.9	31±3.0**	22±1.4*	18 ± 0.9	31± 3.0**	22 ± 1.4*
3	291±5	446±12*	282±4	5.6±0.6	10.1±0.6**	7.4 ± 0.2*	21±1.1	47±2.8**	26±1.4*	21 ± 1.1	47 ± 2.8**	26 ± 1.4*
4	300±8	609±19*	300±4	6.4±0.3	15.7±1.1**	8.6 ± 0.1*	24±1.6	54±4.2**	27±0.8*	24 ± 1.6	54 ± 4.2**	27 ± 0.8*
5	312±6	714±14*	319±3	7.1±0.4	27.0±1.1**	9.9 ± 0.2*	27±0.9	68±3.8**	29±1.6*	27 ± 0.9	68 ± 3.8**	29 ± 1.6*
6	330±3	807±13**	330±4	8.4±0.3	36.8±1.2**	11.6±0.4*	28±1.1	72±4.2**	31±0.9*	28 ± 1.1	72 ± 4.2**	31 ± 0.9*
7	**333**±7	920±23**	337±7	8.8±0.5	48.5±2.3**	12.1±0.7*	30±2.6	96±7.5**	35±1.8*	29±3.4	104±8.0**	37±1.5*

CO and SPC represent the groups fed cholesterol diet only and soy protein plus cholesterol diet defined in Materials and Methods. Values are mean ± SEM of triplicate determinations. * $p < 0.05$ and ** $p < 0.01$ vs. corresponding control value.

TAG and TC concentrations respectively for the three groups. Animals fed cholesterol diet only (CO group) show significantly higher levels in liver, kidney and heart TAG and cholesterol concentrations compared to those fed control and soy protein supplemented diets at the end of the feeding trial. Cholesterol and TAG contents in the three tissues were not significantly changed in both control and soy protein groups. Table 5 shows the serum levels of the enzymes: LDH, ALT, AST and GGT, which are used to assess tissue function, for the respective groups over the six weeks period. Rats in the CO and SPC groups showed elevated levels of ALT, AST and GGT activities when compared with the control. These increases were statistically significant ($p < 0.05$) and were greater in CO group ($p < 0.01$) than in SPC group ($p < 0.05$). Serum LDH activity was significantly increased in the CO group compared with the control and SPC groups. There was no significant ($p > 0.05$) in LDH levels in the serum of the control and SPC rats.

Changes in the liver, kidney and heart LDH activities observed for the respective groups are presented in Table 6. In the liver, rats fed cholesterol diet only (CO) showed significant increase ($P < 0.05$) in LDH activity when compared to control group and soy protein + cholesterol (SPO)-fed group. In the kidney however, significant increases ($p < 0.05$) in LDH activity were observed in rats fed cholesterol diet only and those fed soy protein + cholesterol diet compared with control. The increase observed in the cholesterol only-fed rats was also significantly higher ($p < 0.05$) than that of the soy protein treated rats. The activity pattern of the enzyme in the heart showed that rats fed cholesterol diet without soy protein had significantly higher LDH activity compared with the control ($p < 0.01$) while those fed soy protein plus cholesterol diet were also significantly higher ($p < 0.05$) than the control values.

Liver, kidney and heart ALT and AST activities are presented in Tables 7and 8, respectively. According to the results, the two enzymes showed the same pattern of changes in the three tissues. The animals fed cholesterol diet without soy protein had significantly elevated ALT and AST activities ($p < 0.01$) than those fed with control

Table 6. Liver, kidney and heart LDH activities (U/L) of rats fed cholesterol and soy protein diet.

Time (weeks)	Liver			Kidney			Heart		
	Control	CO	SPC	Control	CO	SPC	Control	CO	SPC
0	9.4±0.3	9.4±0.2	9.7±0.3	4.7±0.1	4.5±0.3	4.7±0.2	2.2±0.1	2.0±0.3	2.0±0.1
1	9.9±0.1	10.1±0.1	10.0±0.3	5.6±0.1	4.9±0.2	5.5±0.2	2.7±0.1	3.3±0.1	2.3±0.1
2	12.3±0.2	14.0±0.3	12.2±0.1	5.9±0.1	5.7±0.2	6.3±0.1	3.5±0.2	5.5±0.2	4.1±0.1
3	12.5±0.1	22.1±0.3*	12.7±0.1	6.6±0.3	10.5±0.2*	9.9±0.1*	4.2±0.1	7.1±0.3	7.3±0.1
4	13.5±0.1	37.4±1.6*	13.9±0.1	7.5±0.3	25.3±0.5**	18.2±0.5*	4.6±0.1	11.4±0.1*	11.7±0.2*
5	14.2±0.3	52.0±2.0**	15.0±0.2	7.8±0.1	38.3±1.2**	24.4±0.3*	5.4±0.1	23.2±0.5**	15.4±0.2*
6	17.0±0.3	73.3.±2.0**	17.9±0.1	9.2±0.3	54.0±2.0**	25.7±0.9*	5.5±0.2	33.2±1.2**	16.2±0.3*
7	19.6±0.1	77.8±3.1**	20.2±0.1	9.5±0.3	60.2±2.4**	28.5±1.1*	5.7±0.2	45.0±1.0**	16.5 ±0.3*

CO and SPC represent the groups fed cholesterol diet only and soy protein plus cholesterol diet defined in Materials and Methods. Values are mean ± SEM of triplicate determinations. * $p < 0.05$ and ** $p < 0.01$ vs. corresponding control value.

Table 7. Liver, kidney and heart ALT activities (U/L) of rats fed cholesterol and soy protein diet.

Time (weeks)	Liver			Kidney			Heart		
	Control	CO	SPC	Control	CO	SPC	Control	CO	SPC
0	3.3±0.1	3.3±0.3	3.3±0.3	2.5±0.2	2.3±0.1	2.7±0.1	2.0±0.1	2.0±0.2	2.1±0.1
1	3.5±0.1	3.7±0.2	3.7±0.1	3.2±0.2	5.6±0.2	4.3±0.1	2.3±0.1	2.5±0.1	2.3±0.2
2	4.8±0.2	5.5±0.3	4.9±0.2	3.1±0.1	5.5±0.2	5.0±0.2	4.2±0.1	5.1±0.1	4.5±0.2
3	5.5±0.2	12.4±0.1*	6.0±0.3	4.8±0.1	10.0±0.2*	6.9±0.2	4.2±0.2	8.8±0.1	5.7±0.1
4	6.2±0.2	33.3±1.2**	10.0±0.2*	5.5±0.1	19.5±1.2**	13.0±0.2*	5.6±0.1	19.9±0.5*	8.5±0.3
5	6.9±0.3	56.3±2.4**	12.3±0.3*	6.9±0.1	28.0±1.0**	19.5±0.3*	5.8±0.1	30.6±1.0**	8.6±0.3
6	7.5±0.1	66.0±3.1**	19.2±0.3*	7.2±0.2	37.3±1.5**	25.4±0.3*	5.9±0.2	33.3±1.3**	10.5±0.3*
7	7.8±0.3	72.1±3.0**	28.0±1.0*	7.3±0.1	43.5±2.0**	30.2±0.7*	6.4±0.3	45.5±2.0**	10.8±0.3*

CO and SPC represent the groups fed cholesterol diet only and soy protein plus cholesterol diet defined in Materials and Methods. Values are mean ± SEM of triplicate determinations. * $p < 0.05$ and ** $p < 0.01$ vs. corresponding control value.

Table 8. Liver, kidney and heart AST activities (U/L) of rats fed cholesterol and soy protein diet.

Time (weeks)	Liver			Kidney			Heart		
	Control	CO	SPC	Control	CO	SPC	Control	CO	SPC
0	5.0±0.1	5.3±0.3	5.5±0.3	3.5±0.1	3.5±0.1	3.5±0.3	2.7±0.1	2.7±0.1	2.6±0.1
1	5.3±0.2	6.2±0.1	6.1±0.3	4.3±0.1	4.0±0.2	4.5±0.1	2.7±0.1	2.9±0.2	3.1±0.2
2	5.5±0.2	6.8±0.1	6.0±0.1	4.5±0.1	5.5±0.1	4.8±0.1	3.1±0.1	4.5±0.1	3.2±0.2
3	6.8±0.3	13.5±0.1*	8.6±0.2	5.0±0.2	9.2±0.1	6.6±0.3	3.3±0.1	7.5±0.3	4.5±0.2
4	7.2±0.2	33.5±1.2**	15.3±0.2*	6.5±0.3	17.5±0.5*	7.1±0.3	3.7±0.2	12.0±0.3*	8.8±0.3*
5	7.2±0.2	37.2±0.9**	22.5±0.2*	6.8±0.3	23.0±0.3*	8.5±0.2	4.3±0.1	19.6±0.7**	11.0±0.3*
6	7.7±0.2	45.3±1.5**	23.6±0.3*	7.0±0.3	35.3±0.9**	11.2±0.3*	4.8±0.2	24.4±0.3**	16.2±0.2*
7	8.0±0.3	59.5±3.2**	24.1±0.3*	7.2±0.2	38.6±2.4**	15.0±0.3*	5.2±0.2	26.5±0.5**	18.9±0.7*

CO and SPC represent the groups fed cholesterol diet only and soy protein plus cholesterol diet defined in Materials and Methods. Values are mean ± SEM of triplicate determinations. * $p < .05$ and ** $p < 0.01$ vs. corresponding control value.

diet. The values of the two enzymes in the rats fed with soy protein plus cholesterol diet were also significantly higher ($p < 0.05$) than that of the control. Table 9 represents γ-GT activities in the liver, kidney and heart for the three groups. The results indicate that the enzyme activity was highest in the heart and lowest in the kidney. Rats fed cholesterol diet only showed significantly elevated ($p < 0.05$) activity of the enzyme in the three

Table 9. Liver, kidney and heart γ-GT activities (U/L) of rats fed cholesterol and soy protein diet.

Time (weeks)	Liver			Kidney			Heart		
	Control	CO	SPC	Control	CO	SPC	Control	CO	SPC
0	15.0±0.5	15.6±1.0	15.6±2.0	19.6±3.0	21.5±2.3	21.3±1.2	27.1±1.0	27.1±2.1	27.1±1.0
1	16.2±1.0	16.2±1.5	15.8±1.0	22.5±3.0	22.8±1.3	22.3±1.1	27.6±1.2	28.2±1.2	27.7±2.2
2	16.8±1.0	23.6±1.0*	17.5±1.0	24.0±2.0	26..5±2.0	24.0±2.0	28.5±1.1	30.6±2.0	28.5±1.2
3	16.7±0.3	45.7±2.0**	22.2±3.0*	25.8±1.4	33.2±2.0*	27.5±2.0	30.3±2.0	47.9±1.0**	35.5±2.0*
4	18.3±0.7	53.3±4.0**	28.5±1.0*	27.0±1.0	48.6±2.0**	36.3±1.3*	31.6±2.0	70.5±1.3**	37.0±2.5*
5	19.5±1.0	67.5±3.1**	32.6±1.0*	29.2±2.0	55.0±2.2**	36.8±3.0*	31.6±1.3	83.3±2.5**	39.5±2.0*
6	19.5±1.0	73.8±3.0**	35.3±1.0*	30.5±3.0	58.2±2.0**	38.0±3.0*	32.3±3.0	91.1±5.0**	42.4±3.5*
7	20.0±1.3	79.2±5.1**	37.1±1.0*	30.5±3.0	63.5±5.6**	41.5±3.0*	32.8±3.0	98.5±4.3**	45.3±3.0*

CO and SPC represent the groups fed cholesterol diet only and soy protein plus cholesterol diet defined in Materials and Methods. Values are mean ± SEM of triplicate determinations. * $p < 0.05$ and ** $p < 0.01$ vs. corresponding control value.

tissues compared to the control and soy protein plus cholesterol fed rats.

DISCUSSION

Accumulation of excess fat and free fatty acids in non-adipose tissues, such as the liver, kidneys, heart, skeletal muscles and blood vessels greatly impair their functions and contribute to cell-dysfunction or cell death due to lipotoxicity (Schaffer, 2003; Montani et al., 2004). Lipotoxicity has been demonstrated to raise tissue enzymes activities due to cell necrosis and subsequent enzymes leakage. For instance, Salomen (2003) reported that liver transaminase activity was markedly increased as a result of lipotoxicity resulting from heavy alcohol intake while also observing that high concentration of HDL-cholesterol lose their protective effect against CHD in men with raised liver transaminase activity.

Substantial data from epidemiological studies and nutritional interventions in humans and animals indicate that soy protein reduces serum total and LDL- cholesterol and triglycerides concentrations as well as hepatic cholesterol and triglycerides (Zhan and Ho, 2005; Weggemans and Trautwein, 2003). Similarly, Lovati and co-worker (1987) reported that binding of VLDL-cholesterol to liver membranes of hypercholesterolemic rats when fed with a diet containing soy protein is increased, indicating an altered hepatic metabolism with increased LDL and beta-VLDL removal by hepatocytes. Kirk et al. (1998) in a related study using LDL-receptor deficient (LDLr-null) mouse observed significant reductions in plasma levels of total, LDL- and VLDL-cholesterol in C57BL/6J (wild type) mice fed soy protein isolate. On the contrary, no significant effect of the soy protein isolate on plasma lipids was observed in LDLr-null mice, suggesting that soy isoflavones might reduce lipid levels by increasing LDL receptor activity. A recent study by Gudbrandsen et al. (2006) showed that feeding obese Zuker rats with soy protein concentrate enriched with isoflavones (HDI) for 6 weeks reduced fatty liver and decreased plasma levels of ALT and AST. A similar report by Iritani et al. (1986) also showed that dietary soy protein reduced the concentration of triglycerides in plasma and especially in liver. As observed by these authors, these effects were associated with marked reductions in the activities of hepatic lipogenic enzymes, particularly glucose-6-phosphate dehydrogenase, malic enzyme, fatty acid synthetase as well as acetyl-coA carboxylase. These observations suggest that soy protein reduces liver triglycerides or fat by partially inhibiting hepatic fatty acid synthesis.

The results of our study indicate clearly that soy protein markedly reduced activities of LDH, ALT, AST and γ-GT in the liver, kidney and heart in cholesterol-fed rats. The rise in tissue levels of these enzymes in rats fed cholesterol only diet is suggestive of cell death (Herrera, 1993). This could be as a result of increased deposition of excess fat from the diet in the cells of these tissues. Evidences from epidemiological studies have shown that increased γ-GT activity is associated with development of cardiovascular disease risk factors, including diabetes, hypertension, dyslipidemia (Lee et al., 2002) and the metabolic syndrome (Lee et al., 2003). γ-GT activity has also been demonstrated to positively correlate with other CVD risk factors such as C-reactive protein (CRP), fibrinogen, F2-isoprostanes and inversely with antioxidant stress (Lee et al., 2004).

The substantial lowering effect of the tissue activities of these enzymes by soy protein as shown in our results further justifies earlier reports that soy protein reduces the accumulation of fat in non-adipose tissues (Zhan and Ho, 2005; Weggemans and Trautwein, 2003; Gudbrandsen et al., 2006; Iritani et al., 1986). Soy protein has also been reported to stimulate the nuclear hormone receptor; peroxisome proliferators activated receptor (PPAR) (Tovar et al., 2003). Activation of this receptor leads to stimulation of the differentiation pathway of preadipocytes into lipid-storing adipocytes which then act as a “sink” for excess fat. This increases the number of smaller adipocytes in subcutaneous adipose tissue (SCAT) with a high affinity for fatty acid and triglyceride

uptake. Thus, diversion of excess fatty acids and cholesterol from diet and other sources to non-adipose tissues is prevented.

In conclusion, changes to measurement of tissue enzymes (such as LDH, ALT, AST and γ-GT) activities in clinical trials with lipid lowering drugs should be published. It would be important to analyze how raised tissue enzymes activities might modify the effects of these drugs in preventing against atherosclerotic progression and coronary events. Eventually, assessment of liver, and other tissue function and related genetic variations could be used to predict the efficacy and safety of drugs that reduces serum and tissue cholesterol.

REFERENCES

Aksnes A, Njaa LR (1981). Catalase, glutathione peroxidase and superoxide dismutase in different fish species. Com. Biochem. Physiol. 69B: 893-896.

Anderson JB, Anthony M, Messina M, Gamer SC (1999). Effects of phytoestrogens on tissues. Nutr. Res. Rev. 12: 75-116.

Bell DSH (2000). Cardiovascular disease in South Asians. Lancet. 356: 1108-1116.

Greaves KA, Wilson MD, Rudel LL, Williams JK, Wagner JD (2000). Consumption of soy protein reduces cholesterol absorption compared to casein protein alone or supplemented with an isoflavone extract or conjugated equine estrogen in ovariectomized cynomologous monkeys. J. Nutr. 130: 820-826.

Gudbrandsen OA, Wergedahl H, Mork S, Liaset B, Espe M, Berge RK (2006). Dietary soya protein concentrate enriched with isoflavones reduced fatty liver, increased hepatic fatty acid oxidation and decreased the hepatic mRNA level of VLDL receptor in obese Zuker rats. Br. J. Nutr. 96: 249-257.

Herrera JL (1993). Abnormal liver enzyme levels. The spectrum of causes. Postgrad. Med. 93(2): 113-116.

Iritani N, Nagashima K, Fukuda H, Katsurada A, Tanaka T (1986). Effets of dietary soy protein on lipogenic enzymes in rat liver. J .Nutr. 116: 190-197.

Kirk EA, Sutherland P, Wang SA, Chait A, Leboeuf RC (1998). Dietary isoflavones reduce plasma cholesterol and atherosclerosis in C57Bl/6 mice but not LDL receptor-deficient mice. J. Nutr. 128: 954-959.

Kubowitz F, Otti P (1946). Lactate dehydrogenase activity in urine. Biochemistry J. 314: 94-96.

Lee DH, Gross MD, Jacobs DR (2004). Association of serum carotenoids and tocopherols with γ-GT: the Coronary Artery Risk Development in Young Adults (CARDIA) Study. Clin. Chem. 50: 582-588.

Lee DH, Ha MH, Kim JR, Gross M, Jacobs DR (2002). Gamma-glutamyl transpeptidase, alcohol, and blood pressure. A four year follow-up study. Ann. Epidemiol. 12: 90-96.

Lee DH, Jacobs DR Jr, Gross M, Kiefe CI, Roseman J, Lewis CE, Steffes M (2003). Gamma-glutamyl transpeptidase is a predictor of incident diabetes and hypertension: the Coronary Artery Risk Development in Young Adults (CARDIA) Study. Clin. Chem. 49: 1358-1366.

Lovati MR, Manzoni C, Canaveri A (1987). Soybean protein diet increases low-density lipoprotein receptor activity in mononuclear cells from hypercholesterolemic patients. J. Clin. Invest. 80: 1498-1502.

Montani J, Carroll JF, Dwyer TM, Antic V, Yang Z, Dulloo AG (2004). Ectopic fat storage in heart, blood vessels and kidneys in the pathogenesis of cardiovascular disease. Int .J .Obes. 28: 558-565.

Oluba OM, Adeyemi O, Ojieh GC, Adebisi KE, Isiosio IO, Aboluwoye CO (2008a). Effect of dietary cholesterol on some serum enzymes. J. Med. Sci. 8(4): 390-394.

Oluba OM, Adeyemi O, Ojieh GC, Isiosio IO (2008b). Fatty acid composition of *Citrullus lanatus* (egusi melon) and its effect on serum lipids and some serum enzymes. Internet J. Cardiovasc. Res. 5: 2.

Potter SM (1995). Overview of proposed mechanisms for the hypocholesterolemic effect of soy. J. Nutr. 125(3): 5606-5611.

Reitman S, Frankel S (1957). A colorimetric method for the determination of serum GOT and GPT. Am. J. Clin. Petol. 28: 56-63.

Salomen JT (2003). Liver damage and protective effect of high density lipoprotein cholesterol. Br. Med. J. 327: 1082-1090.

Schaffer JE (2003). Lipotoxicity: when tissues overact. Curr. Opin. Lipidol. 14: 281-287.

Szaz G (1976). A kinetic photometric method for serum Gamma glutamyl transpeptidase. Clin. Chem. 15: 124-136.

Tovar AR, Torre-Villalvazo I, Ochoa M, Elias AL, Ortiz V, Aguilar-Salinas CA, Torres N (2003). Soy protein reduces hepatic lipotoxicity in hyperinsulinemic obese Zucker fa/fa rats. J. Lipid Res. 46: 1823-1832.

Weggemans RM, Trautwein EA (2003). Relation between soy-associated isoflavones and LDL and HDL cholesterol concentrations in humans: a meta-analysis. Eur. J. Clin. Nutr. 57: 40-46.

Young VR (1991). Soy protein in relation to human protein and amino acid nutrition. J. Am. Diet Assoc. 91: 828-835.

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