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Effects of Dietary Cholesterol on Some Serum Enzymes

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The activities of serum enzymes were investigated in rats feeding on 5 and 10% cholesterol. Rat were subjected to feeding over a period of seven weeks on formulated diets containing no cholesterol supplement, 5 and 10% cholesterol respectively. Serum cholesterol, triglycerides, gamma-glutamyl transpeptidase (GGT), lactate dehydrogenase (LDH), alanine transaminase (AST) and Aspartate Transaminase (AST) of rat placed on the formulated diets were measured every week for seven weeks. Relative to the control, the serum parameters studied increased significantly ($p<0.05$) in the test rats. In contrast, the body weight of rats was significantly lower than that of the control rats ($p<0.05$). It eas considered that consumption of the cholesterol based diet may not lead to increase in body weight. However, it could increase the risk of heart related diseases.

Key words: Serum enzymes, rat feeding, cholesterol-based diet

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INTRODUCTION

Cardiovascular disease is the dominant single cause of premature mortality in the world. In men and women dying before 70 years of age in 1995, 17.8% of deaths were due to stroke (Anand *et al*., 2000). Cardiovascular disease in its various manifestations (coronary disease, cerebrovascular disease, peripheral vascular disease, etc.) has a long pre-symptomatic or incubation period, possibly 30-50 years in duration (Bell, 2000).

Cardiovascular disease results from dietary fats and oils. The fats and oil are used almost universally as stored forms of energy in living organisms as they are derivatives of fatty acids. Fatty acids are hydrocarbon derivative, with low oxidation levels as hydrocarbons in fossil fuels (Clifton and Abbey, 1997). Fat are water soluble products complexes in saturated and unsaturated forms as triglycerides and cholesterol.

The average daily intake of cholesterol is about 300-500 mg and much of this provision can be found in animal products such as eggs (yolk) (Mayor, 1999). As the quantity of dietary cholesterol consumed can significantly alter serum levels, it is therefore ideal to maintain the average intake of cholesterol to <300 mg day^{-1} (Mayor, 1999).

Humans and animals show a certain consistency in the response of their serum lipids levels to fat-modified diets. The effects of dietary changes on serum lipid levels differ significantly between individuals (Katan and Beynene, 1987). The differences in responses may be caused by variations in genes regulating serum levels (Clifton and Abbey, 1997). A significant factor underlying the high continuing incidence rate of coronary heart disease is a typical diet high in saturated fat and cholesterol both of which contribute to elevated serum cholesterol (McGill, 1988). A serum cholesterol level >240 mg dL^{-1} is considered to be a high risk and is labeled as hypercholesterolemia (Kris-Etherton, 1988).

Cases of hypercholesterolemia often look intractable and often constitute the primary cause of death in patients suffering from it. An alternative and earlier means of detecting elevated serum cholesterol level is serum enzyme activities. Studies on the effects of dietary cholesterol on serum enzyme are scanty; however, it is an area that looks promising in term of early prediction of hypercholesterolemia and the aim of present study was to design the drugs for lowering serum cholesterol.

MATERIALS AND METHODS

Chemicals: All chemicals and solvents used were of Analar grade and were products of BDH Chemicals Ltd. Poole, England.

Table 1: Diet composition (percentage by weight)

Feed composition	A	B	C
Maize flour	70	65	60
Fish meal	10	10	10
Groundnut cake	20	20	20
Cholesterol	-	5	10

Animals and diet: Twenty-four male albino rats (twenty weeks old and having an average weight of 150 g) obtained from the animal house of the Faculty of Veterinary Medicine, Ladoke Akintola University of Technology, Ogbomosho, Nigeria, were used for the study. The rats were housed in stainless steels cages with raised wire floors at a temperature of about 30°C and fed on rat chow and water *ad libitum* for an initial period of two weeks. The rats were then divided into three groups (one control, A; and two experimental groups, B and C) of eight rats each and fed three different dietary regimes, A, B and C, for a period of seven weeks. Diet A (control) had no cholesterol, whereas diets B and C (the experimental) contained 5 and 10% by weight of cholesterol respectively, incorporated into the normal rat feed. Composition of each diet is presented in Table 1. Before the commencement of the diet, the rats were fasted overnight but allowed water *ad libitum*. The weekly changes in weight of rat s in each group were also monitored. The animal protocol was approved by the Animal Committee of the National Institute of Medical Laboratory Sciences, Nigeria.

Serum preparation: At the end of each week, rats were denied food overnight but allowed water *ad libitum*. One rat from each group was anaesthetized by chloroform and 2.5 mL blood collected 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 2) centrifuge to separate serum from the cells. The supernatant (serum) was carefully decanted and analysis was carried out immediately.

Assays: Serum cholesterol was determined using the method described by Roeschlau *et al*. (1974). In this method cholesterol is determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine is formed from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxidase. Serum triglycerides were determined using the methods described by Tiez (1990). The triglycerides are determined after enzymatic hydrolysis by lipases. The indicator is quinoneimine formed from hydrogen peroxide, 4-aminophenazone and 4-cholophenol under the catalytic influence of peroxidase.

Gamma glutamyl-transpeptidase (λ-GT) enzyme activity was determined according to the method described by Szaz (1976) and Szaz and Persin (1974).

λ-GT activity was measured by monitoring the increases in absorbance at 405 nm of p-nitroaniline formed by the reaction between L-gamma-glutamyl-p-nitroanilide and glycylglycine. Lactate dehydrogenase (LDH) (E.C.1.1.1.27) activity was determined based on the method described by Kubowitz and Otti (1943). The method involves the reduction of pyruvate to lactate with the concomitant oxidation of NADH to NAD^+. The method measures absorbance at 340 nm. Activities of Aspartate transaminase (AST) (E.C.2.6.1.1) and Alanine transminase (ALT) (2E.C.2.6.1.2) were determined using the method described by Reitman and Frankel (1975). The method measures spectrophotometrically the intensity of the red coloured hydrazone formed from the reaction of pyruvate with 2, 4-dinitrophenylhydrazine at 546 nm.

Statistical analysis: Statistical Analysis was done using One Way Analysis of Variance and Duncan Multiple Range Test (DMRT).

RESULTS

The animal consumed their daily rations satisfactorily and showed increased in body weight. The weekly changes in body weight for the respective group studied are shown in Table 2. At the beginning of the feeding trial there was no significant difference ($p>0.05$) among the weights of the three groups of rats. It was observed that the weights of the test animals (group B and C) were significantly lower ($p<0.05$) than that of the control (A) at the end of the feeding period. However, no significant difference ($p>0.05$) was observed in the body weight between groups B and C.

Table 2: Growth response of rats (g)

Week	Control	5% Cholesterol	10% Cholesterol
0	138.88±3.00	139.25±3.0	134.31±3.0
1	142.13±2.80	142.63±2.9	137.75±3.1
2	148.86±3.10	146.29±2.7	144.43±2.5
3	158.17±2.17	156.67±2.6	154.67±2.2
4	173.80±2.50	164.00±1.6*	162.20±2.15*
5	201.25±3.40	175.25±2.25*	172.25±2.25*
6	204.00±5.20	176.67±3.5*	174.00±2.7*
7	215.00±4.50	191.50±5.6*	186.00±4.2*

Results are means of five determinations±SEM. Values carrying different superscripts are significantly deferent ($p<0.05$)

Table 3: Serum cholesterol concentration (mg dL^{-1})

Week	Control	5% Cholesterol	10% Cholesterol
0	150.0±2.140	150.0±2.64	150.0±2.27
1	200.0±5.000	240.0±7.11*	250.0±2.27*
2	200.0±6.170	260.0±10.70*	280.0±11.31*
3	205.0±11.20	340.0±9.80*	440.0±13.41**
4	203.0±7.910	400.0±12.24*	500.0±16.19**
5	210.0±10.53	410.0±15.60*	550.0±14.70**
6	205.0±9.180	440.0±10.40*	630.0±15.11**
7	250.0±14.80	500.0±9.21*	700.0±10.21**

Results are means of five determinations±SEM. Values carrying different superscripts are significantly different ($p<0.05$)

Changes in serum total cholesterol concentration in the respective groups fed the various levels of cholesterol were as shown in Table 3. From the result it was observed that the cholesterol-fed rats, both at 5 and 10% levels, showed progressively higher concentrations of serum cholesterol when compared with the control group. These increases were statistically significant at ($p<0.05$) and the increase was greater in group C than in group B. Significant changes in serum cholesterol between the test groups (B and C) and control (A) became observable from the first week of feeding.

The results obtained from measurement of serum triglyceride concentration for the respective groups fed different levels of cholesterol were as shown in Table 4. At the end of the feeding exercise, the cholesterol-fed rat had significantly higher ($p<0.05$) concentrations of serum triglyceride when compared with the control rats. Serum triglyceride levels were highest in group C fed 10% cholesterol.

Changes in activity of serum LDH of rats in the respective groups placed on the formulated diet over the seven weeks were as shown in Table 5. At the culmination of the feeding experiment, serum LDH activity was

Table 4: Serum triglyceride concentration (mg dL^{-1})

Week	Control	5% Cholesterol	10% Cholesterol
0	125.0±1.19	125.0±2.03	125.0±1.10
1	130.0±4.17	145.0±3.53*	145.0±3.64*
2	132.0±2.55	155.0±2.71*	160.0±2.98*
3	135.0±3.00	165.0±4.61*	190.0±3.32**
4	135.0±2.71	175.0±3.19*	210.0±2.57**
5	140.0±1.81	220.0±4.31*	250.0±3.96
6	135.0±3.43	240.0±5.61*	300.0±4.77**
7	140.0±3.77	255.0±3.86*	320.0±6.28**

Results are means of five determinations±SEM. Values carrying different superscripts are significantly different ($p<0.05$)

Table 5: Specific activity of serum LDH (U/L)

Week	Control	5% Cholesterol	10% Cholesterol
0	161.90±6.42	161.90±5.61	161.90±5.22
1	161.90±5.12	242.85±4.41*	283.33±6.00**
2	161.85±4.81	283.33±6.12*	323.80±7.11**
3	161.90±4.64	323.80±3.24*	404.75±6.34**
4	161.90±4.22	404.75±7.24*	465.51±5.19**
5	161.93±4.71	469.51±11.40*	485.70±8.44**
6	161.90±3.27	485.70±9.31*	647.70±9.26**
7	161.95±3.56	647.60±12.40*	809.50±11.41**

Results are means of five determinations±SEM. Values carrying different superscripts are significantly different $p<0.05$)

Table 6: Specific activity of serum alanine transaminase (ALT)

Week	Control	5% Cholesterol	10% Cholesterol
0	8.0±0.6	8.0±0.3	8.0±0.8
1	9.0±0.8	12.0±0.5*	14.0±0.7**
2	12.0±0.4	17.0±0.6*	17.0±0.5*
3	14.0±0.8	21.0±0.4*	24.0±0.3**
4	12.0±0.7	23.0±0.2*	26.0±0.8**
5	12.0±1.1	25.0±0.7*	30.0±0.7**
6	14.0±0.6	29.0±0.5*	34.0±0.9**
7	14.0±0.8	31.0±0.8*	35.0±1.2**

Results are means five determinations±SEM. Values carrying different superscripts are significantly different ($p<0.05$)

Table 7: Specific activity of serum aspartate transaminase (AST)

Week	Control	5% Cholesterol	10% Cholesterol
0	7.0±0.2	7.0±0.2	7.0±0.1
1	10.0±0.3	10.0±0.2	10.0±0.2
2	10.0±0.2	13.0±0.4*	16.0±0.2**
3	10.0±0.1	16.0±0.7*	19.0±0.3**
4	13.0±0.6	17.0±0.6*	27.0±0.9**
5	10.0±0.3	19.0±1.1*	31.0±2.1**
6	10.0±0.3	24.0±1.4*	36.0±1.8**
7	10.0±0.2	27.0±1.0*	38.0±2.0**

Results are means of five determinations±SEM. Values carrying different superscripts are significantly different ($p<0.05$)

Table 8: Specific activity of serum gamma glutamyl-transpeptidase (λ-GT)

Week	Control	5% Cholesterol	10% Cholesterol
0	11.11±0.88	11.11±1.00	11.11±0.93
1	22.22±1.00	33.33±1.24*	55.55±1.11**
2	33.33±2.00	44.44±1.51*	66.66±1.60**
3	33.33±0.96	55.55±2.33*	88.88±3.17**
4	22.22±3.55	88.88±3.41*	133.32±5.59**
5	33.33±3.48	99.99±2.79*	133.32±5.59**
6	33.33±3.52	111.10±3.84*	155.54±8.11**
7	33.33±3.66	132.32±5.91*	177.76±9.23**

Results are means of five determinations±SEM. Values carrying different superscripts are significantly different ($p<0.05$)

highest in group C followed by group B. The cholesterol-fed groups showed significantly increased ($p<0.05$) LDH activity compared with the control. Significant difference ($p<0.05$) in serum LDH activity was also observed between group B and C.

The changes observed in activity of serum ALT in the respective groups fed different levels of cholesterol were as presented in Table 6. From the result presented a significant increase ($p<0.05$) in serum ALT activity was observed between rats fed cholesterol-enriched diet and that of the control. Similarly, the 10% cholesterol-fed rat had significantly greater ($p<0.05$) levels of serum ALT compared with the 5% cholesterol-fed rats.

Table 7 shows the results obtained from measurement of serum AST activity for the respective groups. As shown in the results, a dose-dependent increase in activity of serum AST was observed between the cholesterol-fed and the control rats. Rats fed cholesterol both at 5% and 10% levels showed significantly higher ($p<0.05$) activity of serum AST compared with the control.

The changes observed in serum activity of λ-GT in the respective groups fed the different levels of cholesterol were as shown in Table 8. From the results obtained, a significantly higher ($p<0.05$) activity of the enzyme was recorded in the cholesterol-fed groups (both at 5 and 10% levels) compared with the control. The increases were found to be dose-dependent.

DISCUSSION

Hypercholesterolemia has become a worldwide epidemic and its prevalence continues to increase at rapid rate in various populations and across all age groups Hypercholesterolemia poses a major public health challenge since it is a well recognized independent predictor of premature mortality (Anand *et al*., 2000) Moreover, it often coexists with other cardiovascular disease (CVD).

Elevated levels of serum cholesterol is considered the most common underlying abnormality in Coronary Heart Disease (CHD) and is influenced by genetic and environmental factors and in particular, changes in diet (Katan and Beynene, 1987; McGill, 1988). Excess fat intake especially in the form of cholesterol leads to the formation and deposition of lipids in various fatty tissues. In addition, it has been reported that accumulation of excess fat and cholesterol in non-adipose tissues, such as the liver, heart, skeletal muscle, kidneys and blood vessels may impair their functions and contributes to cell dysfunction or cell death, a phenomenon known as lipotoxicity (Schaffer, 2003; Montani *et al*., 2004).

The results generated in this study indicate clearly that cholesterol feeding decreases body weight (Table 3) contrary to the misconception that dietary cholesterol will lead to increase in body weight. The resulting decrease in weight was found to be dose-dependent. Thus the higher the percentage of cholesterol incorporated into the diet the greater is the weight reduction. Hence, a non-overweight individual may not necessarily be innocent at high intake of cholesterol-rich diet.

Similarly, our observations from the present study suggest that serum levels of cholesterol and triglycerides correlate positively with dietary cholesterol. It thus implies that the quantity of cholesterol consumed can significantly alter serum cholesterol concentration and other fats, which are transported between the intestine, liver and periphery in soluble complexes, called lipoprotein. They circulate in the form of spherical particles and comprise an envelope of apoprotein and phospholipids and a non-polar core. One of these is Low-Density Lipoprotein (LDL). The greater the concentration of LDL the greater is the risk of developing atherosclerosis (Hevonoja *et al*., 2000). Therefore, the high serum cholesterol and triglycerides levels observed in the present study may predispose to atherosclerosis.

Furthermore, the results of our present study showed that serum enzyme activities increase proportionately with increasing cholesterol concentration. In most cases, increased levels of these enzymes (LDH, ALT, AST and λ-GT) are usually found in cellular death and or leakages from cell. It has been reported that as fat storage cells increase in size and reach their capacity for storing more fat (usually from increased dietary intake), then additional fat is deferred to non-adipose tissues (such as the liver, skeletal muscle, kidney heart and beta cells of the pancreas) intracellularly where they can exert toxic effects

and dysfunction (Schaffer, 2003; Montani *et al*., 2004). The observed increases in activity of all the serum enzymes monitored in this study might not be unconnected with overloading of the liver, kidneys, heart and blood vessels with lipids from the cholesterol-rich diets.

In conclusion, the data generated in the present study revealed that body weight might not be appropriate as an index for monitoring atherosclerosis. Serum enzymes such as LDH, ALT, AST and λ-GT may serve as better biomarkers for assessing atherosclerosis and myocardial infarction.

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