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Effect of Lipid-Based Diet on Some Lipid-Metabolizing Enzymes

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The effects of long-term feeding of lipid-based diet (containing 5 and 10% cholesterol) on some lipid-metabolizing enzymes were examined by determining the activities of Lecithin: Cholesterol Acyl Transferase (LCAT), lipoprotein lipase (LPL) and triglyceride lipase (TGL). The LCAT activity was found to increase significantly ($p < 0.05$) in the cholesterol fed rats when compared to control animals fed the control diet and the increase was found to be dose dependent. The cholesterol fed rats also showed relatively significant ($p < 0.05$) increases in the levels of both cholesterol ester and free cholesterol. Similarly, both LPL and TG lipase activities were also significantly ($p < 0.05$) elevated in the cholesterol fed rats when compared to control. Histochemical evaluation of the liver of the rats fed 10% cholesterol at the end of the feeding period revealed chronic venous congestion and fatty changes. The kidney showed fat embolism with glomerular capillaries distended with fat globules. The aorta lumen was reduced to less than half the normal diameter by a greatly thickened intima which contained cholesterol crystals, typical of arteromatous plaque. The implications of these findings are discussed with respect to arterogenesis, hyperlipoproteinemia and hypertriglyceridemia.

Key words: Cholesterol diet, LCAT, LPL, TG lipase

INTRODUCTION

Complex lipids of humans fall into one of two broad categories. Non-polar lipids such as triacylglycerols and cholesterol esters and polar lipids represented by phospholipids, sphingolipids and ecosanoids. Specific enzymes hydrolyze the non-polar forms of lipids. These enzymes are lipoprotein lipase and triacylglycerol lipase.

Lipoprotein lipase (LPL) is an enzyme located on the surface of endothelial cells of capillaries that hydrolyses fatty acids from position 1 and 3 of triacylglycerols present in chylomicrons and Very Low-Density Lipoprotein (VLDL) to glycerol and free fatty acids (Ranganathan *et al.*, 2001). Triacylglycerol lipase (TG lipase) also hydrolyses triacylglycerol to glycerol and free fatty acids. Triacylglycerol lipase activity is more in the liver than in the adipose tissue (Chung *et al.*, 1980). Lecithin: cholesterol acyl transferase (LCAT) is an enzyme that catalyzes the transfer of fatty acids from the C-2 position of lecithin to cholesterol (Fielding, 1974).

In humans, almost all plasma cholesterol ester is formed by the activity of LCAT (Nordby and Norum, 1975) and individuals devoid of this enzyme show abnormal plasma lipoproteins (Glomset *et al.*, 1970). In the absence of LCAT activity, both cholesterol and triacylglycerol metabolisms are perturbed and the resulting plasma lipoproteins bear little resemblance to their normal counterparts (Onyeneke *et al.*, 1991). Deficiency in LPL activity is a disorder characterized by marked hyperchylomicronemia and a corresponding hypertriglyceridemia. LPL is essential for the hydrolysis of triacylglycerol and conversion of chylomicrons to chylomicron remnants. The massive accumulation of chylomicrons in the circulation indicates an ability to catabolize dietary fats (Ranganathan *et al.*, 2001). The amount of free fatty acids and glycerol present in chylomicrons and VLDL can be determined by the activities of LPL and TG lipase. A group of rare genetic disorders characterized by deficient activity of LPL and TG lipase results in hyperlipoproteinuria, familial chylomicronemia and a corresponding hypertriglyceridemia. These are disease cases arising as a result of high level of cholesterol in the blood (Ranganathan *et al.*, 2001; Lusis, 2000; McCormick *et al.*, 2007). Deficiency in LCAT, LPL and TG lipase is a disorder characterized by marked hypercholesterolemia, hyperchylomicronemia and the corresponding hypertriglyceridemia (Kane and Havel, 1995; Galeone *et al.*, 2006).

The present study is aimed at monitoring the effect of lipid-based diet on the activities of some lipid-metabolizing enzymes; LCAT, LPL and TG lipase, which

are enzymes responsible for the catabolism of cholesterol present in high density lipoprotein, chylomicrons and VLDL.

MATERIALS AND METHODS

Animals and diets: Twenty-four male albino Wistar rats (twelve weeks old and having an average weight of 112 g) obtained from the animal house of the College of Health Science, Ladoko Akintola University of Technology, Osogbo, Nigeria, were used for the study. The rats were divided into three groups of eight rats such that the weight difference between the groups was less than 0.2 g. The animals were housed individually in stainless steel cages with raised wire floors to prevent coprophagy. A group of rats which served as the control were fed a cholesterol free diet (normal rat chow); the other groups of rats were test rats. One test group was fed rat chow containing 5% cholesterol and the other test group of animals was fed the rat chow with 10% cholesterol. The study lasted for a period of 8 weeks during which the animals were allowed food and water freely. The composition of each diet is presented in Table 1. Before the commencement of the diet regime, the rats were fasted overnight but allowed access to water *ad libitum*.

Enzyme assays: At weekly intervals, three rats (one from each group) were sacrificed after ether anaesthesia. The liver and adipose tissue were immediately removed, rinsed with ice cold saline, weighed, kept in a sealed container and stored at -20°C until analyzed.

Portions of the liver tissue was homogenized and then extracted with chloroform/methanol (2:1v/v) mixture. After the addition of 0.9% normal saline solution to the extraction solution and the separation of phases, aliquots of the organic phase were analyzed for liver total cholesterol (Richmond, 1973) and free cholesterol (Roeschlau *et al.*, 1974). Estimation of free cholesterol and LCAT activity in the liver was by the method of Tato *et al.* (1997), while lipase activity was determined by the method of Tietz (1990). The LPL activity was assayed in the adipose tissue, while TG lipase was assayed in the liver. The LCAT activity was expressed as mg dL⁻¹ cholesterol ester formed, while lipase activity was measured in mmol of free fatty acid liberated per minute.

Table 1: Diet composition (% by weight)

Feed composition	Control	Chow+5% cholesterol	Chow+10% cholesterol
Maize flour	70	65	60
Fish meal	10	10	10
Groundnut cake	20	20	20
Cholesterol incorporated (%)	-	5	10

Other analyses: Histochemical preparations of the aorta, kidney and liver of the rats at the end of the feeding period were made. The cholesterol content of the various dietary regimens is determined by the methods of Richmond (1973) and Roeschlau *et al.* (1974).

Statistical analysis: Statistical difference was determined using ANOVA and differences in the means were tested by Duncan's multiple range tests and $p < 0.05$ is regarded as significant (Sokal and Rohlf, 1969).

RESULTS

The rats consumed their daily rations satisfactorily and showed an increase in body weight. The increase in weight was in order of control > test group fed 5% Cholesterol > test group fed 10% Cholesterol.

Changes in the liver cholesterol concentration in the respective groups fed with cholesterol are shown in Table 2. The cholesterol-fed rats, both at 5 and 10% levels, showed progressively higher levels of cholesterol ester when compared with the control group. These increases were statistically significant ($p < 0.05$) and the increase was greater in group fed 10% than in group fed 5% cholesterol.

The total liver cholesterol concentrations in these two groups were also significantly higher ($p < 0.05$) than that of the control (fed normal rat chow) indicating a general increase in the lipid profile of groups fed cholesterol diet. Similarly, there was a corresponding elevation in the free cholesterol levels in these groups and the increase was statistically significant ($p < 0.05$).

The esterifying activity of LCAT expressed as mg cholesterol ester formed per dL g^{-1} liver is as shown in Fig. 1. The result obtained showed that exogenous cholesterol increases the esterifying activity of LCAT ($p < 0.05$) when compared with the control and that the increase is dose dependent. The observed differences in free cholesterol/esterified cholesterol in the liver of these groups of rats indicate that the activity of LCAT is enhanced in the presence of exogenous cholesterol.

Figure 2 and 3 show the activities of lipoprotein lipase (LPL) and triglyceride (TG) lipase, respectively. As observed, the enzymes: LPL and TG lipase, showed a progressively statistically significant higher activities ($p < 0.05$) in the cholesterol-fed rats when compared to the control animals. This increase was also found to be dose dependent.

Histochemical evaluation of the liver of the group fed 10% cholesterol at the end of the feeding period revealed chronic venous congestion and fatty changes (Fig. 4). The kidney showed fat embolism with glomerular capillaries distended with fat globules (Fig. 5). The aorta of the rats at the end of the dietary period showed atheromatous plaque. The lumen as observed was reduced to less than half-normal diameter by a greatly

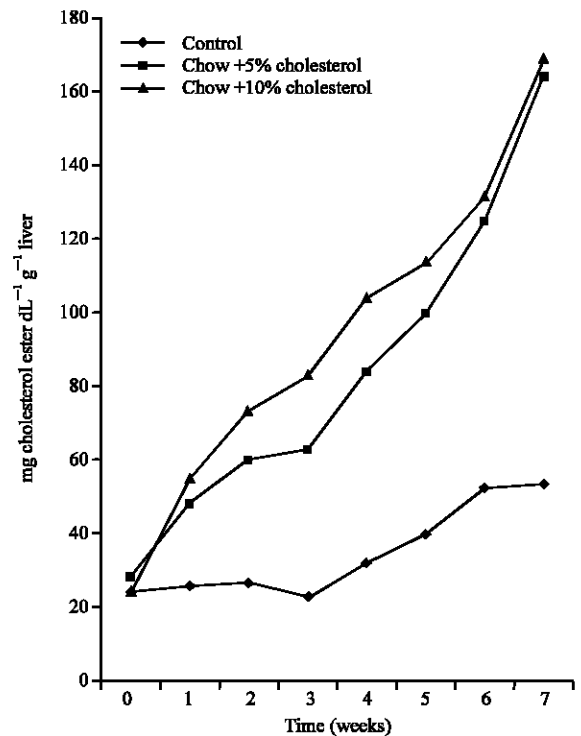


Fig. 1: Cholesterol-esterifying activity of LCAT

Table 2: Changes in liver cholesterol concentration (mg dL⁻¹)

Time weeks	Total cholesterol			Free cholesterol			Cholesterol ester		
	Control	Chow+5% chol.	Chow+10% chol.	Control	Chow+5% chol.	Chow+10% chol.	Control	Chow+5% chol.	Chow+10% chol.
1	60.0±1.3	76.0±2.0	156.0±0.4	136.0±0.6	48.0±1.4	32.0±0.4	24.0±0.5	28.0±0.9	24.0±0.2
2	64.5±0.5	106.5±1.8	125.8±0.8	38.7±0.7	58.1±1.1	71.0±0.7	25.8±1.2	48.4±1.1	54.8±0.4
3	73.3±1.5	133.3±1.5	160.0±1.1	46.7±0.5	73.3±0.8	86.7±0.3	26.6±1.1	60.0±1.3	73.3±2.0
4	80.0±1.1	151.4±1.1	185.7±1.3	57.1±0.8	88.6±0.7	102.9±0.8	22.9±0.9	62.8±1.4	82.8±2.5
5	96.0±0.8	176.0±0.8	212.0±1.5	64.0±1.1	92.0±0.5	108.0±1.6	32.0±1.7	84.0±0.7	104.0±2.8
6	113.3±1.2	206.7±1.0	246.7±1.6	73.3±1.4	106.7±0.9	133.3±1.8	40.0±1.9	100.0±0.8	113.4±3.1
7	127.9±0.3	236.1±1.1	275.4±1.8	75.4±1.1	111.5±1.5	144.3±1.0	52.5±2.5	124.6±0.5	131.1±1.6
8	137.8±1.1	306.7±1.9	320.0±2.1	84.4±0.5	142.2±1.2	151.1±0.3	53.4±3.9	164.5±2.0	168.9±4.2

Values represent mean±SEM. $p < 0.05$ against corresponding control value

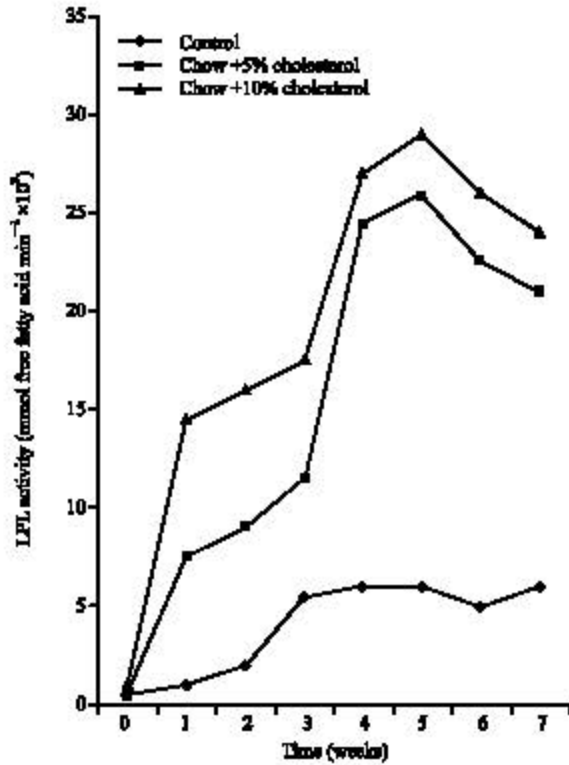


Fig. 2: Lipoprotein lipase activity

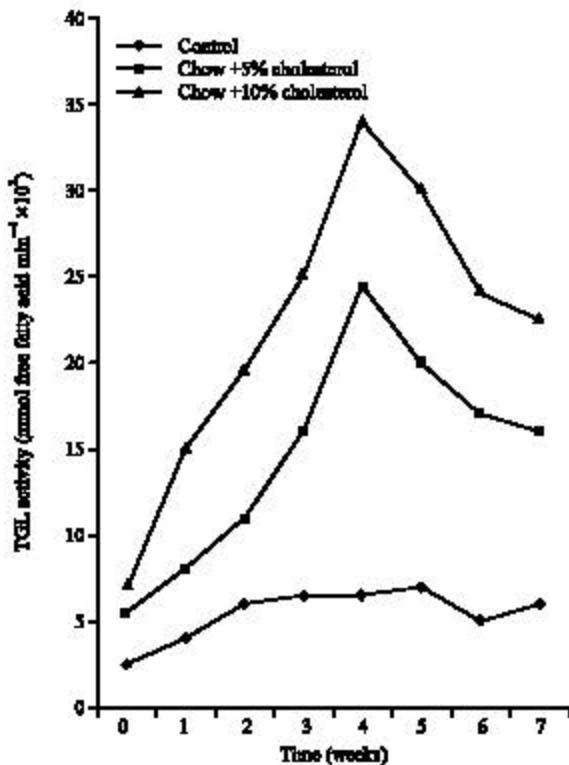


Fig. 3: Triglyceride lipase activity

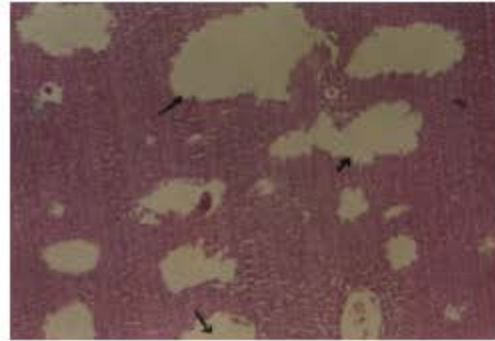


Fig. 4: Histochemical preparation of the liver of the group fed 10% cholesterol at the end of the dietary period. Notice the chronic venous congestion and fatty changes. The liver cells adjoining part of portal tract (left) in which a branch of the portal vein and a small bile duct are visible, are relatively normal but the centrilobular region shows several fatty changes and many hepatocytes are atrophic. Macroscopically, the contrast between the brown colour of the peripheral parts of the lobules and the yellow colour of the fatty central zones produce the nutmeg pattern characteristic of chronic venous congestion. In more severe cases many hepatic cells disappear, fibrous tissue appears to increase and cardiac cirrhosis results.

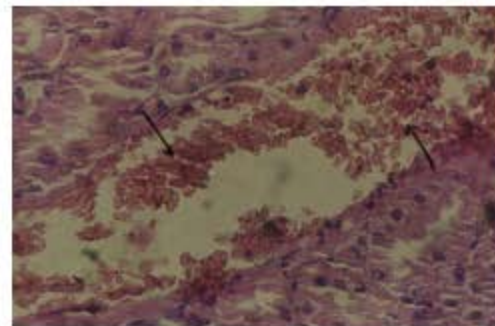


Fig. 5: Histochemical preparation of the kidney of the group fed 10% cholesterol at the end of the dietary period. Notice fat embolism. When fatty tissues such as the marrow of a large bone are severely traumatized, particles of fat may enter the venous circulation. Most are filtered off by the capillaries of the lungs but some get through the lung and form small emboli in the systemic circulation. The glomerular capillaries are distended with fat globules and a small amount of fat is present in the subcapsular space. The gaps in some droplets were caused by fat dissolving in the stain.

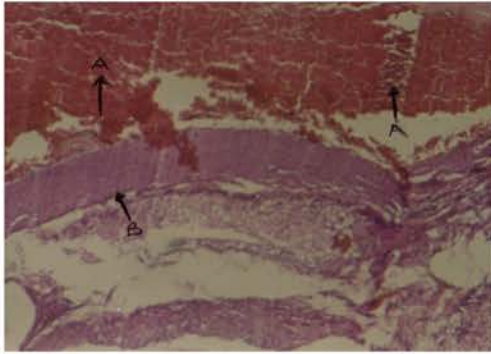


Fig. 6: Histochemical preparation of the aorta of the group fed 10% cholesterol at the end of the dietary period. Notice atheroma. The lumen is reduced to less than half normal by a greatly thickened intima (arrow B). Deep in the intima on the right and pressing on an atrophic media (arrow A) are a crescent-shaped mass of eosinophilic materials and large number of small clefts, which contained cholesterol crystals

thickened intima (arrow B). Deep in the intima on the right and pressing on an atrophic media (arrow A) is a crescent-shaped mass of eosinophilic materials and large numbers of small cleft which contained cholesterol crystals (Fig. 6).

DISCUSSION

Control diet represents the cholesterol dietary requirement of $5.17 \text{ mmol day}^{-1}$ for an average person (65 kg); 5% cholesterol: $8.756 \text{ mmol day}^{-1}$ and 10% cholesterol: $11.385 \text{ mmol day}^{-1}$ excess dietary cholesterol intake.

The hypercholesterolemia that develops when animals are fed a high fat diet is consistently characterized by an increase in the molecular weight of plasma low-density lipoproteins (Berg *et al.*, 1986; Havenoja *et al.*, 2000). Although LCAT esterifies plasma cholesterol solely at the interface of HDL and VLDL, the cholesterol esters thus produced accumulates in all other lipoproteins (Zilversmith *et al.*, 1975). Studies have indicated that there is a correlation between elevations in plasma esterified cholesterol and susceptibility to coronary heart disease (Lusis, 2000). Although elevation of plasma or tissue cholesterol after excessive cholesterol intake depends on the animal species, cholesterol administration has been shown to increase plasma total cholesterol in the rat (Kummerow, 1982; Havenoja *et al.*, 2000).

The results presented here indicate clearly that cholesterol feeding increases the plasma total and

esterified cholesterol concentrations. The increase in plasma esterified cholesterol concentration reflects an increase in the esterifying activity of LCAT, which is responsible for the plasma cholesterol ester level (Nordby and Norum, 1975). Since plasma lecithin is required as the acyl donor for the transesterification reaction of LCAT, an increase in the activity level of the enzyme would result in the lowering of the plasma lecithin level (Onyeneke *et al.*, 1991).

Although exogenous administration of cholesterol is known to increase the plasma cholesterol concentration (Berg *et al.*, 1986; Yokoyama, 2000), the results of our earlier study have shown that this also increased the activity of LCAT. The increase in plasma LCAT found in this work is of interest, since the offending lipid in atherogenesis is the cholesterol ester fraction (Onyeneke *et al.*, 1991; Huxley and Neil, 2003). An increase in the cholesterol ester fraction above the plasma threshold level could possibly initiate atherogenesis (Onyeneke *et al.*, 1991; Overton *et al.*, 2007; Mathew *et al.*, 2003; Gough *et al.*, 2006). It then follows that the hypercholesterolemia that developed when the rats were fed a high-cholesterol diet is due to stimulated activity of LCAT with the result that excess cholesterol ester is produced. If not effectively catabolised due to its relatively high concentration, there would be consequent deposition of the excess in the peripheral and vascular tissues resulting in atherogenesis (Onyeneke *et al.*, 1991; Mathew *et al.*, 2003; Giricz, 2006). Thus, it is evident from the study that LCAT could participate in atherogenesis since the activity level of this enzyme controls the plasma cholesterol ester concentration. It could be argued therefore that those communities that consume a high-cholesterol diet may have elevated levels of LCAT activity, which predisposes them to the development of atherosclerotic cardiovascular disease. This hypothesis supports the earlier finding (Ononogbu and Okpara, 1986) that a gari diet inhibited LCAT activity with the result that those communities that subsist on gari, like Nigeria, have a low incidence of atherosclerotic cardiovascular disease (Ononogbu and Emole, 1978). This regulation of the activity level of LCAT could be beneficial for the control of atherogenesis (Onyeneke *et al.*, 1991).

This study also showed increase in lipoprotein lipase and triglyceride lipase activities for the groups fed cholesterol diet. Lipoprotein lipase is an important enzyme in adipocyte biology and is highly regulated in response to numerous physiological conditions and hormones (Sparks and Pritchard, 1989). Similarly, investigations on hepatic lipase have shown that the lipolytic actions of this enzyme are also sensitive to the cholesterol content

of lipoprotein particles. Lipoprotein lipase and triglyceride lipase activities are high when there is average level of lipoproteins (chylomicrons and very low density lipoproteins) which are the substrates for these enzymes. The activities of these enzymes can be reduced when there is low level of VLDL and that the chylomicrons and VLDL are not broken down normally because of the deficiency of these enzymes. This could result in a build up of the fat-laden chylomicrons in the blood such that the serum appears pale and creamy. Also observed was the higher activity of lipoprotein lipase and triglyceride lipase in the first five weeks than in the last three weeks. This could be because of accumulation of lipoproteins (chylomicrons and VLDL) in the tissues, which leads to a corresponding decrease in the activities of these enzymes.

The histological studies showed deposition of mature fat cells in the hepatocytes with greater concentrations seen peripherally in the test rats (Fig. 4) as compared to the control animals, which had negligible deposits. This confirms the hyperlipidemic condition because of fatty infiltration. In addition, sections of the aorta of the rats at the end of the feeding period showed patchy thickening of the intima of arteries caused mainly by the deposition of lipid and fibrous tissues. The lumen is reduced to less than half-normal diameter by a greatly thickened intima (Arrow B). Deep in the intima on the right and pressing on an atrophic media (Arrow A) are a crescent-shaped mass of ansinophilic materials and large numbers of small clefts, which contained cholesterol crystals (Fig. 6). No such observation was made for group A rats(control), which maintained an intact vasculature. Section of the kidney of 5 and 10% cholesterol fed rats had brown fat cells and mixture of brown fat and mature fat, respectively at the capsule (Fig. 5).

From the results obtained, it could be deduced that the degree of cellular damage in aorta, liver and kidney is proportional to the concentration of cholesterol consumed exogenously. This is also expressed with elevations in the activities of LCAT, LPL and TG lipase found in the experimental rats. Thus, monitoring the activities of some lipid-metabolizing enzymes in the tissues could be a good predictor of atherogenic coronary heart disease and stroke.

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