The effect of feeding **egusi** melon oil as a supplement to a cholesterol-based diet on serum lecithin: cholesterol acyltransferase activity of rats was evaluated. The rats were divided into two groups designated: control and test respectively. Rats in the test group were fed 5% cholesterol diet supplemented with 5% **egusi** melon oil while the control rats received 5% cholesterol diet without **egusi** melon oil. After 6 weeks of diet feeding, the enzyme activity was decreased significantly (p < 0.05) in the **egusi** melon oil-fed rats compared with the control. The test group also showed relative significant decreases in the serum levels cholesteryl ester and lysolecithin (p < 0.05) and increase levels of lecithin (p < 0.05). Significant decreases (p < 0.05) were also observed in serum total and free cholesterol in the **egusi** melon oil treated group compared to the control group. The implications of these results are discussed with respect to hypercholesterolemia.

**Key words:** **Egusi** melon oil, cholesterol diet, cholesteryl ester, LCAT, supplementation.

**INTRODUCTION**

Hypercholesterolemia has become a worldwide epidemic and its prevalence continues to increase at a rapid rate in various populations and across all age groups (Beaglehole et al., 1988). Hypercholesterolemia poses a major public health challenge since it is a well recognized independent predictor of premature mortality (Stamler et al., 1986). Moreover, it often coexists with other cardiovascular risk factors namely, hypertension and diabetes, which further add to the burden of cardiovascular disease. The dramatic increase in the occurrence of hypercholesterolemia over the past several decades is attributed in part to changes in dietary and lifestyle habits, such as rapidly changing diets, increased availability of high fat foods and reduced physical activity of people in both developed and developing countries (Hetzel et al., 1989).

Preventive or therapeutic strategies to control hypercholesterolemia have focused on the manipulation of the amount and nature of dietary fat intakes. In recent years, increased attention has shifted towards the role of nutritional supplement in the management of hypercholesterolemia. **Egusi** melon oil extracted from the seeds of *Citrullus lanatus* that originated in the Western region of Africa is an excellent source of essential fatty acids. The yellow coloured oil which is liquid at room temperature has been reported to contain about 56.9% linoleic acid (Oluba et al., 2008). **Egusi** melon has been shown to lower serum cholesterol concentration in rats when fed as a supplement to a cholesterol-based diet Oluba et al. (2008).

**Lecithin**

Cholesterol acyltransferase (LCAT) (EC 2.3.1.43) is the enzyme responsible for the esterification of plasma cholesterol. In humans almost all plasma cholesterol esters is formed by the activity of this enzyme (Glomset, 1968). It has been suggested that the primary function of this enzyme is related to the maintenance of plasma lipoprotein structure during metabolism (Schumaker and Adams, 1970). A role for the enzyme in the transport of cholesterol from the peripheral tissues to liver (Glomset, 1968) and in maintaining the integrity of the plasma membrane has also been proposed (Glomset and Norum, 1973).
Experiments have shown that the modification of serum lipoproteins by LCAT (Glomset, 1976) 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 (Relimpio and Iriarte, 1981). Since most extra hepatic tissues cannot catabolize cholesterol (Pometta et al., 1986), the mechanism of egress of cholesterol from cells and the involvement of LCAT in this reaction is of utmost importance because most of the cholesterol that accumulates in the aorta during atherosclerotic cardiovascular disease is in the esterified form. The reported response of LCAT to nutritional factors (Fielding and Fielding, 1980) and the earlier report of lowering of LCAT activity by plant protein (Forsythe et al., 1980) prompted us to investigate the effect of egusi melon oil supplementation to a cholesterol diet on serum LCAT activity in rats.

**MATERIALS AND METHODS**

**Chemicals**

All chemicals used were of analytical grade and were products of BDH Chemicals, Poole, England unless otherwise stated.

**Collection and preparation of egusi melon seeds sample**

_Egusi_ melon seeds used for this study were obtained from a local market in Iwaro-Oka Akoko, Ondo State, Nigeria and were identified as _C. lanatus_ (egusi melon) by a taxonomist in the Department of Crop Science, Faculty of Agriculture, University of Benin, Nigeria. The seeds were screened to remove bad ones, shelled manually and further screened. The seeds were then dried to constant weight in an oven at 70°C, ground using mechanical grinder, put in air-tight containers and stored in desiccators for further analysis, some of the seeds was subsequently deposited at the herbarium of the faculty.

**Oil extraction**

Oil from the seeds of egusi melon was extracted by continuous extraction in Soxhlet apparatus (Cehmglass) for 8 h using petroleum ether (60 - 80°C boiling range) as solvent according to the method of AOAC (1980). At the end of the extraction the extraction solvent was evaporated in a rotary evaporator (Cehmglass). The extracted oil was used for feed formulation and the remaining stored in light proof, airtight and moisture proof container at -4°C.

**Animals and diets**

Male Wistar rats (n = 28) eight weeks old and having a mean weight of 120.6 ± 11.2 g were obtained from the Nigerian Institute of Medical Research, Lagos (Nigeria) and housed individually in stainless steel cages with raised wire floor in an environment of 28 - 30°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 and were treated according to the Nigerian guidelines for the care and use of laboratory animals. The experiments were conducted according to the ethical norms approved by the Government of Nigeria and Institutional Animal Ethics Committee Guidelines.

The rats were acclimatized to the facility for 5 days before the start of the experiments. Rats were the assigned to two groups of fourteen animals each designated: control and test respectively and placed on their respective diet for a period of 6 weeks. Composition of each diet is presented in Table 1. Before the start of the diet treatment, the rats were fasted overnight but allowed water ad libitum. Rats have free access to their diet and were weighed weekly.

**Serum preparation**

At weekly intervals two rats from each group was sacrificed and about 2 ml blood collected from each animal by cardiac puncture and pooled together into plain tubes. The blood was allowed to clot at room temperature for 1 h and then centrifuged at 3000 rpm for 10 min the serum was separated into plain tubes and frozen at -20°C and analyzed.

**Assays**

Determination of total and free cholesterol concentrations in the serum was by the method of Searcy and Bergquist (1960), while the esterified cholesterol concentration was calculated as the difference between total and free cholesterol values. Serum lecithin and lyssolecithin concentrations were determined by the method of Stewart (1980) after thin layer chromatography as suggested by Bowyer and King (1977). LCAT activity was determined by the method of Varma and Soloff (1976). The enzyme activity was expressed as mg cholesterol ester formed per 100 ml serum.

**Statistical analysis**

The data are presented as mean ± SEM. Statistical analysis was by one way analysis of variance and Duncan Multiple Range Test (DMRT) using SPSS 11.0.

**RESULTS**

The average daily feed intake for the control and test groups is 23.3 ± 1.3 and 23.0 ± 2.1 g respectively. Both the control and test rats showed increase in body weight with a mean weekly increase in weight of 14.9 ± 1.0 and 18.7 ± 1.4 g respectively.

**Serum lipids**

Table 2 represents the changes observed in serum

<table>
<thead>
<tr>
<th>Feed composition</th>
<th>Control</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize corn</td>
<td>65</td>
<td>60</td>
</tr>
<tr>
<td>Fish meal</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Groundnut cake</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Egusi melon oil</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 1. Diet composition (% by weight).

**Table 2.** Changes observed in serum lipids.

<table>
<thead>
<tr>
<th>Serum Lipid</th>
<th>Control</th>
<th>Test</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol</td>
<td>18.7 ± 1.4</td>
<td>19.2 ± 1.3</td>
<td>0.05</td>
</tr>
<tr>
<td>Triacylglycerol</td>
<td>56.5 ± 2.1</td>
<td>55.0 ± 2.2</td>
<td>0.20</td>
</tr>
<tr>
<td>Phospholipid</td>
<td>22.7 ± 1.5</td>
<td>23.0 ± 1.6</td>
<td>0.08</td>
</tr>
</tbody>
</table>
cholesterol concentration which indicate no significant change \((p > 0.05)\) between the control and test groups in the first 2 weeks of the feeding trial. However, starting from the third week a progressive decrease in serum total, free and esterified cholesterol fractions was observed in the egusi melon oil fed rats compared to control.

Table 3 shows serum lecithin and lysolecithin values. Serum lecithin and lysolecithin concentrations were significantly higher \((p < 0.05)\) in the test rats compared to control rats after 2 weeks of egusi melon treatment.

**Serum LCAT activity**

The esterifying activity of serum LCAT expressed as mg cholesterol formed per 100 ml serum is as shown in Table 4. The results show that exogenous cholesterol increases the esterification rate of LCAT as observed in the control group. On the contrary egusi melon oil suppresses this expected increase in the enzyme activity following cholesterol feeding as seen in the egusi melon fed (test) group. It is important to note that effect of egusi melon supplementation became significant after the third week of feeding.

**DISCUSSION**

Findings in the past several years indicate an important role of dietary fats in influencing the fatty acid profile of serum lipids, including phospholipids which are substrates
of lecithin: cholesterol acyltransferase (LCAT), an important enzyme in lipoprotein metabolism. Although LCAT esterifies serum cholesterol solely at the interface of HDL and VLDL, the cholesteryl esters thus produced accumulate in all other lipoproteins (Zilversmith et al., 1975). Studies have shown that there is a correlation between increases in serum esterified cholesterol and susceptibility to coronary heart disease (Maroko et al., 1979). Although elevation of serum or tissue cholesterol after excessive cholesterol intake depends on the animal species, cholesterol feeding has been demonstrated to increase serum cholesterol in rats (Onyeneke et al., 2007).

The results obtained in this study show that feeding egusi melon oil as a supplement to a cholesterol enriched diet lowers serum total, free and esterified cholesterol levels as well as serum levels of lecithin and lyssolecithin. These observations are in accord with the report of Oluba et al. (2008) on the effect of egusi melon oil on serum lipids in cholesterol-fed rats. The decrease in serum esterified cholesterol concentration observed in this study reflects an inhibition in the esterifying activity of LCAT, which is responsible for the regulation of serum cholesteryl ester level (Glomst, 1962; Nordy and Norum, 1975). Since serum lecithin is required as the acyl donor for the transesterification reaction of LCAT, a decrease in the activity level of the enzyme would result in lowering of serum lecithin level, as is observed in the egusi melon oil fed rats. The increase in lyssolecithin fraction did not correspond to the decrease in the lecithin level, suggesting that the enzyme does not derive its entire acyl moiety from lecithin. The possibility exists that LCAT could utilize other fatty acids within the lipoprotein particles for the esterification process, although this remains to be established.

The decrease in serum LCAT activity leading to a decrease in serum cholesteryl ester fraction observed in this work is of interest, since the offending lipid in atherogenesis is the cholesteryl ester fraction. An increase in cholesteryl ester concentration above the serum threshold level could possibly initiate atherogenesis. It then follows that the hypercholesterolemia that developed when rats are fed a high cholesterol diet is due to stimulated activity of LCAT with the resultant production of excess cholesteryl ester. This is because if the cholesteryl esters produced are not effectively catabolized due to its relatively high concentration, there would be consequent deposition of the excess in the peripheral and vascular tissues thus resulting in atherosclerosis.

The inhibitory role of egusi melon oil on LCAT activity with the result that less amount of cholesteryl ester is produced could be beneficial in reducing the incident rate of atherosclerosis. Egusi melon oil has been reported to contain nutritionally good amount of linoleic acid and other essential fatty acids which have been reported to have protective effect against coronary heart disease (Baylin et al., 2003; Wijendran and Hayes, 2004). Thus, it is evident from this study that regulation of the activity of LCAT (especially by nutritional methods) could be a new target for therapy to prevent atherosclerosis.

REFERENCES