Effect of Egusi Melon Oil on Lecithin: Cholesterol Acyltransferase Activity in Rats Fed a Cholesterol Diet

O Oluba, G Ojieh, G Eidangbe

Abstract
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<.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<.05) and increase levels of lecithin (p<.05). Significant decreases (p<.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.

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 [1]. Hypercholesterolemia poses a major public health challenge since it is a well recognized independent predictor of premature mortality [2]. 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 [3].

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 [4]. Egusi melon has been shown to lower serum cholesterol concentration in rats when fed as a supplement to a cholesterol-based diet [4].

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 [5]. It has been suggested that the primary function of this enzyme is related to the maintenance of plasma lipoprotein structure during metabolism [6]. A role for the enzyme in the transport of cholesterol from the peripheral tissues to liver [5] and in maintaining the integrity of the plasma membrane has also been proposed [7]. Experiments have shown that the modification of serum lipoproteins by LCAT [8] 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 [9]. Since most extra hepatic tissues cannot catabolize cholesterol [10], 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 [11] and the earlier report of lowering of LCAT activity by plant protein [12] 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.

Animals and diets: Male Wistar rats (n=14) 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 28-300C, 50-60% relative humidity and a 12 hour 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 rats were acclimatized to the facility for 5 days before the start of the experiments. Rats were the assigned to two groups of seven 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 one rat from each group was sacrificed and about 2 ml blood collected by cardiac puncture into plain tubes. The blood was allowed to clot at room temperature for 1 hr, and then centrifuged at 3000 rpm for 10 min. the serum was separated into plain tubes and frozen at -200C and analyzed.

Assays: Determination of total and free cholesterol concentrations in the serum was by the method of Searcy and Bergquist [13], while the esterified cholesterol concentration was calculated as the difference between total and free cholesterol values. Serum lecithin and lysolecithin concentrations were determined by the method of Stewart [14] after thin layer chromatography as suggested by Bowyer and King [15]. LCAT activity was determined by the method of Varma and Soloff [16]. 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 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.
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Table 3: Changes in lecithin and lysolecithin concentration (mg/100 ml serum)

<table>
<thead>
<tr>
<th>Time on diet (weeks)</th>
<th>Lecithin</th>
<th>Lysolecithin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Group</td>
<td>Test Group</td>
<td>Control Group</td>
</tr>
<tr>
<td>0</td>
<td>17.5 ± 1.0a</td>
<td>16.9 ± 1.2a</td>
</tr>
<tr>
<td>1</td>
<td>19.1 ± 1.2a</td>
<td>20.2 ± 1.0a</td>
</tr>
<tr>
<td>2</td>
<td>23.3 ± 2.0a</td>
<td>23.6 ± 2.3a</td>
</tr>
<tr>
<td>3</td>
<td>28.3 ± 2.0a</td>
<td>32.2 ± 1.5a</td>
</tr>
<tr>
<td>4</td>
<td>30.0 ± 3.1a</td>
<td>37.2 ± 2.5a</td>
</tr>
<tr>
<td>5</td>
<td>39.1 ± 3.0a</td>
<td>48.7 ± 2.2a</td>
</tr>
<tr>
<td>6</td>
<td>42.5 ± 1.7a</td>
<td>53.5 ± 3.0a</td>
</tr>
</tbody>
</table>

Results are mean ± SEM of triplicate determinations. Values in the same row carrying different superscripts are significant.

Serum LCAT activity: The esterifying activity of serum LCAT expressed as mg cholesterol formed per 100 ml serum is as shown in Table 3. 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.

Table 4: Changes in serum LCAT activity (mg cholesterol ester/100 ml serum)

<table>
<thead>
<tr>
<th>Time on diet (weeks)</th>
<th>Control</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5.9 ± 1.0a</td>
<td>5.6 ± 0.7a</td>
</tr>
<tr>
<td>1</td>
<td>7.7 ± 1.1a</td>
<td>7.8 ± 3.0a</td>
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<td>3</td>
<td>26.5 ± 3.0a</td>
<td>24.5 ± 2.3a</td>
</tr>
<tr>
<td>4</td>
<td>34.5 ± 2.5a</td>
<td>24.0 ± 1.5b</td>
</tr>
<tr>
<td>5</td>
<td>48.0 ± 3.0a</td>
<td>25.9 ± 2.0a</td>
</tr>
<tr>
<td>6</td>
<td>54.2 ± 2.0a</td>
<td>32.7 ± 2.0b</td>
</tr>
</tbody>
</table>

Results are mean ± SEM of triplicate determinations. Values in the same row carrying different superscripts are significant.

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 [17]. Studies have shown that there is a correlation between increases in serum esterified cholesterol and susceptibility to coronary heart disease [18]. 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 [19].

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 lysolecithin. These observations are in accord with the report of Oluba et al. [4] 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
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responsible for the regulation of serum cholesteryl ester level [20, 21]. 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 lysolecithin 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 [23, 24]. 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

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