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Full Length Research Paper

Effects of diet-induced hypercholesterolemia on the lipid profile and some enzyme activities in female Wistar rats

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The effects of a high dietary soybean oil and cholesterol on serum total cholesterol, low density lipoprotein (LDL-C), high density lipoprotein (HDL-C), and triglycerides were investigated. Total protein, albumin, glucose, gamma glutamyl transferase (GGT), aspartate aminotransferase (AST) and alanine amino transferase (ALT) activities were also investigated in weanling female Wistar rats for eight weeks. Two groups of weanling Wistar rats were use in this study. The first group of rats were fed with a control diet made up of the normal rat chow (C), while the second group was given a hypercholesterolemic diet (HPC) enriched with 25% soybean oil and 1% cholesterol for eight weeks. The dietary intake of the HPC diet significantly increased the level of total cholesterol, LDL-C and triglycerides in the serum of animals fed the (HPC) diet. GGT, AST and ALT activities were also markedly elevated in rats fed with the HPC diet. While total protein and glucose level of the animals fed with the HPC diet was remarkably reduced, there was no significant difference in the HDL-C and albumin contents of both groups. This study established that hypercholesterolemia is induced by high soybean oil/cholesterol diet, despite the fact that soybean oil has high content of poly unsaturated fatty acids. Also, the HPC diet led to weight loss in the rats and injury to both heart and liver of the rats.

Key words: Diet-induced, injury, lipid profile, enzyme activities, hypercholesterolemia, soybean oil, cholesterol.

INTRODUCTION

Hypercholesterolemia is a lipoprotein metabolic disorder characterized by high serum low density lipoprotein and blood cholesterol. It has been reported by Rerkasan et al. (2008) as one of the most important risk factors in the development and progression of atherosclerosis that lead to cardiovascular diseases (CVDs). Hypercholesterolemia poses a major problem to many societies as well as health professionals because of the close correlation between cardiovascular diseases and lipid abnormalities (Matos et al. 2005; Ramachandran et al., 2003). Clinical trials have demonstrated that intensive reduction of plasma LDL-C levels could reverse atherosclerosis and decrease the incidence of CVDs (Brown et al., 1990; Ichihashi et al., 1998).

Dietary factors such as continuous ingestion of high amounts of saturated fats and cholesterol are believed to be directly related to hypercholesterolemia and susceptibility to atherosclerosis (Asashina et al., 2005). Furthermore, dietary trials have revealed that the concentration of serum cholesterol is affected by both the content and source of proteins (Serounge et al., 1995; Forsythe, 1995). Lipid structure, composition, configuration, in addition to excessive fat and cholesterol consumption are also believed to affect the lipid profile in the plasma (Zulet et al., 1999).

Hypercholesterolemic animals are useful models for studies on cholesterol homeostasis, and drug trials to better understand the relationship between disorders in cholesterol metabolism, atherogenesis as well as

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Table 1. Composition of the control and hypercholesterolemic diets fed to the female Wistar rats.

Ingredient (g/kg)	Control diet	Hypercholesterolemic diet
Casein	120.00	120.00
Corn starch	729.60	429.60
Cholesterol	0.00	10.00
Cellulose	10.00	130.00
Soybean oil	80.00	250.00
Mineral/vitamin mix	60.40	60.40

possible treatments for the reduction of circulatory cholesterol levels (Pellizon, 2008; Jang and Wang, 2009).

Inducing hypercholesterolemia in rats is often through a high fat, high cholesterol diet, with the fat source varying from lard to canola, coconut, soybean or palm oil. Commercial rations supplemented with cholesterol have also been used for these investigations (Doucet, 1987).

The present study was aimed at investigating the effect of the inclusion of soybean oil and cholesterol in the diet on hypercholesterolemia in Wistar rats. This was with the view of using our findings as a model for subsequent atherogenic studies.

MATERIALS AND METHODS

Animals and diets

Twelve female weanling rats each weighing approximately 66.5 g were obtained from the Animal House at the Department of Biochemistry, University of Ilorin, Nigeria. The animals were housed in groups of six in a controlled environment with 12 h light and dark cycles.

They were allowed free access to different dietary formulations (Table 1) and water *ad libitum* for eight weeks. Six rats were fed with the control diet (C), while the second group was fed with a HPC diet (using a slight modification of the diet of Matos et al., 2005). The animals were weighed gravimetrically every week throughout the period of the experiment.

Assay kits and reagents

The assay kits for cholesterol, HDL-C, LDL-C, triglycerides, total protein, albumin, glucose, gamma glutamyl transferase, alanine and aspartate amino transferases were obtained from Randox Laboratories Ltd., Ardmore, Co. Antrim, UK. All other reagents used were of analytical grade.

Biochemical analysis

Proximate analysis of both the control and hypercholesterolemic diets were determined using the methods described by AOAC, (2002) for crude protein, crude fat, crude fibre, and ash while carbohydrate was determined by subtracting the sum of the other nutrient parameters from 100.

At the end of the eight weeks, the animals were fasted overnight, sacrificed under ether anaesthesia and blood collected from the jugular vein for analysis. The blood samples were centrifuged at 1282 × g for 5 min and the serum collected was stored in Eppendorf tubes at -20°C before analysis.

The heart, liver and kidney of each animal was removed, weighed and stored at -80°C; abdominal fat was also carefully dissected and weighed. The relative organ to body weight was calculated.

Total cholesterol (TC) , HDL-C and triglycerides were determined in the serum of the rats by adopting the protocol outlined in the manufacturer's assay kit from Randox Laboratories Ltd, Ardmore, Co. Antrim, UK. LDL-C was calculated using the Friedewald formula – LDL-C=TC- [HDL-C + TG/5). Total protein, albumin, glucose and enzyme activities were also determined using assay kits and protocol from the same manufacturer.

Statistical analysis

All data were expressed as mean ± S.D and were statistically analyzed using one way analysis of variance (ANOVA). Means were separated by the Duncan multiple test using SAS (2002). Values were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

Table 2 shows the proximate analysis of the control and hypercholesterolemic diets. There was no significant difference in the protein content of both diets, while there was a significant difference $P < 0.05$, in the crude fat content of the hypercholesterolemic diet when compared with the control. This was expected since the HPC diet had higher fat contents (25% soybean oil and 1% cholesterol). However, the ash and total carbohydrate content of the HPC was markedly low.

It has been reported that the relative content of saturated and poly unsaturated fatty acids (PUFA) in the diet affects plasma level of cholesterol (Ohtani et al., 1990). Although metabolic studies have shown that consumption of n-6 PUFA lowers circulating cholesterol level (Ramachandran et al., 2003), in this study however, soybean oil, a rich source of PUFA and cholesterol without cholic acid was successfully used to induce hypercholesterolemia.

Figure 1 shows the body weight gain of the rats during the experimental period. There were significant differences in the weight gain pattern of the animals fed with HPC diet compared to those fed with the control diet. While the animals fed on the control diet maintained a

Table 2. Proximate composition of the control and hypercholesterolemic diets fed to the female Wistar rats.

Parameter (%)	Control diet	Hypercholesterolemic diet
Moisture	6.90 ± 0.14[b]	7.15 ± 0.07[a]
Ash	8.24 ± 0.16[a]	5.73 ± 0.04[b]
Crude protein	17.95 ± 0.14[a]	17.74 ± 0.02[a]
Crude fat	14.64 ± 0.04[b]	29.44 ± 0.14[a]
Crude fibre	2.99 ± 0.14[b]	3.25 ± 0.14[a]
Total carbohydrate	49.53 ± 0.22[a]	37.25 ± 0.13[b]

Values are mean ± SD of 3 replicates. [a - b] Test values along the same row carrying different superscripts for each parameter are significantly different ($P < 0.05$).

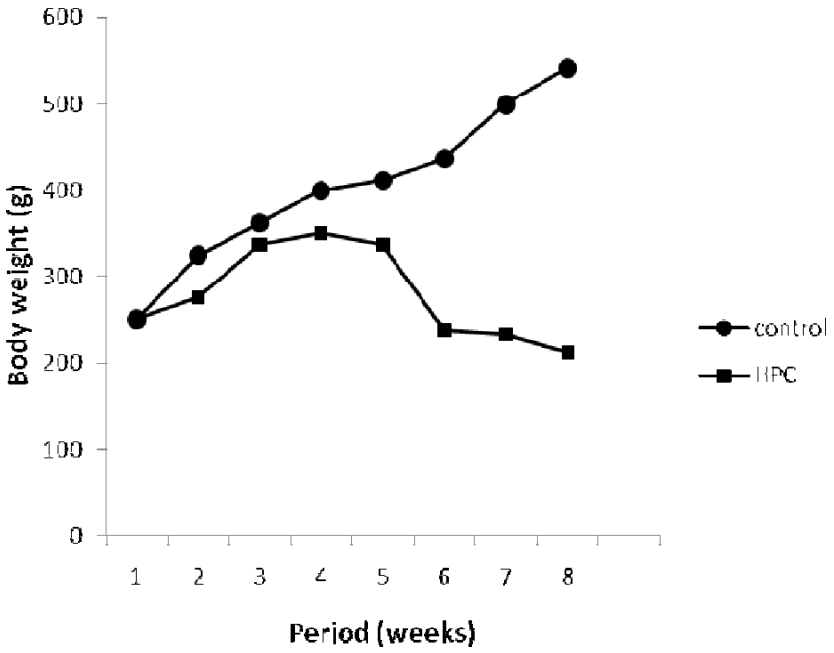

Figure 1. Growth response of female Wistar rats fed with control and hypercholesterolemic (HPC) diet.

consistent weight gain throughout the period, those fed on the HPC only gained weight up till the 4th week, then started a steady decline right till the end of the experiment. The resultant weight loss confirmed by the organ to body weight ratio (Table 3) could be as a result of a reduction in nutrient intake because of the high fat content of the diet which might have impaired the absorption of protein and other nutrients (Zulet and Martinez, 1995; Woo and Henry, 1996; Matos et al., 2005). Records of similar works showed no significant changes in weight gain among the control and hypercholesterolemic animals (Ramachandran et al., 2003), while Harnafi et al. (2009) reported a linear weight increase in all the animals fed with both experimental and control diets. However, Matos et al. (2005), and Hartvigsen et al. (2007) reported similar weight loss as observed in this study with diet-induced hypercholesterolemic rat models. The marked reduction in the abdominal fat observed in the HPC fed rats could be as a result of ketogenic mechanisms (Zulet et al., 1999).

Table 4 shows that there was a significant elevation of serum total cholesterol (TC), low density lipoprotein (LDL-C) and triglycerides in the HPC fed rats, while there was no such difference in the HDL-C of both groups. Increase in LDL-C has been pointed out as one of the risk factors for the development of atherosclerosis and related cardiovascular diseases (Getz and Reardon, 2006). Hence, the result obtained in this study indicated that the dietetic model is suitable for studies in atherogenesis. High serum triglyceride levels has also been reported to

Table 3. Effect of hypercholesterolemic diet on organ to body weight of female Wistar rats.

Organ (g)	Control rats	Hypercholesterolemic rats
Body weight	177.84 ± 4.01[a]	112.50 ± 12.50[b]
Liver	10.00 ± 1.41[a]	7.18 ± 0.25[b]
Heart	3.13 ± 0.18[a]	2.19 ± 0.09[b]
Abdominal fat	5.47 ± 0.69[a]	3.40 ± 0.08[b]
Kidney	3.00 ± 0.00[a]	3.00 ± 0.00[a]

Values are mean ± SD of 3 replicates. [a - b] Test values along the same row carrying different superscripts for each parameter are significantly different (P < 0.05).

Table 4. Effect of hypercholesterolemic diet on serum lipid profile of female Wistar rats.

Parameters (mmol/l)	Control rats	Hypercholesterolemic rats
Total cholesterol	2.50 ± 0.57[b]	5.10 ± 0.28[a]
High density lipoprotein	0.49 ± 0.21[a]	0.49 ± 0.28[a]
Low density lipoprotein	2.00 ± 0.35[b]	4.50 ± 0.28[a]
Triglycerides	1.30 ± 0.28[b]	3.50 ± 0.28[a]

Values are mean ± SD of 3 replicates. [a-b] Test values along the same row carrying different superscripts for each parameter are significantly different (P < 0.05).

Table 5. Effect of Hypercholesterolemic diet on liver and heart functions of female Wistar rats

Parameter	Control rats	Hypercholesterolemic rats
Total protein (g/l)	71.50 ± 1.41[a]	61.00 ± 1.41[b]
Albumin (g/l)	41.50 ± 1.41[a]	41.00 ± 1.41[a]
Glucose (mmol/l)	6.98 ± 0.39[a]	3.85 ± 0.21[b]
Gamma glutamyl transferase (U/l)	17.50 ± 0.00[b]	37.75 ± 0.18[a]
Aspartate amino transferase (U/l)	41.25 ± 0.35[b]	47.25 ± 0.35[a]
Alanine amino transferase (U/l)	8.50 ± 0.35[b]	27.50 ± 0.71[a]

Values are mean ± SD of 3 replicates. [a - b] Test values along the same row carrying different superscripts for each parameter are significantly different (P < 0.05).

be an important risk factor as it influences lipid deposition and clotting mechanisms (Harnafi et al., 2009). Many studies have reported high dietary fat and cholesterol induce hypercholesterolemia in animal models (Cherng and Shih, 2005; Martinello et al., 2006). Similar results were also observed with the HPC fed animals having elevated lipid status than the control. This was an indication that hypercholesterolemia was successfully established in this study.

The data in Table 5 indicates that there was no significant difference P < 0.05 in the albumin level of both the animals fed on the control diet and those fed on the hypercholesterolemic diet. Low serum protein was observed in the HPC fed rats and could be due to poor feed intake and utilization. The high fat diet, because of the energy density, might have led to early satiety, reduced feed intake and impaired absorption of protein and other nutrients (Rolls, 2000). The major reason for the marked decrease (P < 0.05) in serum glucose in the HPC fed rats was uncertain but it could be that the high serum cholesterol increases the level of glucagon-like peptide-1 which enhances insulin secretion from the pancreatic beta-cells leading to hypoglycemia (Xu et al., 1999; Prasad, 2008). It could also be as a result of reduced supply of glucose through the diet and subsequent demand for pentose and glycogen formation. Reports have indicated that saturated fats and polyunsaturated fats may work together to regulate the expression of several enzymes involved in carbohydrate metabolism (Jump et al., 1994).

The enzyme activities shown in Table 5 indicated that all three enzymes – gamma glutamyl transferase (GGT),

aspartate aminotransaminase (AST) and alanine amino transaminase (ALT) were elevated in the serum of the HPC fed rats. This could be as a result of leakage of the enzymes into the serum as a result of damage to the integrity of the heart and liver. Elevated serum activity of these enzymes has been reported to be indicators of calculated risk of cardiovascular disease. According to Pincus and Schaffner (1996), AST and ALT are released into serum when there is severe hepatocellular injury. Elevated serum ALT levels in the absence of viral hepatitis and alcoholism has been reported to lead to a higher risk of cardiovascular disease with the risk greater in women (Ioannou, 2006). The heart also has a high AST content which becomes elevated in myocardial infarction. These reports agree with the present study which showed hepatic injury and cardiovascular distress in the rats fed with HPC.

GGT has been reported to be a very strong risk factor, taking third place, for all forms of heart diseases and a possible indicator for early development of athero-sclerosis. Ruttmann et al. (2005), reported a correlation between GGT and cardiovascular mortality indicating that the higher the elevation of GGT, the greater the risk of death.

Conclusion

This study has revealed that hypercholesterolemia could be induced using soybean oil as a source of fat despite its high content of both mono and polyunsaturated fatty acids; that hypercholesterolemia led to reduced serum level of glucose, had no significant effect on serum albumin, HDL-C and led to weight loss and malnutrition in the rat models. Also, the diet led to impaired hepatic and cardiovascular distress in the rats fed with the hypercholesterolemic diet.

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