DIET-INDUCED HYPERLIPIDEMIA AND ATHEROSCLEROSIS IN WHITE RABBITS.

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ABSTRACT

Improved understanding of causes, effects and treatment of atherosclerotic condition and its severity in the human system for improved health management is essential to the medical scientists. To achieve these, successful induction of atherosclerosis in experimental animals using different modified diets or chemicals provides typical features. However, some of the methods have been criticized to either be less effective or causing untowards pathological effects. This study designed a high lipid diet (atherogenic diet) consisting of a standard diet plus 0.2% cholesterol and 0.6% groundnut oil. Albino rabbits fed a restricted amount (100 g/head/day) of the atherogenic diet (AD) containing 0.2% cholesterol and 0.6% groundnut oil developed mild to severe and persistent hyperlipidemia. The diet was sufficient to induce hypercholesterolemia (180.10±13.50mg/dl), hypertriglyceridemia (64.30±12.70) and atherosclerotic lesion in albino rabbit 8 weeks after atherogenic diet feeding. Mild to severe formation of foam cells were observed in aorta and brachiocephalis of animals given atherogenic diet. Atherosclerotic lesions were measured as presence of fatty streaks and foam cells in the aorta and brachiocephalis. Foam cells and fatty streaks belong to type II lesions. Analyses of lipids levels in the tissues of heart, and liver revealed elevated cholesterol and reduced triglyceride concentrations. This rabbit model of atherosclerosis has such advantages as being able to be produced in a short period and having similar biochemical and pathological characteristics with those in human atherosclerosis. These results should aid investigations of atherosclerosis in albino rabbits by informing the selection of diet to be used.

Keywords: atherosclerosis, lipids, diet, lesions

INTRODUCTION

Over the past few decades, different methods had been adopted in inducing hyperlipidemia and atherosclerosis in animals for the purpose of studying mechanisms of events which include pathological, assessment of drug efficacy. However, some of these methods had been demonstrated to either be less effective or to elicit untoward pathological side effects. For instance, the use of induced mutant mouse models of atherosclerosis to study the mechanisms of lesion formation, diet and drug effects on the extent of lesions, and the role of candidate genes in atherosclerosis susceptibility (1,2) had been criticized. Diets used in the induction have consisted of either chow or Western-type diets,(3) and Western-type or high-cholesterol, high-fat, cholate containing diets(4) have been used to induce hypercholesterolemia and atherosclerosis.5 However, these diets have their short-comings in that (a) while chow diet contained fat content which is not defined and can vary, depending on availability and cost factors, it had also been demonstrated to induces lesions relatively slowly, if at all, in LDLR-/- mice(5); (b) The Western-type diet which usually consists of less than 1% cholesterol and one fifth (wt/wt) of fat, had been demonstrated to induce obesity, with all of the attendant metabolic complications when fed to certain strains of mice, such as C57BL/6J (7). The high-cholesterol, high-fat, cholate-containing diet which usually consists of 1.25% cholesterol, 15% fat (wt/wt), and 0.5% cholic acid (8) had been implicated in the induction of an inflammatory response in mice that may complicate atherosclerosis end points (9). To induce atherosclerosis, Kleeman et al., treated ApoE*3Leiden mice with cholesterol-free (Con), low (LC; 0.25%) and high (HC; 1%) cholesterol diets (10), however, the drawback of the diets included failure of the Con diet to induce early atherosclerosis while the LC diet did so but only mildly. The implication of these is that there is need for further modification of diet to achieve the induction in good time and devoid of any other pathological events that could mask the desired results. The present study tended to induce atherosclerosis in white albino rabbits with modified diet.

METHODOLOGY

Preparation of diets

Both normal and atherogenic diets are prepared according to the table below.

Animals and treatments

Eight rabbits with average weight of 800g were grouped into 2. They were kept in a well ventilated animal house of the Department of Anatomy, Ladoke Akintola University of Technology, Nigeria. The animals had unrestricted access to clean water. Animals in group 1 was given a standard or normal diet (table 1), while those in group 2 were given atherogenic diet containing 0.2% cholesterol and 0.6% groundnut oil in standard diet (table 2). The feeding was done for 8 consecutive weeks. During this period, the weight of each of the rabbits was measured and daily consumptions were monitored. At the 8th week, the animals were sacrificed for collection of samples which included blood and organs for further analyses.

Collection of blood sample

Blood was collected directly from the heart into plain and well-labelled sample bottles and were centrifuged to obtain serum for analyses of biochemical parameters.

Histological study

The heart was quickly excised and immediately placed on blotting paper to remove blood. The tissues were then placed in 10% formalin solution in appropriately labeled sample bottles for histological studies. The tissues of the organ was removed and fixed in Bouin's fluid for 24 h. After fixation, the tissues were dehydrated through ascending grades of ethanol. Thereafter, it was cleared in xylene and finally embedded in paraffin wax. Using a rotary microtome, specimens were sectioned at 5 mm and sections were mounted on clean slides and stained with Sudan black.

Preparation of tissue homogenates

The liver and heart were quickly excised and immediately placed on a blotting paper to remove the blood. Samples of organs were immediately rinsed in 1.15% of potassium chloride solution to remove the hemoglobin. The organ samples were homogenized in aqueous potassium phosphate buffer (0.1M, pH 7.4) in volumes of four times the weight of samples using a Teflon homogenizer. The resultant homogenates were centrifuged at 10,000g for 20 minutes to obtain the post-mitochondrial supernatant fraction (PMF). The PMF was decanted into sample bottles and stored at - 80oC prior to

use. The tissue homogenates of the organs were used to assay for the lipids and lipoproteins.

Analyses of lipids and lipoproteins

Total cholesterol, HDL-C, and triglyceride were analyzed using spectrophotometric methods. Total cholesterol concentration in the serum was determined spectrophotometrically using the cholesterol oxidase method at 546 nm, 37°C. HDL-C was determined by spectrophotometric method of (11) at 500 nm, 37°C. The concentration of LDL-C was determined using the Friedwald equation, i.e., LDL-C = total cholesterol - HDL-C -(0.2triglyceride).

Statistics

All data are presented as means ± SD; where N is the number of experiments. Statistical significance was determined by Student's *t* test for independent samples; P<0.05 was considered statistically significant.

RESULTS

Serum Lipid Contents

Results showed increased but non-significant (p≥0.05) concentrations of total cholesterol, triglyceride, high density and very low density lipoprotein cholesterol in group given atherogenic diet. Mean serum concentration of low density lipoprotein cholesterol was reduced in group given atherogenic, however, this difference was not statistically significant (Table 2).

In the homogenates of heart and liver, total cholesterol and triglyceride showed divergent results. There were significant increases in tissue concentrations of total cholesterol in heart and liver of rabbits given atherogenic diet (p≤0.05). However, the concentrations of triglyceride in tissue homogenates of heart and liver were non-significantly reduced in group given atherogenic diet (p≤0.05) (Table 3).

Within 8 weeks after the start of atherogenic diet feeding, the level of total cholesterol, triglyceride, phospholipid, HDL-C and VLDL-C showed moderate elevations while the level of LDL-C showed moderate reduction (Table 1).

Table 1: Contents of the diets.

compositions	Weight in Kg	Non-atherogenic diet (%)	Atherogenic diet (%)
Maize	8.97	18.00	17.678
Soya bean meal	3.74	8.00	7.37
Brewery dry grain (BDG)	17.50	35.00	34.49
Wheat bran (WB)	5.00	10.00	9.85
Rice bran (RB)	12.50	25.00	24.635
oyster shell (OS)	1.00	2.00	1.97
Bone meal (BM)	0.50	1.00	0.985
Common salt (CS)	0.13	0.50	0.256
methionine	0.50	0.10	0.985
Fish meal	0.50	0.10	0.985
Groundnut oil	0.30	-	0.59
Cholesterol	0.10	-	0.197

Table 2:Concentrations of lipids and lipoproteins in both control animals and those given atherogenic diet.

Biochemical Parameters	Control (N=5)	Atherosclerotic animals (N=5)	p-value
Total cholesterol (mg/dl)	173.00±10.30	180.10±13.50	0.44
Triglyceride (mg/dl)	50.63±6.16	64.30±12.70	0.24
HDL-C (mg/dl)	115.00±32.2	129.80±12.7	0.54
VLDL-C (mg/dl)	10.13±1.23	17.64±6.70	0.20
LDL (mg/dl)	47.84±7.05	40.03±6.46	0.20

Table 3. Concentrations of lipids and lipoproteins in organs of both control animals and those given atherogenic diet.

Biochemical parameters	Organs	Control (N=5)	Atherosclerotic animals (N=5)	p-value
Total Cholesterol (mg/dl)	Heart	27.09±2.24	144.34±1.06	0.0000
	Liver	7.55±1.24	151.45±6.30	0.0007
Triglyceride (mg/dl)	Heart	59.23±8.77	52.00±17.30	0.58
	Liver	63.79±5.29	81.10±15.50	0.21

Pathological Findings of Aortic and Brachiocephalis

Macroscopic findings

Luminal surface of the aorta and brachiocephalis of rabbits fed atherogenic diet were strongly stained compared with those given standard diet. Aorta and brachiocephalis walls of the atherogenic diet fed rabbits were thicker and harder than those fed standard diet (fig. 1-4).

Light microscopic findings

In the aorta and brachiocephalis of the atherogenic diet fed rabbits, there were large number of smooth muscle like cells and focal aggregation of foam cells resulting in intima thickness. Lipid droplets were found in the smooth mucle cells and foam cells in the thickened intima (fig 2 & 4).

There were moderate to severe fat deposition in aorta and brachiocephalic tissues of the artery of the rabbits fed atherogenic diet when compared with normal.
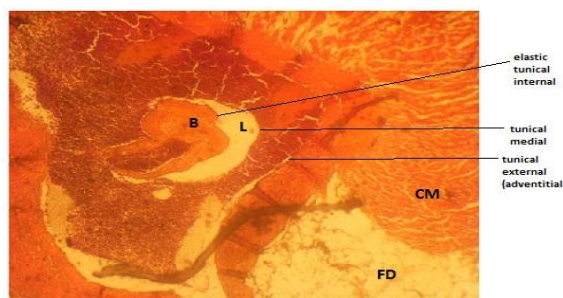
Fig 1: Cross section of aorta of rabbits given normal diet. The features appears normal. The cross section exemplifies a typical elastic artery. Stains: hematoxylin and eosin. Magnification=400 . B = blood; L= lumen; CM = cardiac muscle.
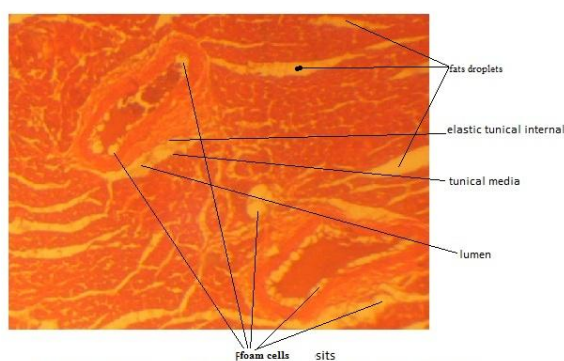

Fig 2: Cross section of aorta of rabbits given atherogenic diet. Degeneration of the blood vessels as a result of fatty deposition at the luminal side of the circular musculature. Stains: hematoxylin and eosin. Magnification=400 . B = blood; L= lumen; CM = cardiac muscle,
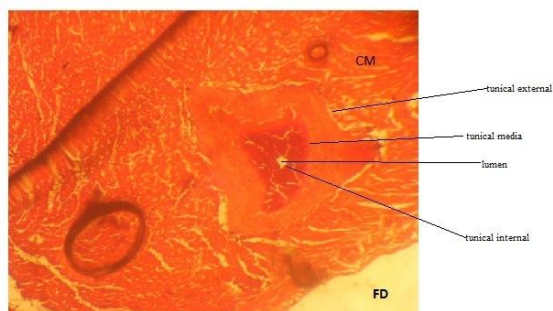

Fig 3: Cross section of brachiocephalis artery of rabbits given normal diet. The features appear normal. Stains: hematoxylin and eosin. Magnification=400 . B = blood; L= lumen; CM = cardiac muscle,
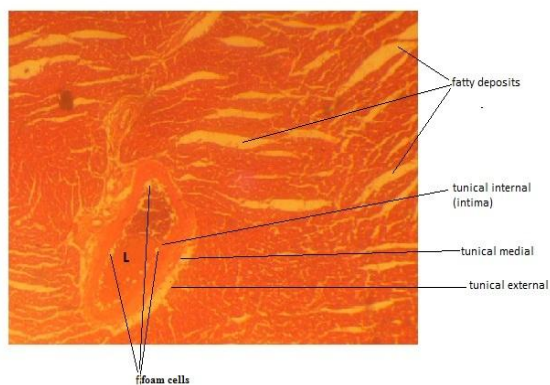

Fig 4: Cross section of brachiocephalis artery of rabbits atherogenic diet. There is severe fat droplets at luminal side of circular musculature. Stains: hematoxylin and eosin. Magnification=400 . B = blood; L= lumen; CM = cardiac muscle,

DISCUSSION

Serum concentrations of lipids in atherogenic diet-fed rabbits were higher (cholesterol(%), triglyceride (%), HDL-C (%), VLDL-C (%)) than those fed the diet containing cholesterol only. In addition to the higher concentrations of serum and arterial lipids in rabbits fed the atherogenic diets compared to those fed the normal diet, higher concentrations of hepatic cholesterol were also found. As in the case of arterial tissue, increases in hepatic concentration of cholesterol were associated with increased serum cholesterol levels.

In this study, severity of arterial lesions was highly associated with serum and tissue cholesterol concentrations (table 1 and figures 1-4). This result is consistent with arterial cholesterol deposition as a distinguishing angiochemical characteristic of atherosclerosis. The arterial lesions were observed with increasing serum levels of total cholesterol. The similarity of the relationship between total cholesterol with this indicator of the severity of atherosclerosis indicates that any causal relationship is largely attributable to the increased serum cholesterol caused by the high lipid diet. The association of elevated levels of arterial lipids with the development of atherosclerosis has been demonstrated previously in many species including pigeon, rabbit and human (12; 13; 14).

Hypertriglyceridemia observed in this study has metabolic consequence. Hypertriglyceridemia that results from either increased production or decreased catabolism of TRL directly influences LDL and HDL composition and metabolism. For example, the hypertriglyceridemia of IR is a consequence of adipocyte lipolysis that results in FFA flux to the liver and increased VLDL secretion. Higher VLDL triglyceride output activates cholesteryl ester transfer protein, which results in triglyceride enrichment of LDL and HDL. The triglyceride content within these particles is hydrolyzed by HTGL, which results in small, dense LDL and HDL particles. Experimental studies suggest that hypertriglyceridemic HDL may be dysfunctional ,(15; 16) that small, dense LDL particles may be more susceptible to oxidative modification (17;18) and that an increased number of atherogenic particles may adversely influence CVD risk (19); however, no clinical outcome trials to date have determined whether normalization of particle composition or reduction of particle number optimizes CVD risk reduction beyond that achieved through LDL-C lowering. An additional complication in hypertriglyceridemic states is accurate quantification of atherogenic particles in the circulation. That is, a high concentration of circulating atherogenic particles is not reliably assessed simply by measurement of TC and/or LDL-C. Moreover, as triglyceride levels increase, the proportion of triglyceride/CE in VLDL increases (ie, >5:1), which results in an underestimation of LDL-C based on the Friedewald formula (20).

The reduction in tissue concentrations of triglyceride could be due to increased lipolysis. In metabolic disorders, increased breakdown of energy store is common. In recent years, high plasma concentrations of lipoproteins rich in triglyceride content have gained attention as strong and independent risk factors for the development of atherosclerosis (21). This association has largely been related to lipoprotein particle size with smaller particles, such as very low density lipoproteins (VLDL) remnants, being primarily implicated in disease development (22). They further work suggested that these smaller particles can enter the artery wall and initiate atherogenesis. Recent work, however, revealed that triglyceride-rich lipoproteins (TGRL), and especially TGRL lipolysis products, can cause vascular injury by other mechanisms. Whereas unmodified TGRL, such as chylomicrons and VLDL, seem to have little effect on endothelial permeability, lipolysis products generated from lipoprotein lipase (LPL)-mediated hydrolysis of TGRL have been shown to decrease barrier function significantly (23; 24).

Atherosclerosis is a complex chronic disease characterized by the accumulation of lipids within arterial walls that eventually go on to form plaques, which can cause narrowing, hardening, and/or complete blockage of arteries. One well known risk factor in humans is hypercholesterolemia (i.e. elevated total cholesterol (TC)) (25), and other important contributors to this disease include inflammation, oxidative stress, and insulin resistance (26; 27). Foods high in dietary saturated fat (SF) and cholesterol (i.e. "Western-type

diets") have been linked to elevations in circulating cholesterol levels (in particular, LDL-C), prompting the recommendation that humans limit the intake of these dietary constituents (25). Like humans, Western-type diets can induce elevated LDL-C and atherosclerosis in certain rodent models (i.e. mice, hamsters, guinea pigs). Therefore, the use of such diets for promoting atherosclerosis in these models has been a valuable tool for both gaining more understanding of this disease and testing therapies that can potentially reverse it. This rabbit model of atherosclerosis has such advantages as being able to be produced in a short period and having similar biochemical and pathological characteristics with those in human atherosclerosis. These results should aid investigations of atherosclerosis in albino rabbits by informing the selection of diet to be used.

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