Streptozotocin-Induced Diabetes Alters The Serum Lipid Profiles Of Adult Wistar Rats

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Citation
O Komolafe, D Adeyemi, S Adewole, E Obuotor. Streptozotocin-Induced Diabetes Alters The Serum Lipid Profiles Of Adult Wistar Rats. The Internet Journal of Cardiovascular Research. 2008 Volume 7 Number 1.

Abstract
This study assessed the changes in serum lipid profiles of experimentally-induced diabetic Wistar rats with view to elucidate the effects STZ induced diabetes on the serum levels of cholesterol and triglycerides of Wistar rats. Twenty adult Wistar rats were randomly assigned into two groups (A and B) of ten rats each. Group A was the control, while Group B was the STZ treated group. The body weight, blood glucose level and serum lipid profiles were monitored in all the animals for four weeks before the commencement of the experiment and throughout the experimental period. Diabetes mellitus was experimentally induced in groups B rats by daily intra-peritoneal administration of multiple doses of 40mg/kg streptozotocin dissolved in 0.1M sodium citrate buffer for 5 consecutive days. The control group was given equivalent volume of citrate buffer. The animals were monitored for four weeks after streptozotocin administration. The data obtained were analyzed using descriptive and inferential statistics. The result revealed a significant (P < 0.05) increase in the serum level of total cholesterol, triglyceride, low-density lipoprotein cholesterol, and very low-density lipoprotein cholesterol of diabetic rats when compared with the control rats while a significant decrease in the high-density lipoprotein cholesterol was obtained. The study revealed that induction of diabetes in rats using STZ result in development of hyperlipidemia in these rats.

INTRODUCTION
Diabetes mellitus is a chronic metabolic disorder characterized by a high blood glucose concentration caused by insulin deficiency, often combined with insulin resistance. Diabetes mellitus is a major cause of disability and hospitalization and it results in significant financial burden. By the year 2010, the total number of people worldwide with diabetes mellitus is projected to reach 239 million. Region with greatest interest are Asia and Africa, where diabetes rates could rise to 2-3 folds than the present. Diabetes mellitus is associated with an increased risk of thrombotic, atherosclerotic and cardiovascular disease. About 70-80% of deaths in diabetic patients are due to vascular disease. Hyperglycemia, the primary clinical manifestation of diabetes, is thought to contribute to diabetic complications by altering vascular cellular metabolism, vascular matrix molecules and circulating lipoproteins. Hyperglycemia increases diacylglycerol levels and activates protein kinase C activity in the aorta of streptozotocin-induced diabetic rats and dogs. Thickening of the basement membranes in renal glomeruli and peripheral capillaries has been observed in STZ-induced diabetic rats and hyperlipidemia is a feature of drug induced diabetes in rats and rabbits.

Streptozotocin (STZ) has long been used as a drug of choice to induce diabetics type II in various animal models. This well-established model is characterized by insulin deficiency associated with insulin resistance. It was reported that a single intravenous injection of STZ could cause increased plasma glucose levels, decrease in body weight and 17% mortality in rats. Hyperlipidemia is a metabolic complication of both clinical and experimental diabetes. Low-density lipoprotein in diabetic patients leads to abnormal metabolism and is associated with increase in very low-density lipoprotein (VLDL) secretion and impaired VLDL catabolism. Ultimately this leads to atherosclerotic plaque. A number of known factors for coronary artery disease such as hypertension, obesity and dyslipidemia are more common in diabetics than in the general population. The World Health Organization (WHO) predicts that the number of cases worldwide for diabetes, as at now is 171 million and it will touch 366 million or more by the year 2030. Patients with DM are more likely to develop microvascular and...
macrovascular complications than the non diabetic population. Dyslipidemia is a frequent complication of DM and is characterized by low levels of high-density lipoprotein-cholesterol (HDL-C) and high levels of low density lipoprotein cholesterol (LDLC) and triglyceride (TG). The specific aim of this study is to characterize the progression of STZ-induced diabetes on the lipid profiles of Wistar rats.

MATERIALS AND METHODS

CARE AND MANAGEMENT OF ANIMALS

Twenty healthy adult male Wistar rats (Rattus norvegicus), weighing between 150 and 250g were used for the experiment. The rats were bred in the animal holding department of Anatomy and Cell Biology Obafemi Awolowo University Ile Ife; these animals were kept in individual cages under natural light and dark cycles at room temperature. They were maintained on standard rat pellet (Ladokun feeds, Ibadan, Nigeria) and water given ad libitum. The animals were randomly assigned into two groups A and B of ten rats each. Group A was the control, while group B was the experimentally-induced diabetic group of rats. There was a pre-experimental period of four weeks during which the body weight, blood glucose level and serum lipid profiles were monitored in the animals before the commencement of the experiment. The animal care conformed to the “Guide for the care and use of Laboratory Animals” published by the US National Institutes of Health (NIH Publication No 85-2 3, 1996).

INDUCTION OF EXPERIMENTAL DIABETICS

Diabetes mellitus was induced in group B animals by intraperitoneal administration of multiple low doses of streptozotocin (Sigma, St. Louis, USA) (40mg/kg body weight) dissolved in freshly prepared 0.1M sodium citrate buffer pH 6.3 for five consecutive days while the animals in group A were given equivalent volume of the citrate buffer intraperitoneally. The rats were fasted overnight before STZ administration.

DETERMINATION OF BODY WEIGHT AND BLOOD GLUCOSE LEVEL

The body weights of the animals were measured using a top loader weighing balance. Blood sample was obtained from the tail vein of the animals and their fasting blood glucose level was determined in mmol/L using a digital glucometer (Accu-chek® Advantage, Roche Diagnostic, Germany). The animals were fasted for a period of 16 hours before their blood glucose level was measured.

BIOCHEMICAL ESTIMATIONS

The serum levels of triglyceride (TGL), total cholesterol (TC) and high-density lipoprotein-cholesterol (HDL-C) were determined spectrophotometrically, using enzymatic colorimetric assay kits (Randox, Northern Ireland) while low-density lipoprotein cholesterol (LDLC), very low-density lipoprotein cholesterol (VLDLC) and antiatherogenic index (AAI) were calculated. Animals were fasted for 12-16 hours before blood samples were obtained. About two milliliters of blood was collected from the tail vein of each rat into an ice-cold centrifuge tubes. The blood samples were centrifuged in a Denley BS400 centrifuge (England) at 5000 R.P.M for 5-minutes. The supernatant (serum) collected was assayed for the serum levels of TGL, TC and HDL-C using the Randox Biochemical kits while LDL-C and VLDL-C were calculated.

ASSAY FOR TRIGLYCERIDES

The serum level of TGL was determined by the method of Treitz. 1000 μl of the reagent was added to 100 μl of the sample and standard. This was incubated for 10 minutes at 20-25 °C and the absorbance of the sample (A_{sample}) and standard (A_{standard}) was measured against the reagent blank within 30 minutes.

\[ TGL \text{ concentration} = \frac{A_{sample}}{A_{standard}} \times 2.29 \text{ mmol/L} \]

Figure 1

ASSAY FOR TOTAL CHOLESTEROL

The serum level of TC was determined after enzymatic hydrolysis and oxidation of the sample as described by Richmond and Roeschlaub et al.. 1000 μl of the reagent was added to 100 μl each of the sample and standard. This was incubated for 10 minutes at 20-25 °C and the absorbance of the sample (A_{sample}) and standard (A_{standard}) was measured against the reagent blank within 30 minutes.

\[ TC \text{ concentration} = \frac{A_{sample}}{A_{standard}} \times 5.17 \text{ mmol/L} \]

Figure 2

ASSAY FOR HDLC

Low-density lipoproteins (LDL and VLDL) and chylomicron fractions in the sample were precipitated quantitatively by the addition of phosphotungstic acid in the presence of magnesium ions. The mixture was allowed to stand for 10 minutes at room temperature centrifuged for 10
minutes at 4000rpm. The supernatant represented the HDLC fraction. The cholesterol concentration in the HDL fraction, which remains in the supernatant, was determined.

**LOW DENSITY LIPOPROTEIN - CHOLESTEROL**

The concentration of LDL cholesterol was calculated mmol/L using Friedewald’s equation \[ \text{LDLC} = \text{TC} - \left( \frac{\text{HDL} + \frac{\text{TGL}}{2.2}}{2} \right) \] as stated below.

**Figure 3**

Very low density lipoprotein - cholesterol

The concentration of VLDL cholesterol was calculated mmol/L using Friedewald’s equation \[ \text{VLDL} = \frac{\text{TGL}}{2.2} \] as stated below.

**Figure 4**

Antiatherogenic Index (AAI)

The antiatherogenic index was calculated according to the method of Guido and Joseph \[ AAI = \frac{\text{HDL} \times 100}{\text{TC} - \text{HDL}} \]. AAI was calculated from total cholesterol and HDL cholesterol using the formula below. The values were expressed as a percentage.

**Figure 5**

**RESULTS**

**CHANGES IN WEIGHT**

Prior to STZ administration, there was no significant difference in the average weights of the control and diabetic group of rats. By the end of the first week after diabetes mellitus was experimentally induced, the weights of diabetic rats were significantly reduced despite the increase in food and fluid intake in these animals. This weight loss continued for four week after STZ administration (fig.1). At the end of the experimental period, there was a significant (p < 0.05) decrease in the body weights of diabetic rats (171.28 ± 5.143) when compared to the control (204.28 ± 8.307) (table 1).

**Figure 6**

Fig. 1 Weekly Changes in the Body Weights of Control and Diabetic Rats

**TABLE 1: Changes in the Body Weight and Blood Glucose Level of Control and Diabetic Groups of Rats after STZ Administration**

<table>
<thead>
<tr>
<th>Group</th>
<th>Body Weight (g)</th>
<th>Blood Glucose Level (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A (initial)</td>
<td>208.64 ± 9.038g</td>
<td>3.39 ± 0.125g</td>
</tr>
<tr>
<td>Group A + Citrate buffer</td>
<td>204.28 ± 8.307g</td>
<td>3.80 ± 0.200g</td>
</tr>
<tr>
<td>Group B (initial)</td>
<td>202.26 ± 6.150g</td>
<td>3.33 ± 0.150g</td>
</tr>
<tr>
<td>Group B + STZ</td>
<td>171.28 ± 5.143g</td>
<td>24.17 ± 2.535g</td>
</tr>
</tbody>
</table>

Values are given as mean ± SEM for ten rats in each group. a, b within column signifies that means with different letters differs significantly at P < 0.05 (two tailed T-test) while means with the same letters does not differ significantly at P <0.05 (two tailed T-test).

**STATISTICAL ANALYSIS**

The data were analysed using descriptive and inferential statistics. All values are presented as mean ± standard error of mean (SEM) for ten rats in each of the two group of rats. The significance of difference in the means of all parameters reported for the two groups of animals was determined using paired sample student t – test and a p – value of < 0.05 (two tailed) was considered as significant.
CHANGES IN THE BLOOD GLUCOSE LEVEL

Prior to STZ administration, the fasting blood glucose level did not differ significantly (p < 0.05) between the control and diabetic groups of rats. The blood glucose level gradually increased during the five days period of STZ administration. One week after administration of STZ, the blood glucose level was significantly (p < 0.05) higher in groups B rats. The blood glucose level of these rats remains elevated over a period of four weeks (fig. 2). Control rats treated with citrate buffer maintains a normal blood glucose level throughout the period of experiment. At the end of the experiment, there was a significant difference (p < 0.05) in the blood glucose level of groups A and B rats (table 1, fig. 2)

Figure 8
Fig. 2 Weekly Changes in the Blood Glucose Level of Control and Diabetic Rats

Tables 2 and 3 illustrate the effects of streptozotocin on the levels of total cholesterol, triglycerides, HDLC, LDLC, VLDLC and AAI in the serum of experimentally induced diabetic rats. The levels of total cholesterol, triglycerides LDL-C and VLDLC were significantly (p < 0.05) increased in diabetic rats whereas the level of HDL-C and the percentage of AAI (ratio of HDL to total cholesterol) were significantly (p < 0.05) reduced in these rats when compared to the control normal rats.

DISCUSSION

When rats are injected with streptozotocin, they provide an animal model of insulin-dependent diabetes mellitus. In this model, severe hyperglycaemia appears throughout the period of induction with a partial deficiency in insulin. In the present study, the streptozotocin induced diabetic rats showed significantly higher levels of fasting blood glucose levels and lower body weight compared to normal control rats. This was consistent with early reports. Diabetes mellitus is a complex metabolic disease caused by impairment of insulin signaling, pathways, and the defect usually results from pancreatic β-cell deficiency and/or a deficiency of insulin. This disease causes many chronic complications such as vascular disease, retinopathy, neuropathy, kidney disease and heart disease. Cardiovascular disease is one of the major causes of death in diabetic patients. Diabetes mellitus is associated with profound alteration in the serum lipid and lipoprotein profile with an increased risk in coronary heart disease. Hyperlipidemia is a recognized complication of Diabetes mellitus characterized by elevated levels of cholesterol, triglycerides and phospholipids; and changes in lipoprotein composition.

The present study supports the results of other investigators.
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who proved significant increase in total cholesterol, triglyceride, LDL-Cholesterol, VLDL-Cholesterol and significant decrease of HDL-Cholesterol in STZ-induced diabetic rats. Mathé 27 reported that hypercholesterolemia in STZ-induced diabetic rat's results from increased intestinal absorption and synthesis of cholesterol. The low levels of HDL-Cholesterol in diabetic rats negatively modulate endothelial function through a lack of oxidation inhibition and a concomitant over expression of adhesive molecules (vascular cell adhesive molecules, VCAM, and inter cellular adhesion molecules ICAM) 28. Natarajan and Nadler 29 suggested that monocyte adhesion to endothelial cells as well as excessive proliferation and migration of vascular smooth muscle cells (VSMC) are key events in the development of atherosclerosis in diabetes 29. These processes are mainly mediated by growth factors, inflammatory cytokines, chemokines and related factors released by various cells in the vessel wall 29. The mechanism of action of these factors are however not clear. These growth factors and cytokines acting on VSMC and endothelial cells can activate phospholipases with the release of lipids such as arachidonic and lipoleic acids. These lipids can be further metabolized by several pathways including the lipoxygenase (LO) pathway. These oxidative pathways may lead to the formation of free radicals and lipid peroxides. LO products have been shown to be associated with oxidant stress that may lead to atherosclerosis, hypertension and related diabetic complications 29. There is increasing evidence that lipid peroxidation plays an important role in the premature development of atherosclerosis 30,31. Abnormally high levels of free radicals, lipid peroxidation and simultaneous decline in antioxidant defense mechanism can lead to damage of cellular organelles and enzymes. Elevated levels of lipid peroxidation in circulation of diabetic rats are one of the characteristic features of chronic diabetes 32.

In conclusion, our findings suggested that administration of multiple low doses of streptozotocin had a potential hyperglycemic activity in rats. In addition to increasing serum glucose level, it also increases serum levels of total cholesterol, triglycerides, and LDL cholesterol which are characteristic features of hyperlipidemia.

References

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