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Research Article

HYPERLIPIDEMIC MODEL: STUDYING LIPID PROFILE IN SMALL EXPERIMENTAL ANIMAL

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ABSTRACT

Fat-enriched diets have been used for decades to model obesity, hyperlipidemia, dyslipidemia and insulin intolerance in rodents. It has been observed that the disorders achieved by high-fat feeding resemble the human metabolic syndrome closely, and this also may extend to the cardiovascular diseases. Hyperlipidemia is an elevation of lipids (fats) in the blood stream. These lipids include cholesterol, HDL, LDL and triglycerides. They're transported in the blood as part of large molecules called lipoproteins. The objective of this study was to create a model by inducing hyperlipidemia in rats by long-term high-fat diet intake, then investigate the lipid profile, liver function test and histological alterations in liver tissues of these animals and discuss their potential significance. Results clearly shows that, high fat diet fed groups, showed significant increase in cholesterol level after 7th day with respect to control attaining the maximum level till 28th day post feeding and then after it the cholesterol starts metabolizing, thus level again decreases (Figure 1). Similarly triglycerides, VLDL and LDL shows significant increase between 21st day and maximum on 28 th day although later on the level decreases due to the metabolism of lipids.

Keywords: Hyperlipidemia, High Fat Diet (HFD), Cardiovascular diseases, Charles foster rats.

INTRODUCTION

No one disagrees that diet is an important part of the management of hyperlipidemia. However, disagreement persists about the best diet for management of hyperlipidemia[1]. The coincidence of obesity, insulin resistance, hypertension and dyslipidemia is commonly referred to as the 'metabolic syndrome'. This condition affects approximately 20–40% of the population in the industrialized nations, and its prevalence is expected to rise further in the next decades[2]. Cholesterol is a soft, waxy substance found among the lipids (fats) in the bloodstream and in all parts of body's cells. It is normal to have cholesterol. Cholesterol is an important part of a healthy body because it is used to form cell membranes, some hormones and other needed tissue. But, too high level of cholesterol in the blood is a major risk for coronary heart disease, which can lead to heart attack. High cholesterol diet is regarded as an important factor in the development of cardiac diseases as it leads to the development of hyperlipidemia, atherosclerosis and ischemic heart diseases[3,4]. The potential for coronary heart disease (CHD), the number one cause of death in western societies, is increased in individuals with elevated concentrations of plasma low-density-lipoprotein (LDL) cholesterol. It has been well established by the National Cholesterol Education Program (NCEP) and European Atherosclerosis Society (EAS) guidelines that the LDL cholesterol (CHOL) concentrations should be reduced in patients at risk for CHD as well as in the general population[5].

With this background information, to study the details of parameters associated with hyperlipidemia (Cholesterol, Triglycerides, HDL, LDL & VLDL) an attempt to develop a hyperlipidemic rat model with 30% fat rich diet is performed[6,7].

MATERIALS AND METHOD

Chemicals

Diagnostic kits used like SGOT and SGPT were obtained from Bayer Diagnostics, Germany. Other kits like Cholesterol, Triglyceride, HDL and Bilirubin used in the whole experiment were of GR grade and obtained from E-Merck India Limited.

Animals

Twenty-eight adult female *Charles Foster* (CF) rats of 8 to 10 weeks of age were chosen for the study. They were housed in steel cages and maintained under conventional condition (12h light: 12h dark cycle) at a temperature of 22±2 °C and humidity control. The animals were divided into four groups with seven animals per group. The grouping of animals is as:

Group I (Control): Normal diet [standard rat feed] + Normal water

Group II: Normal diet + 3% Ethanolic water[8]

Group III: High Fat Diet (HFD) + Normal water

Group IV: High Fat Diet (HFD) + 3% Ethanolic Water

The Normal diet is obtained from M/s Dayal Feed, Dayal Industries Ltd., Lucknow, India and drinking water is administered *ad libitum*. Animals of Group 3 and 4 were given High fat diet (Diet containing 30% vegetable oil + 3% pure cholesterol from Himedia). All animals used for this study were maintained in accordance with the guidelines recommended by the Committee for the Purpose of Control and Supervision of Experiments in Animals (CPCSEA) [9,10].

In-life observation

All the experimental animals of group 1 & 2 were given normal diet and animals of group 3 & 4 were provided high fat diet along with 3% ethanolic water mentioned for group 2 & 4. Animals were observed every day for general signs and symptoms. Daily carefully monitored examination of animal after and before experiment such as vomiting, skin loosening, changes in secretion, respiration rate, furring were noted. Changes in gait, posture and response to handling were also recorded[11].

Collection of Blood Samples

The blood samples were collected from the retro-orbital plexus after interval of every seven day for estimation of lipid profile. Serum was subjected for the estimation of clinical chemistry parameters [Hepatic function test: Serum Glutamate Pyurvate Transaminase (SGPT), Serum Glutamate Oxaloacetate (SGOT); Lipid Profile: Cholesterol, triglycerides and HDL] using biochemical Kits (Merck Specialties Private Ltd. and Bayer Diagnostics, Germany).On 63rd day, all the animals were sacrificed and target organs such as liver and heart were collected and weighed[12]. Small pieces of liver and heart tissues from control and experimental groups were homogenized in normal saline solution (10% homogenate). The homogenates thus obtained were centrifuged in a Biofuge Stratus at 3000 rpm for 20 min at 4°C to obtain supernatants which were stored at -20°C for tissue lipid estimation[13,14].

Statistical Analysis

Effects of High fat diet in experimental condition were analyzed by using Graph Pad Prism 5 software program for the results (Mean ± SEM). For non-parametric comparison, Turkey's test was employed using same programs.

RESULTS

In-life observation

All the animals (Control and experimental) during the experimental period showed no observable changes. No mortality or morbidity was observed during the entire experimental period. At the start of the experiment, animals fed with High fat diet showed a remarkably high consumption of diet but later on their consumption capacity becomes stable.

Changes of body, liver and heart weight in rats fed with high fat diet

The hyperlipidemia was induced by feeding rats with high-fat diet for 63 days and the general condition of the rats remained satisfactory. All rats showed a steady increase in body weight. The gain in liver and heart weight in the high fat group was significantly greater than that in the normal diet group.

Changes in Clinical Chemistry Parameters:

Changes in lipid profile

Results clearly shows that, high fat diet fed groups, showed significant increase in cholesterol level after 7th day with respect to control attaining the maximum level till 28th day post feeding and then after it the cholesterol starts metabolizing, thus level again decreases. Similarly triglycerides, VLDL and LDL shows significant increase between 21st day and maximum on 28 th day although later on the level decreases due to the metabolization of lipids. Decrease in HDL level was observed as compared to control groups (Fig. 2).

Changes in Liver function profile

Collected blood samples for separation of serum after every week for liver function test, shows a slight increase in SGPT and SGOT level during the 7th and 14th day of experiment, later on the level of both activities decreases with respect to control groups. The Bilirubin level remains normal in experimental groups.

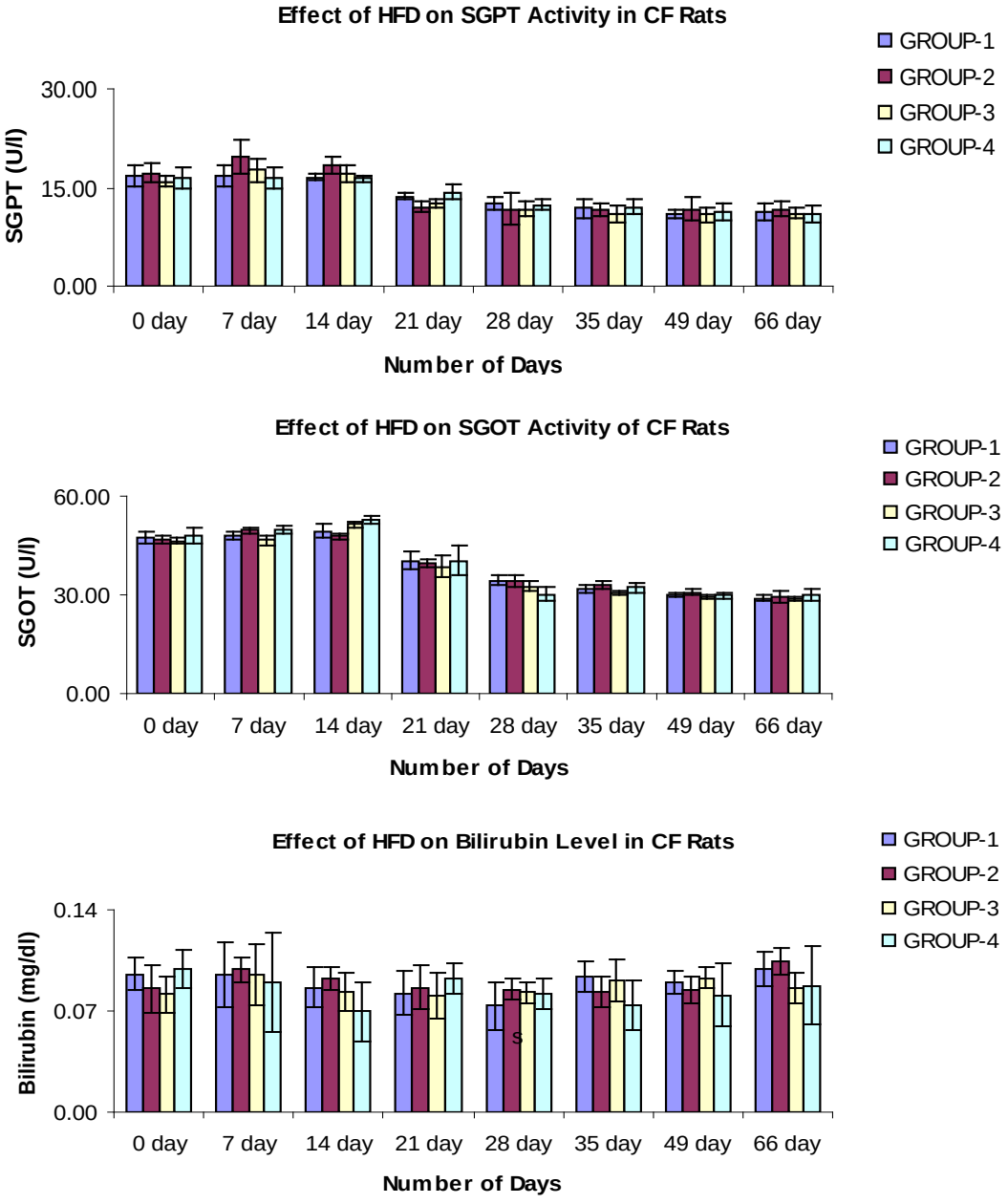

Fig. 1: Effect of HFD on Liver function test (SGPT, SGOT and Bilirubin).

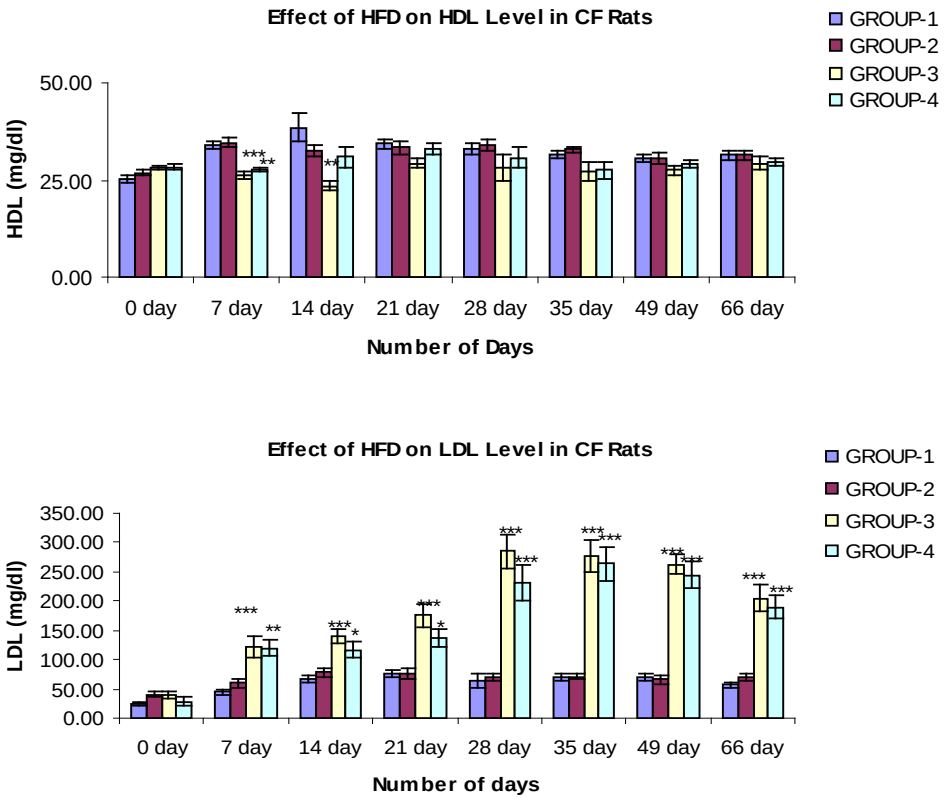

Fig. 2: Effect of HFD on HDL, Bodyweight and LDL level in CF rats.

DISSCUSION

The average food intake per day was found to be 32±5 g. Results clearly indicate that feeding HFD increased plasma and tissue lipids and lipoprotein levels[15]. Hypercholesterolemia was induced in rats by feeding them with a cholesterol-enriched diet consisting of 3% cholesterol. In this experiment, it was observed that feeding rats with high fat diet for 28 days is sufficient in elevating the serum cholesterol level to 329.52 mg/dl and similarly significant increase in all the lipid parameters such as triglycerides, LDL, VLDL and decrease in HDL levels (Fig. 2) were observed. Increase in lipid profile were observed from 21st day post feeding attaining maximum level on 28th day and then starts metabolizing, yet the level remains significant to control group for a period of 63 days. At the start of experiment, observed initial increase in SGPT and SGOT level signifies that physical stress due to handling of animals may be responsible for this, although later on no rise in the level was observed during experiment. Also there is significant change in body weight was observed[16]. Parallel studies have shown that diets supplemented with 1% cholesterol and 0.5% cholic acid for 5 days is sufficient in elevating the serum total cholesterol level to 238 mg/dl[17].

CONCLUSION

Summarizing the above studies we can conclude that feeding rats with diet consisting of 3% cholesterol, shows induction of hyperlipidemia which is maintained from 21st day to 63rd day post feeding showing significant maximum increase on 28th day feeding.

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