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## High fat simple carbohydrate-fed male Wistar rats: a useful model to study metabolic syndrome

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**Metabolic syndrome represents a combination of cardiovascular risk determinants and lipid abnormalities. The syndrome is associated with a five-fold higher risk of developing type-II diabetes and two to three-fold higher risk of cardiovascular disease. The etiology of the syndrome at the molecular level is not yet understood. Therefore, there is a need to study the syndrome in an animal model. In the present study, the effect of high fat simple carbohydrate (HFSC) diet on male Wistar rats was investigated. The rats were fed HFSC diet for a period of five months. Anthropometric parameters were measured and serum triglycerides, total cholesterol, HDL and LDL were analysed. Glucose intolerance was studied using oral glucose tolerance test. The formulated (HFSC) diet succeeded in developing an animal model for metabolic syndrome within 17 weeks characterized by obesity, hypertriglyceridemia, impaired glucose tolerance, fatty liver and abnormal cardiac histology.**

**Keywords:** High fat simple carbohydrate diet, metabolic syndrome, oral glucose tolerance test.

METABOLIC syndrome (MetS) represents a combination of cardiovascular risk determinants such as obesity, insulin resistance, hypertension and lipid abnormalities. This syndrome has reached epidemic proportions worldwide. It affects one in five people and prevalence increases with age. According to the population studies, from the age of 20 to 25 years and upwards, the prevalence of metabolic syndrome varies from 8% (India) to 24% (the United States) in men and from 7% (France) to 46% (India) in women<sup>1</sup>. Metabolic syndrome is a major public health burden<sup>2</sup> and contributes to cardiovascular disorders, cognitive decline and reduces the expected lifespan by an average of 15 years in the aged. Besides morbidity and mortality, these disorders impact the socio-economic status of individuals as well as the state<sup>3</sup>.

Metabolic syndrome seems to have three potential etiological categories: obesity and disorders of adipose tissue, insulin resistance and a constellation of independent factors (e.g. molecules of hepatic, vascular and

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immunologic origin) that mediate specific components of the metabolic syndrome. Other factors such as ageing, pro-inflammatory state and hormonal changes have been implicated as contributors as well<sup>4</sup>. Obesity contributes to hypertension, high serum cholesterol, low high density lipoprotein (HDL) cholesterol and hyperglycemia; it otherwise associates with higher cardiovascular disease (CVD) risk<sup>4</sup>.

Beyond obesity and insulin resistance, each risk factor of the metabolic syndrome is subject to its own regulation through both genetic and acquired factors that lead to variability in the expression of risk factors<sup>4</sup>. Most of the studies on metabolic syndrome are based on clinical data and therefore, there is a need to undertake studies in animals at various stages before and during the emergence of the obese and insulin-resistant phenotype, to establish definitively which genes and tissues have a causal role in the development of metabolic disease.

There are several animal models which are susceptible to diet/nutrition-induced changes. For example, Israeli Sand rat (*Psammomys obesus*), Spiny mouse (*Acomys calirinus*), C57/BL6J (*Mus musculus*), *Ctenomys talarum*, etc. Male Wistar rats are easily available and therefore, in the present work we have attempted to induce metabolic syndrome in male Wistar rats by feeding them a specially formulated diet comprising high fat and simple carbohydrates (HFSC).

All chemicals and kits were purchased locally and were of analytical grade (Merck, India). The kits used were from Agappe diagnostics (Kerala, India).

Twelve three-month-old, healthy, male Wistar rats weighing approximately 120 g were used. They were housed in rat cages and maintained under controlled room temperature (25–27°C) with 12 h light and dark cycles according to the CPCSEA guidelines with approval from Institutional animal ethics committee (Sanction No. SAC/IAEC/108/2011 dated 30-3-2011). Four control rats and eight test rats were maintained. The control rats were fed with standard diet and water *ad libitum*, while the test rats were fed with HFSC diet and water *ad libitum* daily for a 150 days.

All food components for the formulation of the diet were purchased locally.

Formulation of the HFSC diet was done with modifications<sup>5</sup>. The diet composition was as follows: simple carbohydrates – 26%, proteins – 14% and fats – 60%. The source of carbohydrates was corn starch and sucrose and for fat it was porcine fat. Casein was the main source of protein. The proportion of these ingredients was ideal for inducing metabolic syndrome as it ensures a progressive increase in body weight, lipid profile as seen in human beings, but not suppressing the growth of the animal. The diet also ensures the availability of sufficient amount of each food group, as unrealistic diets do not depict the correct picture. The test animals were fed with HFSC diet daily at the rate of 5 g per animal during 3–4 pm.

The control and test rats were weighed monthly and the percentage of increase in the body weight per month was determined. Lee index is an index for adiposity and is a validated model to predict the risk of cardiovascular events<sup>6,7</sup>.

The Lee index of control and test rats was calculated using the formula<sup>6</sup>

$$\text{Lee index} = \frac{\sqrt[3]{\text{Final body weight (g)}}}{\text{Anal - nasal length (cm)}} \times 1000$$

Oral glucose tolerance test (OGTT) was performed at 143 days post-feeding<sup>8</sup>. After starving the rats overnight, D-glucose solution (1 g/ml) was fed (1 g/kg body weight). The rats were bled by tail vein bleeding at intervals of 30 min for 150 min. Blood glucose was determined using glucose kit (Agappe Diagnostics, Kerala, India).

Rats were sacrificed by cervical dislocation after feeding with respective diets for 150 days. Organs were dissected out and washed in physiological saline, blot-dried and wrapped in aluminium foil and stored at –20°C till further use.

Blood from the control and test rats was obtained from 90 days on a monthly basis by tail vein bleeding method and centrifuged at 2000 g for 10 min to obtain serum. This was further used to determine triglycerides, total cholesterol and HDL cholesterol using suitable Agappe kits (Kerala, India). Low density lipoprotein (LDL) cholesterol was determined using the formula

$$\text{Low density lipoprotein} = \text{Total cholesterol} - \frac{\text{HDL cholesterol} + \text{triglycerides}}{5}$$

Triglycerides, total cholesterol content, HDL and LDL cholesterol were expressed as mg/dl.

The serum glutamic oxaloacetate transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) were determined on sacrifice using suitable kits (Agappe Diagnostics, Kerala, India). The estimations were performed according to the manufacturer's instruction. The concentration of SGOT and SGPT was expressed as U/l.

The heart and liver tissues of the experimental rats were weighed using an electronic balance (Denver Instrument, Germany). Subsequently, the tissues were fixed in buffered formalin, and then the tissue was dissected and embedded in paraformaldehyde and sectioned. The slides were deparaffinized with xylene, dehydrated in descending grades of alcohol, stained with haematoxylin, rinsed in running tap water and differentiated with acid alcohol. The slides were then stained with eosin, rinsed with distilled water, dehydrated and observed under Olympus Microscope (BX 41TF, Singapore) and photographed using Jenoptek camera (Germany). The triglyceride content was studied using Oil Red staining<sup>9</sup>. The slides were deparaffinized with xylene, dipped in 60% isopropanol

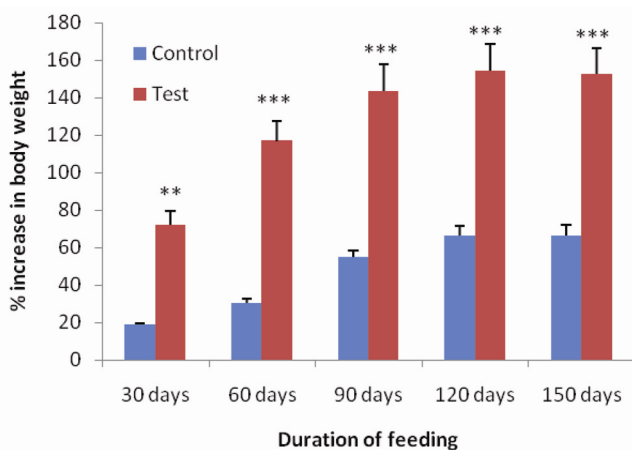
and stained with Oil Red solution for 15–30 min. They were then rinsed with water, dried and observed.

The estimation of triglycerides was performed using Agappe Diagnostics kit as per the manufacturer’s protocol. Briefly, 200 mg of tissue (heart and liver) was weighed and homogenized in 1% Triton X prepared in phosphate buffer saline. The homogenate was centrifuged at 1000 g for 10 min. The supernatant was used for triglyceride analysis.

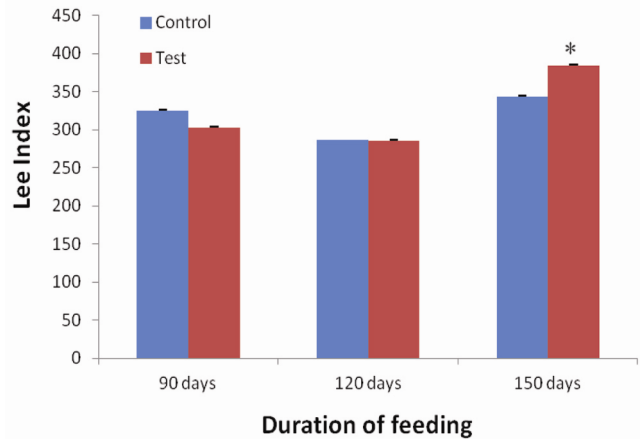
Statistical valuations of the data were done by ANOVA using Graph Pad INSTAT (ver. 3) computer program by two-tail unpaired test. The degree of significance is mentioned as obtained from the analysis. It ranges from significant (\*) to highly significant (\*\*\*)

The test rats weighed significantly more than age-matched control rats fed with a standard diet (Figure 1). In the present study, 71.9% increase in body weight of test rats at 30th day was observed compared to 18.9% increase in body weight of control rats at 30th day ( $p < 0.05$ ). Also, 152.41% increase in body weight of test rats at 150th day was observed compared to 66.43% increase in the body weight of control rats at 150th day ( $p < 0.001$ ). Monitoring of body weight is important since both humans and rodents tend to gain weight with high calorie intake<sup>7</sup>. Similar results were seen in a study done<sup>5</sup>, wherein adult C57BL/6J mice fed with high fat diet for same period showed a noticeable obesity with significant 10% increase in total body weight.

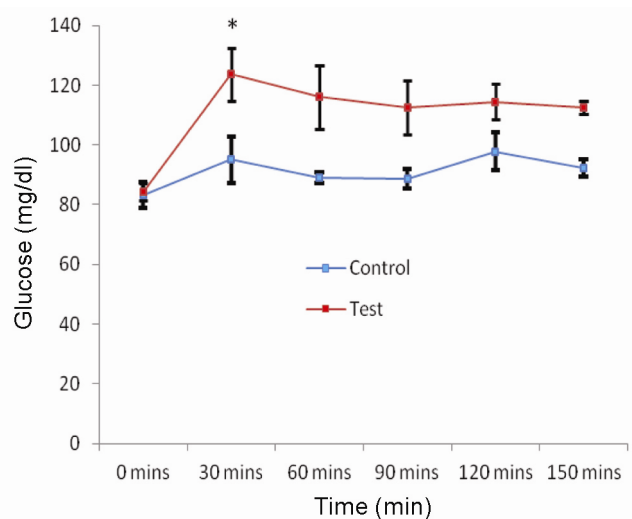
In another study<sup>10</sup>, male Sprague–Dawley rats were fed with high fat diet containing 24% fat, 24% protein and 41% carbohydrate. A 14% increase in body weight of test male Wistar rats was observed in that study<sup>10</sup> compared to control within 30 days, while a 50% difference was observed in the present study. Our results were found to



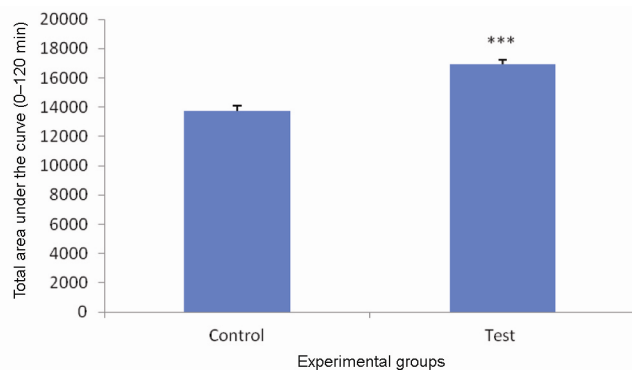
**Figure 1.** Percentage of increase in body weight of male Wistar rats. (Test group showed significant (\*\* $p < 0.01$ ) percentage of increase in body weight within 30 days of feeding of high fat simple carbohydrate (HFSC) diet compared to control fed with standard diet for 30 days. The gain in percentage of body weight was found to increase and also maintained throughout the study period (\*\*\*) from 60 to 150 days of feeding within the test and control groups.)



**Figure 2.** Effect of HFSC diet on Lee index of male Wistar rats. (Test group showed significant increase( $p < 0.05$ ) in Lee index after 150 days of feeding of HFSC diet as compared to control fed with standard diet for 150 days.)

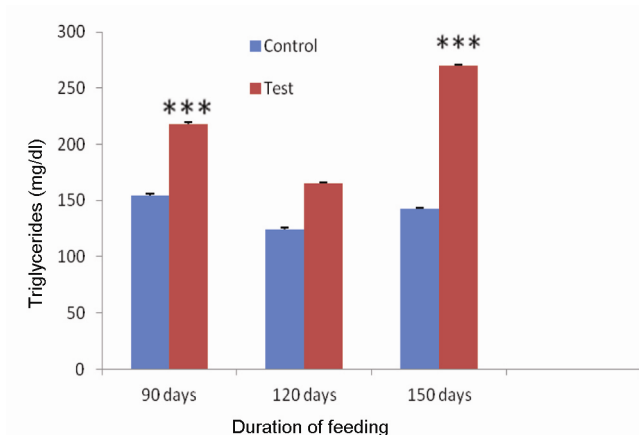


**Figure 3.** Effect of HFSC diet on oral glucose tolerance of male Wistar rats. (On performing oral glucose tolerance test after 143 days of feeding the test group with HFSC diet and control group with standard diet, significant increase ( $p < 0.05$ ) in the test blood glucose was observed compared to control fed at 30 min post-glucose load.)

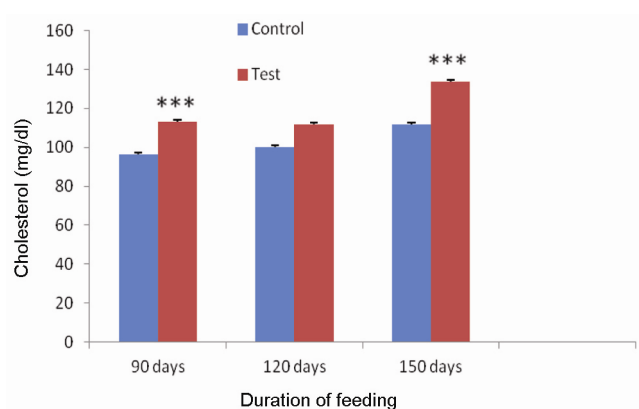


**Figure 4.** Total area under the curve of oral glucose tolerance test of male Wistar rats. (Total area under the curve (0–120 min) of the test is significantly higher compared to the control (\*\*\*)

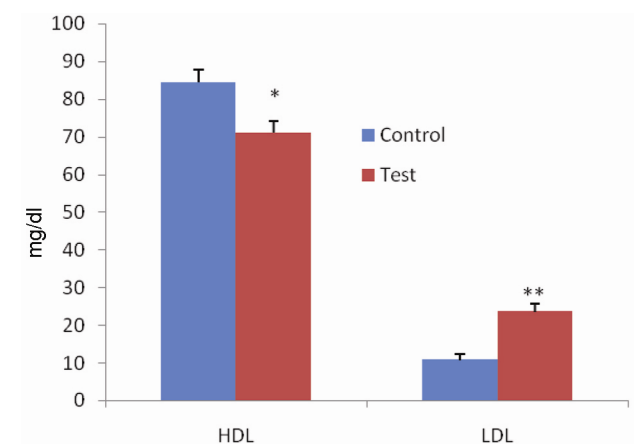
be very significant compared to 40% increase in test rats obtained in another study<sup>6</sup> in which male Wistar rats were fed with high fat diet (68% energy as carbohydrate, 20% as protein and 12% as fat).



**Figure 5.** Effect of HFSC diet on triglyceride of male Wistar rats. (The test group showed very significant increase (\*\*\*)  $p < 0.001$ ) in triglyceride levels at 90 and 150 days as compared to the control.)



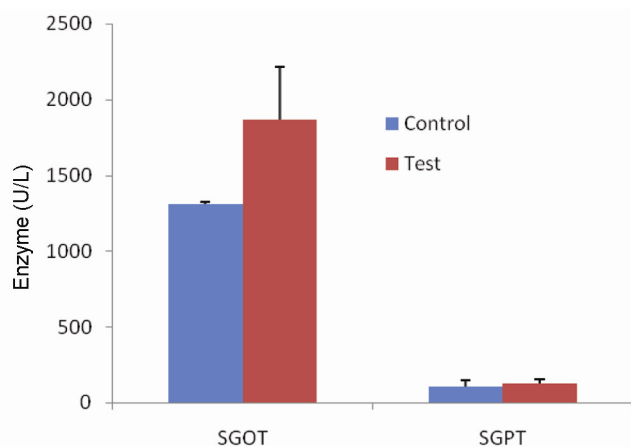
**Figure 6.** Effect of HFSC diet on total cholesterol of male Wistar rats. (The test group showed very significant increase (\*\*\*)  $p < 0.001$ ) in total cholesterol levels at 90 and 150 days as compared to the control.)



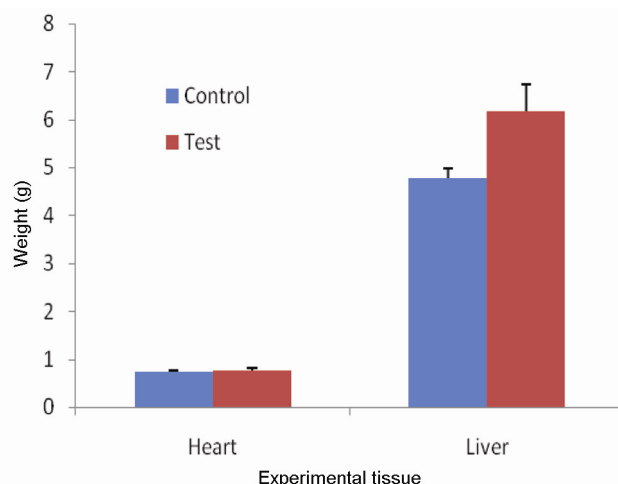
**Figure 7.** Effect of HFSC diet on HDL and LDL cholesterol of male Wistar rats. (Significant decrease ( $*p < 0.05$ ) in the test HDL cholesterol and significant increase in the test LDL cholesterol (\*\* $p < 0.01$ ) was observed compared to control after 150 days of feeding.)

Energy intake is an important factor in regulation of body weight. In our study, Lee index of the test rats was found to show significant increase ( $p < 0.05$ ) as compared to the control 150 days post-feeding (Figure 2).

The test animals were also found to show significant ( $p < 0.05$ ) impaired glucose tolerance (Figure 3). Blood glucose levels peaked within 30 min in control and test rats. For the next 120 min, blood glucose levels remained high in the test rats, whereas they returned to normal levels in the control rats within 60 min. Similar results were obtained<sup>10</sup> in Wistar rats fed with high fat diet for a period of 32 weeks. However, the present study has shown glucose impairment in the test rats within a period of 17 weeks post-feeding. The total area under the curve (0–120 min) of the test rats was significantly higher

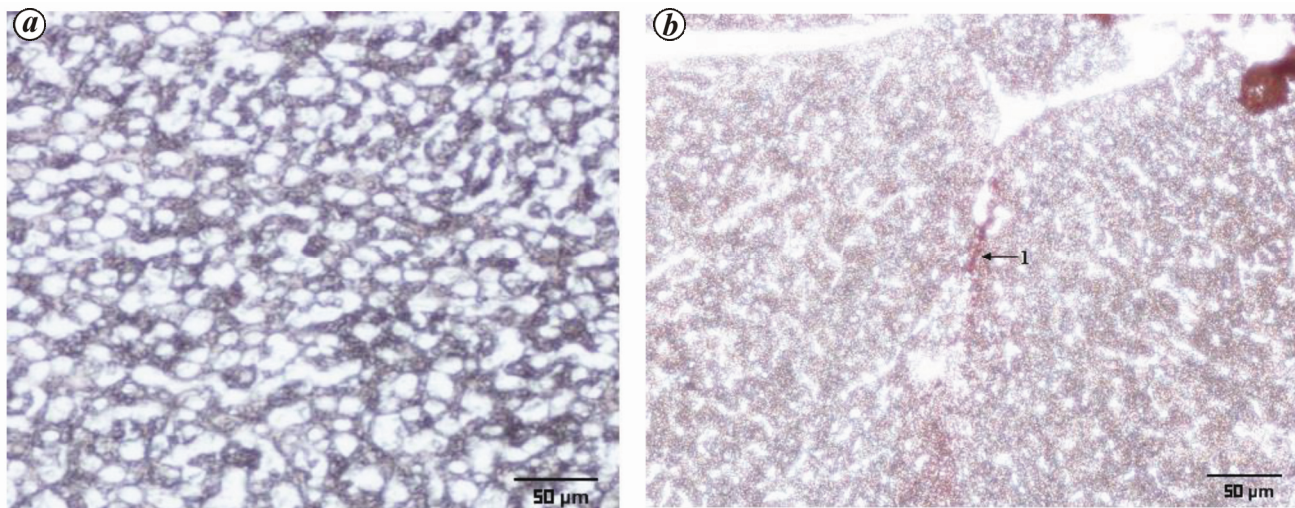


**Figure 8.** Effect of HFSC diet on SGOT and SGPT of male Wistar rats. (Increase in the test SGOT levels was observed as compared to the control after 150 days of feeding HFSC diet. The SGPT levels were almost equal in both the groups.)



**Figure 9.** Effect of HFSC diet on weight of heart and liver of male Wistar rats. (No difference was observed in the weight of heart of both test and control groups. However, test liver weighed higher compared to control after 150 days of feeding HFSC diet.)





**Figure 10.** Oil red staining of liver (100 $\times$ ). *a*, Control; *b*, Test – (1) Increased lipid accumulation as observed by intensity of oil red staining.

compared to the control (Figure 4), indicating higher glucose levels in the test even in the late phase of OGTT compared to the Control. A more in-depth understanding could be obtained by performing insulin analysis to ascertain the cause of the observed glucose intolerance. However, review of the literature shows that impaired glucose tolerance could be due to insulin resistance<sup>6,11</sup>. In a parallel study using C57BL/6J mice in our laboratory, we measured insulin resistance and it correlated well with impaired glucose tolerance<sup>12</sup>.

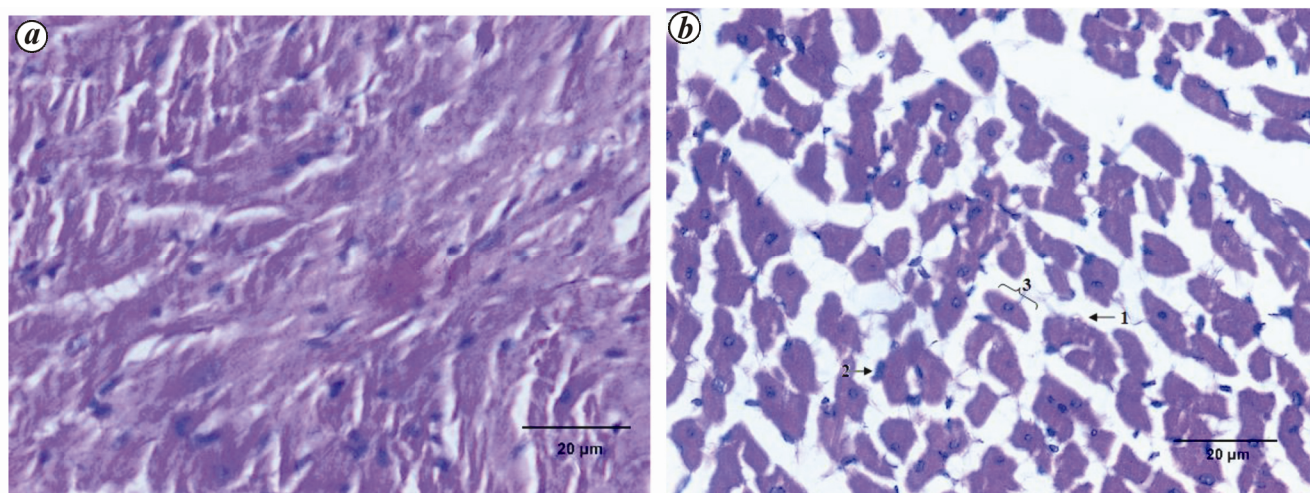
The plasma triglyceride levels were also found to significantly increase ( $p < 0.001$ ; Figure 5) in the test rats compared to the control after 90 days post-feeding, which is in agreement with another study<sup>13</sup> where Wistar rats were fed with moderately fat diet for 10 weeks. In the present study, the triglyceride levels of the test rats showed an extremely significant ( $p < 0.001$ ) increase compared to the control rats, indicating the possible onset of hypertriglyceridemia after 90 days post-feeding. A decline in the control and test triglycerides was observed after 120 days of feeding. This may be due to the change in eating habits of the rats in terms of weight of the feed consumed during the peak of the summer season. This result has been repeatedly observed during the repeats carried out in the study. Elevated triglyceride levels are mainly due to regular intake of high calorie-rich food, more than what is burnt in the body. Increased secretion and severely impaired clearance of triglyceride-rich VLDL1 along with increased plasma levels of apolipoprotein C-III were linked with impaired clearance of triglycerides from serum in obese hypertriglyceridemic men<sup>14</sup>.

A similar extremely significant increase ( $p < 0.001$ ) was seen in the cholesterol levels (Figure 6). Further analysis of HDL and LDL was conducted. The HDL levels of the test rats were found to be significantly low

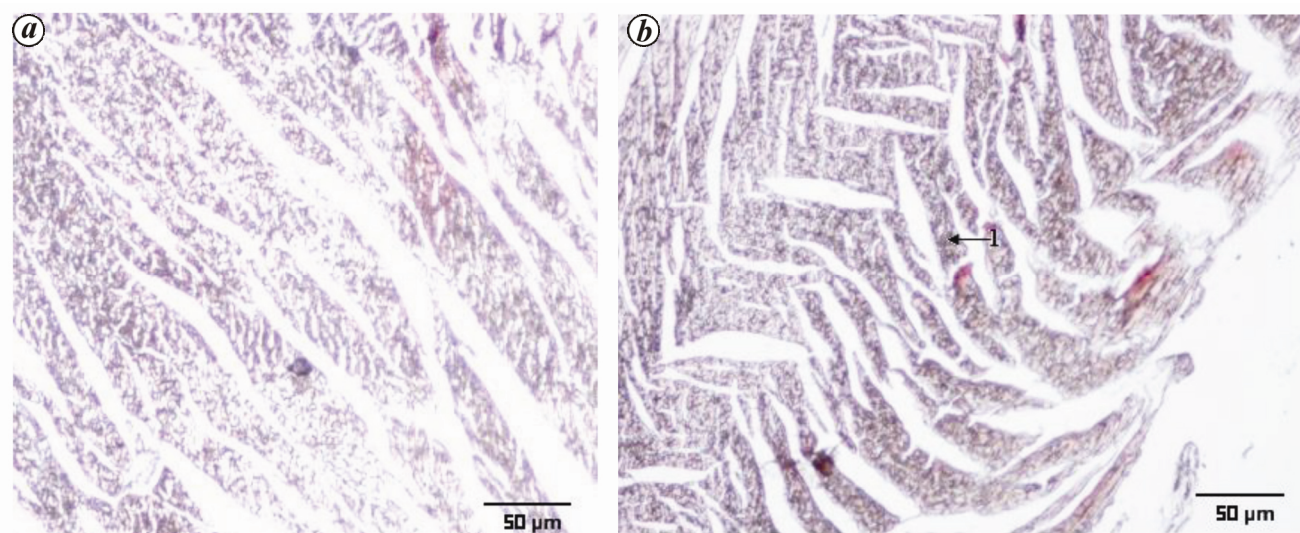
( $p < 0.05$ ) compared to the control (Figure 7) coupled with a significant increase ( $p < 0.01$ ) in the LDL levels of test rats compared to control (Figure 7). Similar studies have been carried out<sup>15</sup> on male Wistar rats using high calorie diet (high carbohydrate), which showed an increase in LDL cholesterol concentration and a decrease in HDL cholesterol. Obesity is the most prevalent cause of metabolic syndrome<sup>16</sup>.

SGOT and SGPT are transaminases used for liver function tests. Glutamic oxaloacetic transaminase is present in the cardiac muscle, hepatocytes, skeletal muscle and kidney cells, increased levels being found in serum in hepatic diseases and myocardial infarctions<sup>17</sup>. Excessive triglyceride accumulation in hepatocytes leads to hepatic steatosis, which boosts SGOT and SGPT levels in the serum<sup>18</sup>. In the present study, an increase in SGOT levels of test rats was observed, though not significant, compared to the control. However, no significant increase was observed in the SGPT values of test rats compared to control (Figure 7). Although SGOT is a known marker for liver function, it is also present in heart, skeletal muscle, kidney and red blood cells. The increasing trend for SGOT and not SGPT may be due to possible damage of other organs not considered in our study (Figure 8). Therefore, the present model did not develop severe hepatic/cardiac damage in the given period of study. Hence the model could be further used for the systematic analysis of various components of the syndrome.

There was no significant increase in the gross weight of heart tissue, whereas there was an increase in the weight of liver as a result of feeding HFSC diet (Figure 9). Though hypertrophy of heart has been previously reported<sup>19,20</sup> in obese spontaneously hypertensive rats (SHR-ob) and genetically obese Zucker rats at 6 and 12 months of age<sup>21</sup>, we have not observed such a change at 5 months post-feeding.



**Figure 11.** Haematoxylin eosin staining of heart (400×). *a*, Control; *b*, Test – (1) Increased interstitial spaces; (2) Macrophage infiltration; (3) Shrunken cardiomyocyte.



**Figure 12.** Oil red staining of heart (100×). *a*, Control; *b*, Test – (1) Increased lipid accumulation as observed by intensity of oil red staining.

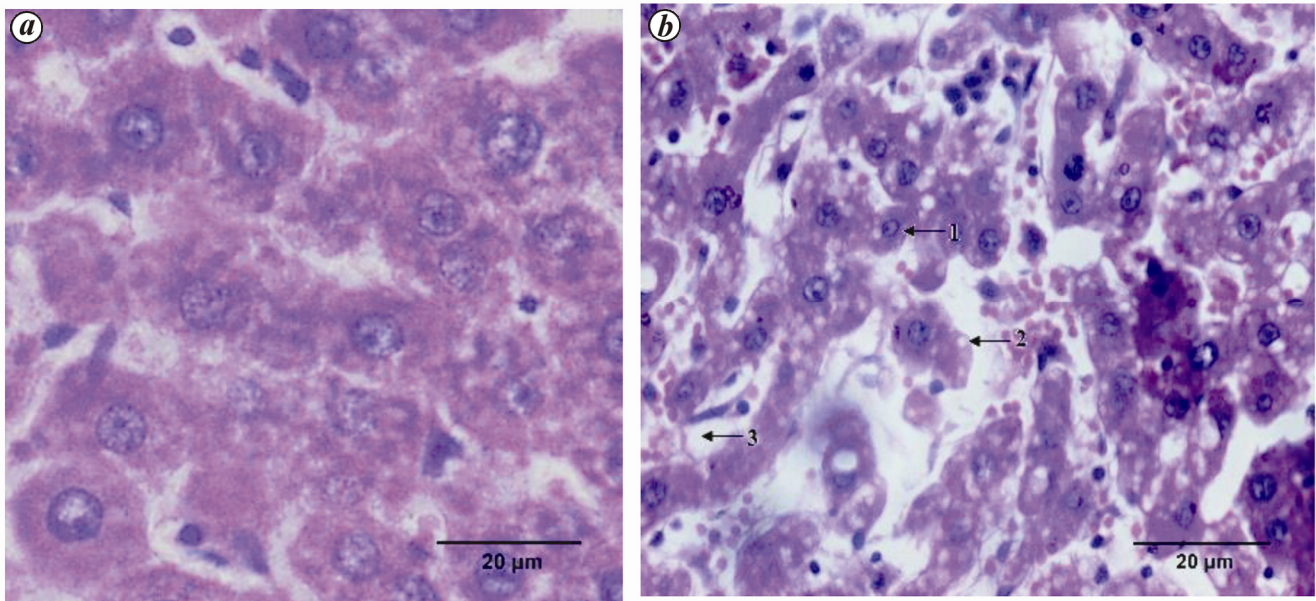
The enhancement of liver weight might be due to accumulation of triglycerides as evidenced by histological examination in the present study (Figure 10). MetS is a strong predictor of non-alcoholic fatty liver disease (NAFLD), which is characterized by increased lipid deposition in the liver<sup>22,23</sup>.

Haematoxylin eosin staining of heart tissue showed an increase in interstitial space between the cardiomyocytes (Figure 11). This could be perhaps due to deposition of lipids, collagen or any fibrous tissues. Oil Red staining of cardiac tissue (Figure 12) has ruled out extracellular deposition of lipids. Obese spontaneously hypertensive rats (SHR-ob) are reported to have increased interstitial collagen deposition<sup>19,20</sup>. The same studies reported cellular hypertrophy and increase in cardiomyocyte diameter.

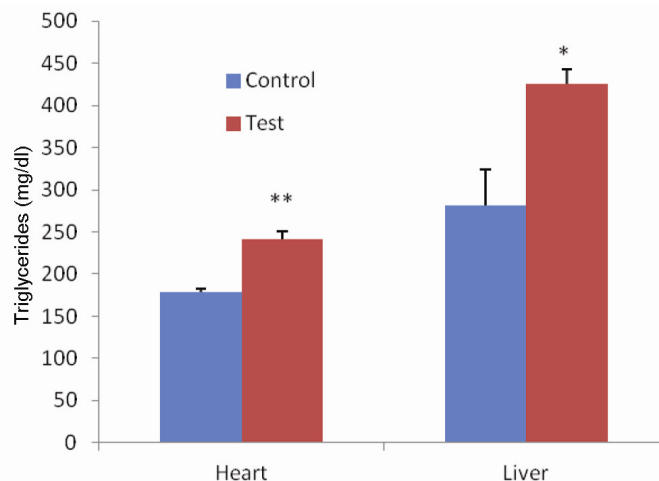
However, in our study we found shrinkage of cardiomyocytes. Haematoxylin eosin staining of liver tissue showed that hepatocytes of the test animals possessed shrunken nuclei, vacuolated cytoplasm, ambiguous cell boundaries and dilated sinusoids. Tissue also showed lymphocyte infiltration (Figure 13 *a* and *b*). The results indicate signs of NAFLD shown by the hepatocytes.

NAFLD is defined as fat accumulation in the liver exceeding 5–10% by weight, as determined from the percentage of fat-laden hepatocytes under light microscopy<sup>24</sup>. Increased fat accumulation in the liver is a marker of hepatic insulin resistance and a close correlate of all components of the metabolic syndrome independent of obesity. The fatty liver resists the action of insulin to suppress hepatic glucose production, resulting in





**Figure 13.** Haematoxylin eosin staining of liver (400 $\times$ ). *a*, Control; *b*, Test – (1) Shrunken nuclei; (2) Vacuolated cytoplasm; (3) Dilated sinusoids.



**Figure 14.** Effect of HFSC diet on triglyceride content of heart and liver of male Wistar rats. (Significant increase in triglyceride content of test heart (\*\* $p < 0.01$ ) and test liver (\* $p < 0.05$ ) was observed compared to control after feeding HFSC diet for 150 days.)

hyperglycemia and hyperinsulinemia<sup>25</sup>. Oil Red staining confirmed increased fat accumulation in the liver of test animals compared to the control (Figure 10 *a* and *b*).

There was significant increase in the cardiac ( $p < 0.01$ ) and hepatic ( $p < 0.05$ ) triglyceride content of the test rats compared to the control (Figure 14). The heart mainly utilizes fatty acids. However, since the capacity for triglyceride storage is low, the uptake and oxidation of fatty acids is tightly coupled<sup>26</sup>. The reasons for triglyceride accumulation may be impaired fatty acid oxidation in the heart<sup>27</sup> along with hypertriglyceridemia as observed in obesity<sup>28</sup>.

The increase in hepatic triglyceride content may be due to insulin resistance as evidenced by impaired glucose

tolerance. Hepatic insulin resistance results in liver fat accumulation<sup>29</sup>. The proposed mechanism is elevated plasma concentrations of glucose and fatty acids may promote hepatic fatty acid and triglyceride uptake and synthesis and impair  $\beta$ -oxidation. As a result, an accumulation of triglycerides in the liver will occur, leading to hepatic steatosis<sup>30</sup>.

The results obtained satisfied most of the criteria laid down by the International Diabetes Federation for metabolic syndrome. There was a change in the lipid profile within a period of 90 days and derailment of glucose profile within 150 days. The present study further confirms the presence of various components of the metabolic syndrome like fatty liver. However, during the study severe

## RESEARCH COMMUNICATIONS

**Table 1.** Comparison of different diets and models used to study metabolic syndrome and parameters

Food type	Duration of study	Parameters studied in context of metabolic syndrome	Model used	Remarks
High fat diet <sup>31</sup>	12 months	Oral glucose tolerance, insulin resistance	C57BL/6J mice female	C57BL/6J is a good model to study impaired glucose tolerance and early type-2 diabetes.
High fat fructose diet <sup>32</sup>	8 months	Body weight, food/water consumption, energy intake, fat/lean mass ratio, plasma glucose, high density lipoprotein (HDL), low density lipoprotein (LDL), triglycerides and cholesterol levels	Male C57BL/6J mice	Body weight increase was observed at week 3; increase in triglycerides observed at week 4; however, decreased compared to week 4 till 8 months; type-2 diabetes at week 8; decreased plasma HDL at week 12; plasma LDL significantly higher at week 16; minimal renal injury.
High fat diet <sup>33</sup>	11 months	Fasted glucose, insulin, triglycerides	Male Wistar rats	Impaired fasting glucose, higher levels of insulin, high levels of triglyceridemia.
Moderate high fat diet; low fat diet <sup>13</sup>	Short duration (3 weeks); long duration (10 weeks)	Body weight, triglycerides, very low density lipoprotein (VLDL), low density lipoprotein, cholesterol, systolic blood pressure	Male Sprague Dawley rats	Body weight higher at week 3; triglycerides and VLDL increased at week 3; LDL cholesterol increased after week 3, but blunted at week 10; systolic pressure increased at week 10.
High fat simple carbohydrate diet <sup>12</sup>	5 months	Body weight, triglycerides, cholesterol, blood glucose, oral glucose tolerance, insulin resistance, low density lipoprotein and high density lipoprotein	Male C57BL/6J mice	Significant increase in body weight at 3 months till end of the study; significant increase in blood glucose at 1 month till end of the study; significant increase in triglycerides at 4 months; significant increase in cholesterol at 3 months till end of the study; significant impaired oral glucose tolerance in test was observed at 5 months along with significant insulin resistance in test compared to control.
High fat diet <sup>34</sup>	11 weeks	Fasting blood glucose, plasma insulin, oral glucose tolerance, insulin tolerance, insulin tolerance test, triglycerides, free fatty acids	Nfr2 <sup>-/-</sup> ob/ob mice	Decreased rate of body weight gain, hyperglycemia, increase in fasting insulin levels, hyper-triglyceridemia compared to Nfr2 <sup>+/+</sup> ob/ob mice.
High fat diet <sup>35</sup>	8–12 weeks	Body weight, triglycerides, cholesterol	ADP-KO mice	ADP-KO mice had more fat mass and lesser lean mass compared to wild-type mice, higher triglycerides, non-esterified fatty acids and cholesterol in ADP-KO mice as compared to wild-type.

hepatic and cardiac damage was not observed. Also, the blood pressure measurements could not be carried out in the present study. We had measured Lactate dehydrogenase and no change was noted. Hence it could be concluded that no severe cardiac pathology developed during the period of the study. We have compared our results with various models and diet regimens used to study metabolic syndrome (summarized in Table 1)<sup>12,13,31–35</sup>. Our results indicate that HFSC-fed male Wistar rats could therefore be a useful model to further elucidate pathophysiological alterations to develop new interventional and pharmacological treatment strategies for metabolic syndrome.

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