Nutritional profile of High Fat Simple Carbohydrate Diet used to induce metabolic syndrome in C57BL/6J mice

Serena Stephen D'Souza, Sri Charan Bindu B., Mohammed Ali M., Alex Tisha, K.V. Deepthi, Silveira Siona, Fernandes Santya, Asha Abraham

Purpose: Many diet based studies on metabolic syndrome lack an in-depth study of feed nutrient profile post preparation. We analyzed the nutritional profile of formulated High Fat Simple Carbohydrate (HFSC) feed and studied its effect on target organs.

Methods: Control feed and HFSC feed were analyzed for macro and micronutrient profile and fed to one month male C57BL/6J mice for 5 months during which feed intake, energy intake and feed efficiency was monitored. The effects of feeds were preliminarily studied on kidney, liver, adrenal gland and pancreas by histological staining.

Results: The HFSC feed had significantly lower carbohydrate, moisture, crude fiber, ash content, mineral, vitamin content, higher ω-6/ω-3 fatty acid ratio as compared to control feed. The HFSC fed mice had higher food intake; energy intake; feed efficiency; lymphocyte infiltration in the liver; hypertrophy of the kidney, adrenal medulla and degenerated islets of pancreas as compared to the control mice after 5 months of feeding.

Conclusion: Food with a higher ω-6/ω-3 fatty acid ratio and simple carbohydrate leads to deterioration of structural integrity of vital organs.

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1. Introduction

Lack of time, stress and increased per capita income has increased the consumption of nutritionally poor food. Availability of junk food rich in fat, carbohydrate or both in large portions and at an affordable cost has made it a daily occurrence [12]. Overconsumption of such food has led to an increasing epidemic of Obesity [3–5], Insulin resistance [6] and Dyslipidemia [7]. The International Diabetes Federation has defined clustering of lipid abnormalities and insulin resistance as Metabolic syndrome [8]. Its incidence has become increasingly prevalent in children and adolescents leading to a specialized definition for this age group [9]. Subjects born with a higher than average birth weight were found to have a greater risk of developing MetS in childhood and adolescence [10].

Therefore, knowledge of nutritional content of food consumed is of utmost importance. For example, Walker et al., (2014) [11] reported fructose content in sugar sweetened drinks made with High Fructose Corn Syrup had higher fructose to glucose ratio than expected. Dietary habits play an important role in development of syndrome [12–15]. Few studies elaborating the relation of nutritional content of food and metabolic syndrome exist. We formulated a High Fat Simple Carbohydrate (HFSC) Diet mimicking fast foods according to Fraulob et al., (2010) [16] with modifications and fed it to one month male C57BL/6J mice which led to the development of dyslipidemia and Type 2 diabetes inducing metabolic syndrome after five months [17]. In the present investigation, we have attempted to determine the nutritional content of the HFSC feed and its effect on some vital organs.

2. Materials and methods

2.1. Formulation of feed

The HFSC feed was formulated in reference to Fraulob et al., (2010) [16] with modifications which included addition of sucrose.
(16% w/w) and reduction of cornstarch (10% w/w). All components for the formulation of the feed were purchased locally.

2.1.1. Control feed
Roasted Wheat flour (79% w/w), Amul Infant Spray (16% w/w) (nutritional details as per manufacturers details: Protein (22 g/100 g), Fat (18 g/100 g), Carbohydrate (50 g/100 g), Added sugar (18 g/100 g), Vitamin mix (2.24 g/100 g) and Mineral mix (1.41 g/100 g)) and pre-warmed Soyabean Oil (4.67% v/w) was mixed. Adequate amount of pre-boiled warm water was added to hold the mixture in the form of a pellet.

2.1.2. HFSC feed
Warmed lard (13% w/w), Melted Sucrose (10% w/w), Cornstarch (7% w/w) and Amul Infant Spray (70% w/w) was mixed and molded into a pellet as described above.

2.2. Food analysis

2.2.1. Caloric content
It was determined by multiplying the weight of each macronutrient with its respective energy density per gram (For Protein and Carbohydrate: 4 kcal/g and Fat 9 kcal/g [18]). The total caloric content was obtained by the sum of the individual calorie content. The percent energy (kilocalorie) obtained from each macronutrient in the weights (V-Wa) represented the weight of crude fat obtained by following the Bligh and Dyer method [19]. Fatty acids were transmethylated using 2 M methanolic sodium hydroxide followed by 2 M methanolic hydrochloric acid to obtain Fatty Acid methyl Esters (FAME). FAMEs were analyzed by GC (Shimadzu GC 2014; M/s Shimadzu, Kyoto, Japan) fitted with a flame ionization detector for identifying fatty acids. FAME dissolved in hexane was analyzed using GC with the following conditions: Column-Omegawax TM 320 fused silica capillary column (30 m × 0.32 mm × 0.25 μm), Split ratio - 1/10, Injection temperature used was - 250 °C, Detector (FID) temperature - 260 °C, Column temperature - 200 °C. The peaks were identified by comparing with authentic standards (Supelco® 37 Component FAME Mix, Bangalore, India). Peak areas above 1% of total were only considered for calculation of % composition of fatty acids.

2.2.2. Carbohydrate content
The total carbohydrate content of the sample was obtained by multiplying the weight of each macronutrient with its respective energy density per gram (For Protein and Carbohydrate: 4 kcal/g and Fat 9 kcal/g [18]). The total carbohydrate content was obtained by dividing the individual calorie content and the total kilocalorie content multiplied by 100.

2.3. Proximate analysis

2.3.1. Moisture content
5 g of feed was dried in pre-weighed crucible in an oven at 100 °C and cooled. The process of heating and cooling was repeated till a constant weight was obtained [19].

\[
\text{Moisture content} (\%) = \left(\frac{\text{Initial weight (g)} - \text{Final weight (g)}}{\text{Weight of sample (g)}}\right) \times 100.
\]

2.3.2. Protein content
100 mg of sample, 1 g of digestion mixture (Copper sulphate: Selenium: Potassium sulphate in a ratio 1:1:20) and 20 ml of concentrated sulfuric acid was digested in a Kjeldahl flask and. Post dilution, 10 ml of the sample and 10 ml of 40% Sodium hydroxide was distilled. The liberated ammonia was collected into a receiver containing 25 ml of 4% Boric acid and methylene blue. This was back titrated using 0.02 N Hydrochloric acid. A reagent blank was similarly treated. The protein content was determined by using the following formula [19].

\[
\text{Protein content } (\frac{\text{g}}{100\text{g}}) = \frac{6.25 \times \text{Acid required to neutralize sample (ml)}}{\text{weight of sample (g)}} - \text{酸 required to neutralize blank (ml)} - \text{final volume (ml)}
\]

2.3.3. Fat content
1 g of sample, methanol and chloroform (2:1) was vortexed for 20 min. To this, 1 ml of chloroform and 1.8 ml of distilled water was added. On centrifugation, organic layer was evaporated. The fat content was determined by subtracting difference in weights [20].

2.3.4. Fatty acid profile analysis
The fat obtained by following the Bligh and Dyer method was further analyzed by Gas chromatography (GC) [21]. Fatty acids were transmethylated using 2 M methanolic sodium hydroxide followed by 2 M methanolic hydrochloric acid to obtain Fatty Acid methyl Esters (FAME). FAMEs were analyzed by GC (Shimadzu GC 2014; M/s Shimadzu, Kyoto, Japan) fitted with a flame ionization detector for identifying fatty acids. FAME dissolved in hexane was analyzed using GC with the following conditions: Column-Omegawax TM 320 fused silica capillary column (30 m × 0.32 mm × 0.25 μm), Split ratio - 1/10, Injection temperature used was - 250 °C, Detector (FID) temperature - 260 °C, Column temperature - 200 °C. The peaks were identified by comparing with authentic standards (Supelco® 37 Component FAME Mix, Bangalore, India). Peak areas above 1% of total were only considered for calculation of % composition of fatty acids.

2.3.5. Crude fibre
2 g of moisture and fat free samples and 200 ml of 0.255 N Sulfuric acid was boiled for 30 min and filtered. The residue was rinsed with hot water and boiled with 200 ml 0.313 N Sodium hydroxide for 30 min. The residue was rinsed with hot water followed by washing with alcohol and ether (crucible labelled as We). It was dried overnight at 80–100 °C in a crucible and weighed. The crucible was heated in a muffle furnace at 600 °C for 2–3 h, cooled and weighed again (crucible labelled as Wa). The difference in the weights (V-Wa) represented the weight of crude fibre [19].

\[
\text{Crude fibre} = \left(\frac{\left(100 - (\text{Moisture (g)} + \text{Fat (g)})\right) \times (\text{We} - \text{Wa})}{\text{Weight of moisture and fat free sample (g)}}\right).
\]

2.3.6. Ash content
5 g of feed was heated in a muffle furnace for 3–5 h at about 600 °C in a crucible. This was repeated till constant weight was obtained and the ash was almost white or grayish white in color [19].

2.3.7. Carbohydrate content
Carbohydrate content was determined as follows [19].

\[
\text{Carbohydrate content} = 100 - [\text{Moisture content (g/100g)} + \text{Protein content (g/100g)} + \text{Fat content (g/100g)} + \text{Ash content (g/100g)} + \text{Crude fibre content (g/100g)}]
\]

2.4. Mineral analysis
2.5 g of feed and 25 ml of concentrated Nitric acid was boiled for 45 min 10 ml of 70% Perchloric acid was added to the cooled solution and boiled till the solution became colorless. This was filtered and made up to 100 ml with deionized double distilled
2.5. Vitamin analysis

2.5.1. Vitamin A analysis

5 g of powdered feed and 2 N Potassium hydroxide prepared in 90% alcohol was refluxed for 20 min at 60 °C. On cooling, 20 ml of water and 10 ml of petroleum ether was added in a separating funnel. The petroleum ether layer was pooled and the process was repeated again. To the pooled petroleum ether layer, anhydrous sodium sulphate was added and kept for 60 min 5 ml of aliquot was dried at 60 °C. The dried residue was dissolved in 1 ml of chloroform. 2 ml trichloroacetic acid was added and absorbance measured at 620 nm. This was plotted against standard ascorbic palmitate (1.5–7.5 μg/ml) solution to obtain unknown concentration [23].

2.5.2. Vitamin C analysis

5 g of feed was ground with 50 ml of 4% Oxalic acid solution, centrifuged and filtered. 10 ml of filtrate was dehydrogenated with bromine water till the solution became orange yellow. Excess of bromine was displaced with air. The solution was made up to 25 ml with 4% oxalic acid solution. 0.5 ml of this solution was made up to 3 ml with distilled water, treated with 2,4-dinitrophenylhydrazine and thiourea followed by incubation at 37 °C for 3 h. The orange red osazone crystals were dissolved by adding 7 ml of 80% sulfuric acid. 0.5 ml of this solution was made up to 25 ml bromine was displaced with air. The solution was made up to 25 ml.

2.6. Feeding calorifically dense feed to male C57BL/6J mice

2.6.1. Animals used

One month male C57BL/6J mice thus obtained by breeding were used for the study (Stock animals were procured from National Institute of Nutrition, Hyderabad, India). They were housed in polypropylene cages and maintained at 25 ± 2 °C. Male mice were chosen as hormonal variation of sex differences of body fat and less abdominal fat as compared to males [27]. They were divided into two groups control and test (n = 30 each) and were fed with control feed and HFSC feed respectively at the rate of 5 g/day as per Indian National Science Academy regulations [28]. The mice were provided with fresh food and water; both ad libitum daily. The unconsumed food was measured daily. The animals were sacrificed by cervical dislocation. All the experiments were carried out according to the CPCSEA guidelines with approval from Institutional animal ethics committee (Sanction No. SAC/IAC/110/2011 dt. 30th March, 2011).

2.6.2. Food intake, energy intake and feed efficiency

They were calculated as follows [16,29].

\[
\text{Food intake (g)} = \text{weight of food provided (g)} - \text{weight of food unconsumed (g)}
\]

Energy intake = Mean food consumed (g) × dietary metabolizable energy (Kcal)

Data is represented as Mean ± S.E.M. of 3 independent values (except energy content which is value obtained from one single determination). ***p < 0.001 HFSC feed as compared to the Control feed.

Feed efficiency = \( \frac{\text{Weight gain (g)}}{\text{Kcal consumed}} \) \times 100

Biochemical and anthropometric parameters of the mice consuming these feeds were studied and results are published [17] and will be discussed.

2.7. Histological analysis

2.7.1. Haematoxylin- eosin staining

The tissues were harvested from the mice at the time of sacrifice. The tissues were then fixed in buffered formalin, dissected, embedded in paraformaldehyde and sectioned. The slides were deparaffinised 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 41 TF, Singapore) and photographed using camera (Jenoptek, Germany).

3. Results and discussion

3.1. Calorific content

The HFSC feed was found to be more energy dense (5064.581 kcal/kg) as compared to control feed (3582.369 kcal/kg). The control feed provided 10% calories from Protein, 6% from Fat and 84% from Carbohydrates whereas the HFSC feed provided 12% calories from protein, 46% calories from Fat and 42% calories from Carbohydrate (Table 1).

3.2. Proximate analysis

3.2.1. Moisture, protein, fat, carbohydrate, Crude Fibre and Ash content

The HFSC feed had significantly lower (p < 0.001) moisture content as compared to the control feed. The difference in moisture content could be due to the volume of water required to form a pellet for both feeds. The HFSC feed had significantly higher protein content (p < 0.001), Fat content (p < 0.001) and lower Carbohydrate content (p < 0.001) as compared to control feed. Even though the HFSC feed has lower carbohydrate content as compared to Control

### Table 1

<table>
<thead>
<tr>
<th>Moisture content (g/100 g)</th>
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<th>Carbohydrate content (g/100 g)</th>
<th>Crude fibre (g/100 g)</th>
<th>Ash (g/100 g)</th>
<th>Energy content (Kcal/kg)</th>
<th>Protein (%)</th>
<th>Fat (%)</th>
<th>Carbohydrate (%)</th>
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<tr>
<td>Control feed 5.28 ± 0.04</td>
<td>8.75 ± 0.14</td>
<td>2.42 ± 0.01</td>
<td>75.35 ± 0.45</td>
<td>13.0 ± 0.00</td>
<td>6.87 ± 0.25</td>
<td>3582.36</td>
<td>10</td>
<td>6</td>
<td>75.35</td>
</tr>
<tr>
<td>HFSC feed 3.15 ± 0.04***</td>
<td>15.76 ± 0.35***</td>
<td>25.61 ± 0.08***</td>
<td>53.21 ± 0.54***</td>
<td>0.03 ± 0.00***</td>
<td>2.21 ± 0.10***</td>
<td>5064.58</td>
<td>12</td>
<td>46</td>
<td>15.76</td>
</tr>
</tbody>
</table>

Data is represented as Mean ± S.E.M. of 3 independent values (except energy content which is value obtained from one single determination). ***p < 0.001 HFSC feed as compared to the Control feed.

Proximate analysis, food composition and energy content of feed.

### Table 1

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<td>12</td>
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<td>15.76</td>
</tr>
</tbody>
</table>
Data is represented as Mean ± S.E.M. of 3 independent values.

**p < 0.01; ***p < 0.001 HFSC feed as compared to Control feed.

### Table 2
Fatty acid profile of feed.

<table>
<thead>
<tr>
<th></th>
<th>Control feed (% of total fatty acid)</th>
<th>HFSC feed (% of total fatty acid)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capric Acid</td>
<td>0.85 ± 0.06</td>
<td>0.84 ± 0.17</td>
</tr>
<tr>
<td>Lauric Acid</td>
<td>1.78 ± 0.04</td>
<td>1.20 ± 0.11**</td>
</tr>
<tr>
<td>Myristic Acid</td>
<td>6.84 ± 0.03</td>
<td>4.35 ± 0.03***</td>
</tr>
<tr>
<td>Pentadecanoic Acid</td>
<td>1.02 ± 0.17</td>
<td>0.69 ± 0.05</td>
</tr>
<tr>
<td>Palmitic Acid</td>
<td>24.35 ± 0.19</td>
<td>30.82 ± 0.45***</td>
</tr>
<tr>
<td>Palmitoleic Acid</td>
<td>2.94 ± 0.004</td>
<td>0.93 ± 0.10***</td>
</tr>
<tr>
<td>Stearic Acid</td>
<td>11.08 ± 0.28</td>
<td>9.31 ± 0.08**</td>
</tr>
<tr>
<td>Oleic Acid/Elaidic Acid</td>
<td>34.29 ± 0.21</td>
<td>25.81 ± 0.54***</td>
</tr>
<tr>
<td>Linoleic Acid</td>
<td>8.25 ± 0.07</td>
<td>27.10 ± 0.39***</td>
</tr>
<tr>
<td>α-Linolenic Acid</td>
<td>0.48 ± 0.04</td>
<td>1.23 ± 0.04***</td>
</tr>
<tr>
<td>Arachidic Acid</td>
<td>0.90 ± 0.005</td>
<td>0.62 ± 0.002***</td>
</tr>
</tbody>
</table>

Data is represented as Mean ± S.E.M. of 3 independent values.

### Table 3
Mineral and vitamin analysis of feed.

<table>
<thead>
<tr>
<th></th>
<th>Control feed (mg/100 g)</th>
<th>HFSC feed (mg/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zinc</td>
<td>119.06 ± 0.80</td>
<td>54.92 ± 2.70***</td>
</tr>
<tr>
<td>Iron</td>
<td>256.56 ± 1.98</td>
<td>147.72 ± 0.91***</td>
</tr>
<tr>
<td>Copper</td>
<td>12.81 ± 2.66</td>
<td>5.90 ± 0.16</td>
</tr>
<tr>
<td>Manganese</td>
<td>30.53 ± 0.17</td>
<td>1.74 ± 0.34***</td>
</tr>
<tr>
<td>Magnesium</td>
<td>529.78 ± 13.43</td>
<td>411.74 ± 9.71***</td>
</tr>
<tr>
<td>Sodium</td>
<td>1307.35 ± 34.63</td>
<td>1601.73 ± 17.31**</td>
</tr>
<tr>
<td>Potassium</td>
<td>1908.44 ± 54.90</td>
<td>2109.74 ± 18.30**</td>
</tr>
<tr>
<td>Calcium</td>
<td>613.15 ± 6.78</td>
<td>1834.02 ± 13.56**</td>
</tr>
<tr>
<td>Vitamin A</td>
<td>0.27 ± 0.001</td>
<td>0.19 ± 0.0003***</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>4.67 ± 0.21</td>
<td>3.028 ± 0.24**</td>
</tr>
</tbody>
</table>

Data is represented as Mean ± S.E.M. of 3 independent values.

* p < 0.05; **p < 0.01; ***p < 0.001 HFSC feed as compared to Control feed.

### Table 4
Effect of HFSC feed on food intake, energy intake and feed efficiency of male C57BL/6j mice during the 5 month study.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 M</td>
<td>2 M</td>
</tr>
<tr>
<td>Food intake (g/month)</td>
<td>750</td>
<td>750</td>
</tr>
<tr>
<td>Energy intake (Kcal/month)</td>
<td>3582.36</td>
<td>3582.36</td>
</tr>
<tr>
<td>Feed efficiency</td>
<td>0.02</td>
<td>0.47</td>
</tr>
</tbody>
</table>

Statistical analysis was not possible for this data as food for the entire group and not individual mouse was recorded.

Data is represented as a single value obtained from 6 mice.

feed, it was more calorifically dense due to High Fat content. This was due to the decrease in the energy provided on catabolism by each macronutrient group with fat providing the most energy (9 kcal/g) as compared to protein and carbohydrate (4 kcal/g) [17]. The HFSC feed had significantly (p < 0.001) lower Crude Fibre and Ash content as compared to the control feed (Table 1).

Dietary carbohydrate is shown to be the main dietary determinant of blood glucose [30]. Protein and fat are also known to induce satiety and reduce orexigenic signals to the brain [31]. The proportion in which these three macronutrients are present in a diet plays a key role in obesity. For example, Low Carbohydrate High Protein diets (LCHP) are popular for weight loss as high demand for glucose by sole glucose consuming organs accelerates protein turnover leading to increased energy expenditure furthering weight loss [32]. Humans are found to become obese only if diets are high in both fat and carbohydrate indicating that the determining factor is carbohydrate [33]. This further validates the use of the formulated HFSC feed. The nature of carbohydrate and fatty acids consumed in the diet also has differential effects. Consumption of simple carbohydrates such as sucrose, fructose coupled with high fat is found to be detrimental [34,35]. A reduction in mono-unsaturated fatty acids coupled with an increase in starchy foods was found to further deteriorate insulin sensitivity [36].

#### 3.2.1. Fatty acid profile.

To get a better understanding of the nature of fatty acids present in both the feeds, fatty acid profile was analyzed by Gas Chromatography (GC). In our study, a marked difference in the saturated, mono and polyunsaturated fatty acid profile was observed in both the feeds. The saturated fatty acids detected in the control feed include Capric acid, Lauric acid, Myristic acid, Palmitanoic acid, Palmitic acid, Stearic acid, Oleic acid and Arachidic acid. The HFSC feed contained Heptadecanoic acid in addition to the above mentioned fatty acids. Palmitoleic acid—a mono unsaturated fatty acid was also detected in the both feeds. Polyunsaturated fatty acids (PUFA) namely Linoleic acid (ω-6) and α-Linolenic acid (ω-3) were also detected in both feeds. The HFSC feed contained higher ω-6
and ω-3 as compared to control feed. The HFSC feed also had a higher ω-6/ω-3 ratios as compared to the control feed. The HFSC feed had significantly lower Lauric acid \( (p < 0.01) \), Myristic acid \( (p < 0.001) \), Palmitoleic acid \( (p < 0.001) \), Stearic acid \( (p < 0.01) \), Oleic acid \( (p < 0.001) \), Arachidic acid \( (p < 0.001) \) and higher Palmitic acid \( (p < 0.001) \), Linoleic acid \( (p < 0.001) \) and \( \alpha \)-Linolenic acid \( (p < 0.001) \) as compared to control feed (Table 2). There was no significant difference between the Capric acid content between the two feeds. Thus, in our study, the two feeds were found to be significantly distinct from each other in terms of fatty acid profile.

Consumption of mono and polyunsaturated fatty acids are shown to be more beneficial as compared to saturated fatty acids especially in MetS cases [36]. Palmitic acid is known to alter central nervous system control of insulin secretion and suppression of leptin and insulin [37]. However, some saturated fatty acids also positively correlated with good health. For example, Lauric acid was found to increase total High Density Lipoprotein (HDL) compared to other fatty acids [38]. ω-3 fatty acid improved insulin action whereas monounsaturated fatty acids and ω-6 fatty acids were found to impair it lesser than saturated fatty acid [39]. These were also found to have an inverse relation with plasma insulin concentration [40]. Among the polyunsaturated fatty acids, higher levels of ω-3 fatty acids are preferred as compared to ω-6 fatty acids as the latter promotes the formation of thrombus and atheroma. Lower ratio of ω-6/ω-3 is therefore preferred [41]. The results of our study were in agreement with these studies as the HFSC feed contained lower Lauric acid and higher ω-6/ω-3 ratios. In our study, the HFSC fed mice were found to have increased body weight and hypertriglyceridemia as compared to the control mice [17]. The fatty acid profile of the feed might have potentiated the induction of MetS.

3.2.1.2. Mineral analysis.

In our study, the HFSC feed was found to have significantly lower \( (p < 0.001) \) Zinc, Iron, Manganese, Magnesium content and significantly higher \( (p < 0.01) \) Sodium, Potassium and Calcium content as compared to control feed (Table 3). The minerals present in feed play an important role in health and disease. Magnesium is an important co-factor for several enzymes in carbohydrate metabolism [42]. Zinc is involved in synthesis, storage and release of insulin [43]; Sodium and Potassium play an important role in hypertension in human subjects [44].
Also, deficiency, either biologically or due to dietary habits, in some minerals has been reported in patients with MetS and individual abnormalities. Low Zinc levels and high serum Calcium levels were reported female MetS patients [45,46]. Increased dietary intake of Magnesium has been reported to reduce the risks of MetS [47,48]. Lower intake of Magnesium, Manganese and Copper and increased Sodium intake was reported in women with high blood pressure [49,50]. Thus, it is evident that dietary minerals play a role in carbohydrate and lipid metabolism. In our study, altered carbohydrate metabolism and impaired glucose tolerance were observed in the test group fed with HFSC feed as compared to the control [17]. Perhaps the altered mineral content in the HFSC feed in synergy may have contributed to the development of the syndrome.

3.2.1.3. Vitamin profile.
The HFSC feed had lower (p < 0.001) Vitamin A and Vitamin C (p < 0.05) content as compared to the control feed (Table 3). Oxidative stress has also been linked to obesity, diabetes and cardiovascular disease. The antioxidants, Vitamin C and Vitamin E were found to be lower in MetS patients [51]. Chronic dietary Vitamin A supplementation was found to reduce weight gain in obese mutant WNIN/Ob rat model [52]. This supports our study.

3.2.1.4. Food intake, energy intake and feed efficiency.
Hyperphagia was observed in the test mice as compared to the control throughout the study. Interestingly, after 4 months and 5 months of feeding, the food intake of test mice decreased as compared to its own food intake in the prior months (Table 4). The energy intake of test mice increased throughout the study as compared to the control. Again, the energy intake of test decreased after 4 and 5 months of feeding as compared to its own energy intake in the prior months (Table 4). These changes indicate derailment in regulation of food intake. Despite this, the percentage body weight of the test mice [17], were still found to increase indicating lack of energy expenditure. We also observed an increase in the adipose tissue during the progression of the syndrome (Figs. 1 and 2).

3.3. Histological analysis
Gradual changes in lymphocyte infiltration along with dilated sinusoids, dilated central veins, disrupted hepatocytic cords and shrunken nucleus was observed in the test liver after 5 months of feeding [Figs. 3 and 4]. The results indicate that hepatocytes showed morphological features of Non Alcoholic Fatty Liver Disease. Widened Bowman space, increase in interstitial space, distortion of cellular continuity, thickening of basement membrane and hypertrophy was observed in kidneys of test mice after 5 months of feeding [Figs. 5 and 6]. Hypertrophy of the adrenal medulla [Figs. 7 and 8] and degradation of the islets of pancreas [Figs. 9 and 10] was observed after 5 months of feeding. Very few studies have been carried out on the effect of diets containing 10–12% protein calorifically on histology of organs. Diets which utilize 8% protein have reported loss of weight in rat kidney and
Fig. 5. Control kidney stained with haematoxylin eosin staining after 5 months of feeding (100X).

Fig. 6. Test kidney stained with haematoxylin eosin staining after 5 months of feeding (100X) widened Bowman space (1), Increase in interstitial space (2), Cellular continuity distorted (3) Hypertrophy (4).

Fig. 7. Control adrenal gland stained with haematoxylin eosin staining after 5 months of feeding (100X).

Fig. 8. Test adrenal gland stained with haematoxylin eosin staining after 5 months of feeding (100X) Fat accumulation (1), Hypertrophy of the medulla (2).

Fig. 9. Control pancreas stained with haematoxylin eosin staining after 5 months of feeding (400X).

Fig. 10. Test pancreas stained with haematoxylin eosin staining after 5 months of feeding (400X) destruction of islet cells (1).
liver among other organs without any effect on the histology of the organs [53]. A study carried out on the effect of maternal low protein diet during pregnancy reported cellular swelling, hydropic degeneration characterized by pale cytoplasm in liver wistar rats [54]. Diets with 45% or more protein were found to cause glomerular enlargement in NMRI mice kidney which is VEGF dependant and can be ameloriated by VEGF supplementation in high protein diet [55]. Maternal low protein diets (8%) were found to cause fetal pancreas to have lower beta cell mass and vascu- larization which could be overcome by supplementation with taurine in rats [56]. Thus from these reports it is clear that intake of protein (8%) during pregnancy has a detrimental effect on the fetus. Perhaps long term low protein intake post birth would cause histological changes in the organs.

3.4. Concluding remarks

High Fat Simple Carbohydrate Diet used in our study is repre- sentative of the fast foods consumed by an increasing number of people both in the developed and a fast catching up developing countries. It was found to contain decreased levels of fiber, the “good fats”, minerals and vitamins. This effect is only magnified by other compounding factors such as stress and bad life style habits. The HFSC diet was also found to induce hyperphagia in the HFSC fed mice as compared to the control mice. The importance of the knowledge of nutritional content of food consumed is highlighted by this study. The histological studies show that metabolic syn- drome induced by HFSC feed causes simultaneous deterioration in many organs indicating a need for holistic research in this area for possible amelioration.

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Conflict of interest

We declare that there is no conflict of interest in this study.

Contribution of authors

D’Souza Serena Stephen has contributed towards the literature search, design, experimental studies, data acquisition, data analysis, statistical analysis of this entire study, manuscript preparation and editing of this manuscript. Sri Chanand Bindu and Mohammed Ali M have contributed to the data acquisition of the fatty acid profile part of the manuscript. Tisha Alex, Deepthi KV, Siona Silveira, Santy Fernandes have contributed towards the data acquisition of the histological experiments of Liver and kidney of the mice. Dr Asha Abraham has contributed towards the concepts, design, definition of intellectual content, literature search, manuscript editing and manuscript review of this work.

All authors have approved the final article.

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