FRUCTOSE; IS IT AN IDEAL SWEETENING AGENT?

Dr. Zehra Naz¹, Dr. Abdul Khaliq Naveed², Dr. Mehdi Raza³

ABSTRACT… Objectives: To study and compare the effects of fructose and galactose on, blood glucose, insulin, HbA₁c, and lipids and anthropometric measurements. Data Source: Ninety, healthy adult male rats of Sprague-Dawley strain. Design: Experimental study. Setting: Department of Biochemistry and Molecular Biology, A.M.C, Rawalpindi, in collaboration with N.I.H, Islamabad. Period: Twelve months. Subjects and Methods: Rats weighing 180-350 grams were selected by random sampling and were divided into three groups, 30 each. Group I was given standard diet, Group II was given high fructose diet (HFD), i.e., 1.5gms/Kg body weight/day of fructose, along with standard diet for two weeks and Group III was given high galactose diet, i.e., 0.83gms/Kg body weight/day, along with standard diet for two weeks. Results: There was a significant increase in height of galactose group while fructose group has significant weight loss; BMI decreased in both but more in former. The blood levels of cholesterol, HDL-c, LDL-c, TG, and insulin were significantly higher in fructose group than in galactose group. There was no significant difference between blood glucose and HbA₁c among these groups yet their higher levels indicate the chances of developing insulin resistance. Conclusions: Fructose due to its less hyperglycaemic effects should not be used in diet and must not be prescribed in diabetes, as in the long run it can lead to obesity, hypertension and cardiovascular risk. Non-significant effects of galactose on above parameters (except lipoproteins), does not mean that it can be used as an alternative to fructose and this area needs exploration.

Key words: HFD=High fructose diet, HGD=High galactose diet, BMI=Body mass index, HDL-c=High density lipoprotein cholesterol, LDL-c=Low density lipoprotein cholesterol, TG=Triglycerides, HbA₁c=Glycated haemoglobin.

INTRODUCTION

According to data from Pakistan National Health Survey, there is a growing increase in the incidence of coronary heart diseases (CHD), hypertension (HTN), obesity¹,² and diabetes³. Moreover, internationally obesity has become an epidemic worldwide, and most of the population has become obese and rest of them are heading towards it⁴.

One of the main contributing factors for the above mentioned diseases is diet. As a result, of changing life styles, the dietary habits have also changed e.g. the consumption of liquid beverages like soft drinks, fruit juices etc which contain high fructose corn syrup has increased and they are becoming the part of normal regular meal.

The aim of the present study was to compare the effects produced by high fructose and galactose diet on the biochemical markers and anthropometric measurements. The study provided important information which may be used as dietary guidelines for our population. In addition the results would be able to contribute in the better understanding of impact of fructose and galactose on growth especially the height, weight and body mass index (BMI). It will also help in finding the answer to questions like whether fructose is more harmful or galactose and whether
galactose can be used as a replacement of fructose or not.

**SUBJECTS AND METHODS**

This experimental study was conducted from Jan 2010 to Dec 2010, after the approval of Research and Ethical Review Committee of College of Physicians and Surgeons, Pakistan, at the Department of Biochemistry and Molecular Biology, Army Medical College. Chemical analysis was done in the department of Chemical Pathology, Army Medical College, Rawalpindi in collaboration with National Institute of Health (N.I.H.), Islamabad.

Ninety normal, healthy, male rats of Sprague-Dawley strain, weighing 180-350 grams, were selected by random sampling and body mass index (BMI) of every rat was calculated by measuring height (in meters) and weight (in kilograms). They were housed in metallic cages at Animal House, NIH, Islamabad, under standard conditions i.e. 12h light and 12h dark cycle and room temperature of 25±3°C. They were given free access to water and were fed ad libitum with standard diet or high fructose diet (HFD) or high galactose diet (HGD) respectively. Group I: It comprised of those thirty rats which were given standard diet, composed of skimmed milk 20%, wheat bran 28.5%, wheat flour 28.5%, molasses 1%, fish meal 15%, vegetable oil 5%, salt 0.5%, vitamins premix 0.5% and minerals premix 0.5% for two weeks. They were included in the study to provide a baseline for chemical parameters. Group II: It was comprised of those thirty rats which were given HFD i.e. 1.5 gms/Kg body weight/day of fructose along with standard diet for two weeks. Group III: It comprised of those thirty rats which were given HGD i.e. 0.83 gms/Kg body weight/day along with standard diet for two weeks. Regarding the preparation of HFD, average body weight was calculated and then the consumption of fructose at a rate of 1.5 gms/kg body weight/day was calculated for 14 days. Analyte fructose i.e., D-Fructose manufactured by Merck®, U.S.A, was used.

When the period of fourteen days feeding was completed blood samples were collected after an overnight fast of twelve hours (from 9:00 p.m. to 9:00 a.m.). The rats were given open ether inhalation anaesthesia. When rat was completely anaesthetized, 10 cc blood was collected by cardiac puncture. Glucose, lipids and HbA1c levels were determined immediately whereas insulin samples were frozen (-20°C) till analyzed. All estimations were done by using ready to use commercially available kits (glucose kit manufactured by Globe Diagnostics S.r.l., Milan, Italy; HDL-c kit manufactured by Human Gesellschaft für Biochemica und Diagnostica mbH®, Weisbaden, Germany; TG kit manufactured by Globe Diagnostics S.r.l., Milan, Italy; cholesterol kit manufactured by Pioneer Diagnostics®, New York, USA; HbA1c kit manufactured by Human Gesellschaft für Biochemica und Diagnostica mbH®, Weisbaden, Germany and insulin by Access, Ultra sensitive insulin kit manufactured by Beckman Coulter®, California, USA) on automated analyzers (Vitalab Selectra E, Microlab 200 and Beckman Coulter, Access 2, Immunoassay Systems). LDL-c which was estimated by using the following formula:

\[
\text{LDL cholesterol} = \left[ \text{Total Cholesterol} \right] - \left[ \text{HDL Cholesterol + Triglycerides/2.2} \right]
\]

Data was analyzed by using Statistical package for Social Sciences (SPSS version 16). Descriptive statistics was used to describe the data. Mean and standard deviation (S.D.) was used to describe numeric variables like age, height, weight, glucose, TG, HDL-c, LDL-c, cholesterol, insulin and HbA1c.

Paired t-test was applied to compare the anthropometric measurement among the two groups i.e. fructose and galactose. Analysis of
Variance (ANOVA) was applied for the comparison of numeric variables like triglycerides, cholesterol, high density lipoprotein (HDL-c), low density lipoprotein (LDL-c), glycosylated haemoglobin (HbA1c) and insulin. For multiple comparisons Post–Hoc (Tukey HSD) was applied. P value <0.05 was considered as significant.

RESULTS
Table-I shows as the mean and standard deviation of anthropometric measurements along with their significance in fructose and galactose groups before and after diet.

In case of fructose group, there was a significant decrease in weight after diet (p<0.01); there was no significant change in height after diet, however, there was a significant decrease in the BMI of this group (p<0.001). Whereas, in case of galactose group, there was no significant change in weight after diet, however height was significantly increased (p<0.001) whereas BMI was significantly decreased (p<0.01).

Table-II shows the mean and standard deviation of chemical parameters and their significance.

Regarding average mean, among all the three groups, it was highest, in fructose fed group in case of cholesterol (1.68±0.30 mmol/L), TG (1.17±0.34 mmol/L), LDL (0.71±0.18 mmol/L), insulin (1.87±3.97 μIU/mL), HbA1c (5.39±0.91%) and in controls in case of glucose (13.76±6.28 mmol/L).

Regarding average mean, among all the three groups, it was lowest, in galactose fed group in case of TG (0.74±0.19 mmol/L), HDL (0.41±0.07 mmol/L), insulin (0.19±0.29 μIU/mL), glucose (11.78±3.94 mmol/L), HbA1c (4.97±0.81%) and in controls in case of LDL (0.41±0.12 mmol/L).

Table-III shows the comparison of chemical parameters between the three groups i.e. Control, fructose fed and galactose fed groups (Post-Hoc, Tukey HSD). Significant differences were observed in cholesterol, TG, LDL, HDL, insulin parameters in between these three groups whereas no significant difference was observed in the levels of glucose and HbA1c of these groups.

DISCUSSION
With a growing increase in the diabetes, cardiac diseases, hypertension, renal diseases and obesity in our society it has become necessary to prevent this process of national progression.
## ANOVA

<table>
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<th>Parameters</th>
<th>Controls (n=30)</th>
<th>Fructose fed (n=30)</th>
<th>Galactose fed (n=30)</th>
<th>P</th>
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</thead>
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<tr>
<td>Cholesterol (mmol/L)</td>
<td>1.33±0.26</td>
<td>1.68±0.30</td>
<td>1.37±0.20</td>
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<td>TG (mmol/L)</td>
<td>0.90±0.26</td>
<td>1.17±0.34</td>
<td>0.74±0.19</td>
<td>0.0001*</td>
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<td>LDL-c (mmol/L)</td>
<td>0.41±0.12</td>
<td>0.71±0.18</td>
<td>0.67±0.20</td>
<td>0.0001*</td>
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<td>HDL-c (mmol/L)</td>
<td>0.51±0.10</td>
<td>0.43±0.06</td>
<td>0.41±0.07</td>
<td>0.0001*</td>
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<tr>
<td>Insulin (mIU/mL)</td>
<td>0.27±0.49</td>
<td>1.87±3.97</td>
<td>0.19±0.29</td>
<td>0.009*</td>
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<tr>
<td>Glucose (mmol/L)</td>
<td>13.76±6.28</td>
<td>12.31±3.90</td>
<td>11.78±3.94</td>
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<tr>
<td>HbA1c (%)</td>
<td>5.06±0.87</td>
<td>5.39±0.91</td>
<td>4.97±0.81</td>
<td>0.15NS</td>
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</tbody>
</table>

Table-II. Chemical parameters of control rats and rats fed on high fructose diet and high galactose diet (expressed as mean±S.D.)

*NS = Non-significant, **P < 0.05, ***P < 0.01, ****P < 0.001

Key:
- HbA1c = Glycated haemoglobin
- HDL-c = High density lipoprotein
- LDL-c = Low density lipoprotein
- TG = Triglyceride

<table>
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<th>Parameters</th>
<th>Controls vs. Fructose fed</th>
<th>Controls vs. Galactose fed</th>
<th>Fructose fed vs. Galactose fed</th>
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<tbody>
<tr>
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<td>Mean difference</td>
<td>P</td>
<td>Mean difference</td>
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<td>Cholesterol (mmol/L)</td>
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<td>LDL-c (mmol/L)</td>
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<td>HDL-c (mmol/L)</td>
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<td>Insulin (mIU/mL)</td>
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<tr>
<td>Glucose (mmol/L)</td>
<td>1.44</td>
<td>0.48NS</td>
<td>1.97</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>0.32</td>
<td>0.31NS</td>
<td>0.09</td>
</tr>
</tbody>
</table>

Table-III. Comparison between chemical parameters of control rats and rats fed on high fructose diet and high galactose diet

*NS = Non-significant, **P < 0.05, ***P < 0.01, ****P < 0.001

Key:
- HbA1c = Glycated haemoglobin
- HDL-c = High density lipoprotein
- LDL-c = Low density lipoprotein
- TG = Triglyceride
towards ‘metabolic syndrome’, which is a combined term used for all these nowadays, one has to investigate the possible causes associated with it. From past to present, a great deal of change has been observed in our life style and dietary habits. In recent past, sucrose was used as a sweetener in our foods but nowadays it has been replaced by other sweetening agents like fructose and its liquid form that is high fructose corn syrup, which is a growing industry. As a result of these slow changing processes in our diet we are heading towards a sweetly wrapped dead end of life.

In present study, we have found a significant increase (p <0.0001) in height in galactose fed group; weight was significantly decreased in fructose fed group (p<0.001) after diet was given. But there was a significant decrease (p<0.0001) in BMI in both the groups, however, greater decrease was observed in galactose fed group.

Twenty three male rats of two and a half months of age, were divided into no-fructose diet control group and high fructose diet group, in the study done by Shapiro et al. Later on, the groups were switched to high fat diet. Initially the body weight of second group was 324±3.9 grams they slowly gained weight in six months to nearly 375 grams (by graph), though fructose fed group was slightly lesser in weight as compared to other group. Results were not similar to ours, as there was no significant difference in two groups. The gain in weight could be due to the age of animals as they used younger rats while ours were adult.

Sánchez-Lozada and colleagues took twelve, male Sprague-Dawley rats of 150 grams weight. Two groups were made, one received 60% sucrose (n=6) while second group received 30% glucose plus 30% fructose (FG) (n=6), six more rats of same strain and weight were taken as controls. After four months body weight of controls was 634±63 grams whereas that of FG group was 647±53 grams. Results were contrary to our study. It could be because of different environmental factors like, age (younger in their study) and diet.

Sugawa-Katayama and Morita high fructose diet group's (fructose 69%) final weight in male rats was 152±8.7 grams (weight gain was 5.0±1.8 g/4 d), in female rats 136±7.4 grams (weight gain was 2.3±2.1 g/4 d). Results do not match with ours. Reason might be the age and the duration of the study which was shorter than ours. Had they conducted the study for a longer period there might have been a decrease in weight rather than gain.

According to Cryer and Hartley when a 70% galactose diet is consumed by rats first they lost and later gained weight at a rate of one sixth to their normal rate. This increase is in agreement with our study.

Strother et al gave 50% galactose diet to male Sprague-Dawley rats. Weight of controls was 342±4 gms, while that of galactose fed group was 274±5 gms. Significant decrease in weight was observed in the galactose fed group which is contrary to present study. Reason might be the quantitative difference in the galactose diet.

Rats of Xue et al weighing 50-60 grams were given fructose and galactose. After nine months galactose fed rats had a highly significant weight loss which is an observation opposite to our study. Reason might be the duration of the study in both the cases.

Higher BMI of our study does not match with controls of Altunkaynak and Özbek which was 24.53±0.22 Kg/m².

The observed weight loss in case of fructose group might be because of the direct relationship between the levels of insulin with the secretion of leptin, a satiety hormone. Higher level of insulin might have caused increased secretion of leptin as a result there was a decreased food intake leading to weight loss and ultimately affecting body mass index. Whereas decrease in BMI in case of galactose group might be because of the mobilization of fatty acids and increased oxidation of lipids.
In present study, significant differences were observed in case of cholesterol, HDL-c, LDL-c, TG and insulin. There was no significant finding in case of glucose and HbA1c among the groups.

The study of Shapiro et al. included rats of two and a half months age, which were first divided into fructose free controls and fructose fed rats. Later the group was switched to high fat diet. All biochemical parameters were measured in non-fasting rats except glucose which was measured both in fasting as well as non-fasting state. Controls glucose levels (both fasting and non-fasting) of this study matches the ones obtained in our study whereas rest of the parameters are higher. Whereas in case of high fructose diet group insulin appears logically less than ours as we were unable to find the standard conversion factor for it. Investigations in this study were not done at baseline level and were done at the end of the experiment thus not providing an opportunity to monitor any changes but high levels exhibited in lipid profile are most probably be due to high fat diet to which rats were switched on at the later stage of experiment.

Sugawa-Katayama and Morita have given high carbohydrate diet for four days to both male and female Sprague-Dawley rats of five weeks age. In group 1 of this study, which was high fructose diet group (fructose 69%), serum insulin levels were quite higher then the levels seen in our study. This might be due to the use of different method of estimation i.e. radioimmunoassay.

Botzelli et al. have taken twenty one days old, forty five male wistar rats. Rats were divide into three groups (fifteen per group) controls, L who ingested coca cola light (fructose poor) and R who drank regular coca cola (fructose rich) for eight weeks. Glucose in all groups lower than ours, TG higher then ours in all groups, cholesterol similar to ours in case of L and R groups, LDL-c and HDL-c were higher in all groups then our.

Strother et al. took 175-200 gms male Sprague-Dawley rats which were divided into four groups, we have considered only controls and 50% galactose fed group. All the parameters were lower then ours.

Xue et al. in their study have given fructose and galactose to rats with marginal or adequate copper levels. HbA1c, glucose, cholesterol and TG were measured after nine months. Plasma cholesterol was increased in fructose fed group, a finding similar to ours whereas HbA1c was significantly increased in galactose fed group, a finding negating our results. Duration and copper levels can be the reasons.

Fructose, the only keto sugar of physiological importance does have a catalytic effect when consumed in small amounts as it bypasses the regulatory step of phosphofructokinase but when taken in excessive quantity this leads to excessive production of substrates that are the part of lipid metabolism causing a rise in the concentrations of TG, LDL and cholesterol. TG is considered as the first indicator of disturbance in lipid profile the reason is that with fructose ingestion the substrates for the production of fatty acids are available in an increased amount; increased availability of fatty acids results in decreased lipolysis as malonyl Co-A is produced, which decreases their entry inside the mitochondria; with rise in lipid levels there is an increase in the formation of VLDL and decrease in the breakdown of apoB, moreover, there is decrease in the rate of removal of TG from the plasma too. The same was indicated by the fructose group of this study. Fructose can form glucose so when excess is taken its production can be increased although it has less hyperglycemic effects. It increases the hepatic uptake of glucose but as the capacity of liver to take up the glucose is saturated, the level of insulin rises to cover and insulin resistance develops. The above mentioned observation holds true in our case as we have given the amount of fructose that is considered as the high amount, it might have produced a catalytic effect for the duration which we studied i.e. two weeks, because our rats even though were healthy but their glucose levels were on higher side, Also a rise in insulin levels in our study indicates the start of insulin resistance.
In galactose group it appears that the utilization of TG is more as compared to the preservation of LDL. In case of galactose there is another possibility that is the involvement of adiponectin, from the adipose tissue. This hormone has an inverse relationship with the insulin levels\textsuperscript{20, 21}. As observed in our galactose group there was a decrease in insulin levels though not that significant, but might have increased the secretion of adiponectin with an increased fatty acid breakdown leading to a decreased level of TG. Thus there is an increased uptake of glucose in the tissues as a result decreased blood glucose levels were seen. Further studies are required to confirm these findings.

As fructose increases, protein glycation products also increases\textsuperscript{22} and we have observed the similar in case of fructose (though non-significant). But its decrease in case of galactose indicates that galactose might not be that reactive to proteins or it might have a harmful effect on red blood cells decreasing their number as in case of other cells of the human body\textsuperscript{23} and thus decreasing the amount of glycated proteins or excessive galactose might have halted the production of glycated proteins resulting in decreased HbA\textsubscript{1c}.

Limitations of the study were time constraint, non-availability of rats, cages, chemicals especially galactose and last but not the least was cost effectiveness due to which insulin samples were frozen, otherwise they would have been analyzed on the respective day like others samples.

CONCLUSIONS

It is concluded from the present study that the galactose increases height significantly, whereas, fructose causes more weight loss than galactose. Though, both fructose and galactose show decrease in BMI but more was observed in case of galactose. Fructose causes more hypercholesterolemia, hyperlipoproteinemia and hypertriglyceridemia than galactose. Though the comparisons among the groups were not significant in case of blood glucose and HbA\textsubscript{1c}, yet their levels indicate the chances of developing insulin resistance (as indicated by significantly increased insulin levels in fructose group). Therefore, it is recommended that fructose due to its less hyperglycaemic effects i.e. low levels of blood glucose and HbA\textsubscript{1c} should not be used in the dietary items especially liquid beverages and must not be prescribed to diabetics, as in the long run it may lead to obesity, hypertension, cardiovascular risk, and diabetes. The non-significant effects of galactose (except lipoproteins), does not mean that it has no impact and can be used as an alternative to fructose. This area need to be explored further and a study with a greater sample size for longer duration is required to show a visible impact.

ACKNOWLEDGMENTS

Financial Assistance in the form of grant was provided by the National University of Science and Technology, Islamabad.

Dr. Muhammad Hussain, Incharge Animal House, N.I.H., Islamabad, helped in obtaining the animals.

Dr. Hussain Ali, Assistant Incharge, Animal House, N.I.H., Islamabad, helped in collecting samples and for taking good care of study subjects.

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REFERENCES


