The impact of high fructose diet on development of metabolic syndrome in male and female rats.

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Introduction

Metabolic syndrome (MetS) is a combination of many risk factors that lead to chronic conditions such as heart disease, type 2 diabetes, etc. and therefore is a growing health concern (*P. Huang, 2009*). 1 in every 4 European has been diagnosed with MetS. The National Heart, Lung, and Blood Institute (NHLBI) and American Heart Association (AHA), define MetS as a combination of a triad of the following conditions (*KG. Alberti, 2009*):

- Large waistline: Excess fat in abdominal area, Waist circumference >102cm (40 inch) in men or >88cm (35 inch) in women.
- High triglyceride level: >150mg/dL (or with drug therapy for hypertriglyceridemia)
- A low HDL-cholesterol level: <40mg/dL in men, <50mg/dL in women (or with drug therapy to reduce HDL-C.)
- High blood pressure: >130/85mmHG (or with drug therapy for hypertension)
- Fasting glucose: >100 mg/dL (or with drug therapy for hyperglycaemia)

MetS can lead to microvascular dysfunction resulting in hypertension. Dysfunction in adipose tissue leads to obesity-related insulin resistance (*E. Tasali, 2008*), involving adipose cell enlargement and infiltration of macrophages and the release of proinflammatory cytokines.

Fructose is a major component of modern diet, over the years use of high fructose content in diet has to development of MetS in population. Few theories have been to proposed to explain the role of fructose in inducing MetS e.g.

- Studies have shown that high fructose diet (HFD) stimulates increased production of proinflammatory cytokines e.g. TNF alpha, which in turn causes inflammation. Increased inflammatory response leads to induction of MetS.
- HFD causes increased plasma uric acid levels and several studies have proven that high plasma uric acid level is a significant causative agent of MetS. Rats with high plasma uric acid levels and consequent MetS showed improvement in MetS when treated with allopurinol which helps to reduce uric acid levels.
- HFD causes metabolic and hormonal dysregulation which a major contributor towards MetS. Fructose reduces insulin sensitivity as well as does not stimulate insulin secretion like glucose. *(A. Miller et al, 2008.)*

The aim was to observe the effect of HFD and consequent development of MetS in male and female rats and to identify any protective role of female sex hormones. We chose Wistar rats as subject for this experiment because they show similar characteristics to humans. Any results obtained from Wistar rats would be applicable to humans and would provide vital research information.

Materials and Methodology

Laboratory animals and procedures:

The experiment was carried out according requirements of the institutional and national regulations and European Directive of 22.09.2010 (2010/63/EU) concerning the protection of animals used for scientific and experimental purposes.

For the experiment 30 adult Wistar rats (18=male, 12=female) weighting 190 - 220g. The rats were kept in plexiglass cages with the cage floor covered in sawdust. The rats were exposed to a controlled environment with 12/12 hours light/dark cycle and a temperature ranging at 18-25°C throughout the experimental period. The animals had free access to food (standard lab rat chow) and tap water ad libitum.

After a habituation period of one week, the rats were randomly divided into four groups, based on the type of diet and supplementation and gender:

- Female Control group (FC, n = 6) – they received tap water throughout the experiment;
- Male Control group (MC, n = 9) – they received tap water throughout the experiment;
- Female fructose fed group (FF, n = 6) – they received a 15 % solution of fructose in drinking water ad libitum for 12 weeks;
- Male fructose fed group (MF, n = 9) – they received a 15 % solution of fructose in drinking water ad libitum for 12 weeks;

The experiment length was 12 weeks. The fructose solution was prepared using 150 mg fructose (Totem, Stara Zagora, Bulgaria) dissolved in 1-liter tap water.

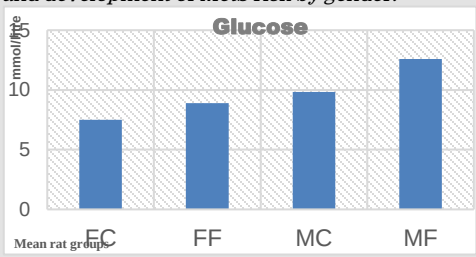
The amounts of consumed water and 15 % solution of fructose were measured daily. At the end of the experimental period (week 12) the rats were anesthetized with Nembutal 50 mg/kg intraperitoneally and after opening of the thoracic cavity they were exsanguinated by cardiac puncture. Blood samples were collected by cardiac puncture.

Fasting serum glucose, triglycerides (TG) levels, total cholesterol (TC), high-density lipoprotein levels (HDL), low-density lipoprotein levels (LDL), were measured by standard laboratory methods for all groups. To verify the metabolic disturbances we calculated lipid indexes such as TC/HDL, LDL/HDL and lipoprotein combined index (LCI): TC*TG*LDL/HDL.

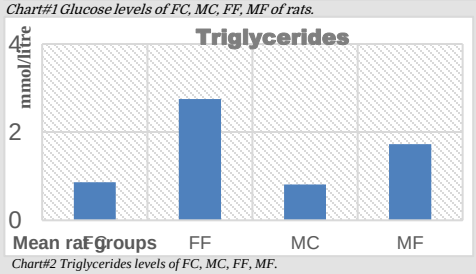
All quantitative data were processed by Statistics for Windows software (Statistics 64 Version 12) and presented as mean and SD. The t-test was performed and P less than or equal to 0.05 was considered as statistically significant.

Results and Discussion

Charts 1-4 and table 1 represent association between fasting glucose, lipid parameters and development of MetS risk by gender.



Chart#1 Glucose levels of FC, MC, FF, MF of rats.



Chart#2 Triglycerides levels of FC, MC, FF, MF.

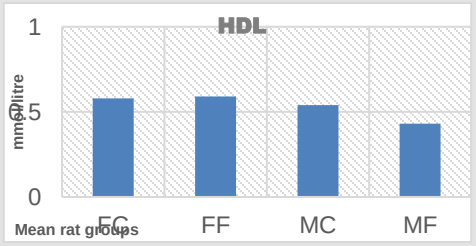


Chart #3 -HDL levels of FC, MC, FF, MF.

Groups	Chol/HDL	SD	LDL/HDL	SD	Tr/HDL	SD	non-HDL/HDL	SD	LCI	SD
FC	2,20	0,28	0,67	0,19	1,49	0,51	1,55	0,26	0,75	0,36
FF	2,21	0,18	0,56	0,10	4,69	2,25	1,65	0,21	2,19	1,52
MC	2,50	0,26	0,81	0,25	1,52	0,40	1,69	0,18	0,86	0,26
MF	2,92	0,39	0,74	0,50	3,74	1,69	2,18	0,41	1,15	0,60

Table #1 - Lipid Indices

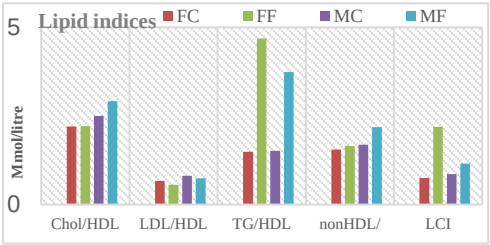


Chart 1 shows both female groups (FC,FF) had lower FSG levels in comparison to the male counterpart groups (MC, MF), MF/FF show higher glucose levels compared to the control groups MC/FC. This is an indicates the development of insulin resistance which is one of the criteria of IR syndrome. Low levels of FSG in both female groups in comparison to male counterpart groups also indicates the protective role of female sex hormones in females; several studies have shown that post-menopausal women are at higher risk of developing coronary heart disease and type 2 diabetes.

Chart 2,3 compares the levels of TG and HDL cholesterol among all experimental groups showing similar trends reaffirming the protective role of sex hormones in females, as well as indicating an increased risk of developing MetS in HFD groups.

Table 1 for Chol/HDL shows that there is a larger increase in MC to MF (0.42) in comparison to FC to FF (0.01).

On the other hand LDL/HDL for both genders fructose groups have shown a decrease in comparison to their control counterparts indicating increased LDL level hence increased risk of developing MetS in HFD groups.

However there are some discrepancies in this data-set, FF showed the highest TG/HDL and LCI (4.69 and 2.19 respectively) compared to all experimental groups including MF. These exceptionally high values can be explained by standard deviation 2.25 and 1.52, high standard deviation shows skewing of results due to one experimental subject with higher parameters.

Another explanation has been proposed by *Teff et al. 2004.* In their study they found that plasma triglyceride levels were high in experimental groups with HFD, in comparison to the experimental group with glucose diet (*figure1.*)

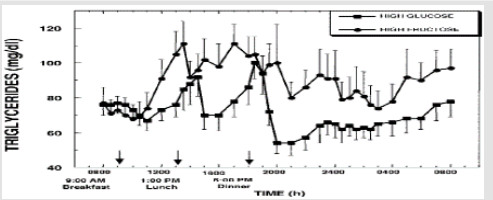


Figure1- Triglycerides levels in glucose & fructose group. (Teff et al, 2004)

Conclusion

HFD induces disturbances in blood parameters of lipid and glucose metabolism. Data obtained from this study has shown that there is an effect on both genders of rats inducing characteristics of MetS, however MF group have shown comparatively higher levels of glucose, triglycerides and LDL in comparison to FF. Therefore MF had higher tendencies to develop metabolic syndrome. This observation also indicates the protective role of female sex hormones.

Acknowledgements

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