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THE EFFECT OF LONG TERM HIGH FRUCTOSE HIGH FAT (HFHF) DIET ON BODY WEIGHT, GLUCOSE HOMEOSTASIS AND LIPID PROFILE IN ADULT WNIN RATS

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ABSTRACT:

This study was aimed at investigating the effect of high-fructose high-fat (HFHF) diet for longer duration (10 months) on body weight, glucose homeostasis and lipid profile in WNIN rats. Two months old male Wistar NIN (WNIN) rats were maintained either on AIN-93M diet alone (control group) or high fructose (32%) with high fat (24%) contains AIN- 93 diet (HFHF group) to WNIN rats developed insulin resistance associated pre-diabetes at 3 months, maintained pre-diabetes over a period of 10 months. Body weight after one month of feeding show that significantly higher in rats fed HFHF diet compared to rats fed AIN-93 diet. Rats fed HFHF diet exhibited significantly higher plasma triglycerides and significantly lowered HDL levels at the end of the experiment, but total cholesterol levels marginally reduced and LDL levels slightly higher in HFHF fed rats compared to control. These results suggest that a HFHF diet ideal for obesity and consequence of complications.

KEY WORD: *Effect, High Fructose High Fat (HFHF), Body weight, Glucose homeostasis, Lipid profile, WNIN rats.*

INTRODUCTION:

High fat (HF) diet mediates progression of impaired glucose tolerance, insulin resistance and associated cardiovascular complications. It does so by promoting hypothalamic resistance to main anorexigenic hormones leptin and insulin, leading to loss of balance between food intake and its metabolic breakdown thus resulting in obesity (Klockener et al. 2011, Sahu 2011, Quennell et al. 2011). Similarly, T2D is associated with insulin resistance, high triglycerides, hyperinsulinemia, hypertension, and micro and macroangiopathies. In fact, almost 10-30% of Indian adults are hypertensive and the country is home to almost 30 million confirmed diabetics. However unlike western counterparts, although Asian Indians exhibit lower BMI and a lean physique are more susceptible to insulin resistance which is attributed to ethinicity, genetic predisposition, abdominal obesity and/or food habits (Gupta et al. 2009, Khoo et al. 2011, Lele, Joshi and Gupte 2006, Lee, Brancati and Yeh 2011, Vasudevan et al. 2011, Wulan, Westerterp and Plasqui 2010). Additionally, Indians are known to develop diabetes a decade earlier than Caucasians.

High-fat and high-fructose diets are usually used to induce animal model of T2D mellitus (Huang et al. 2004). This is most commonly used model for the study of metabolic syndrome (Panchal et al. 2011). As this model is known to resemble most of the characteristics of human T2D we evaluated this model for the study of T2D associated complications.

In this present study we used adult WNIN rats to study the effect of long term HFHF diet on body weight, glucose homeostasis and lipid profile.

MATERIALS AND METHODS:

Male WNIN rats (6-8 weeks old) with an average body weight of 195±21 g (received from the NCLAS) were kept on AIN-93 diet (control group; n=6-9) or AIN-93 diet with 34% fructose with 24% fat (HFHF group, n=6-9); (Table 1) Animals were maintained with their respective diets and water *ad libitum* for a period of ten months in individual cages with a 12 h light-dark cycle. This study was approved by the Institutional Animal Ethics Committee (P29/IAEC/NIN/2012/7/PS/Rats-WNIN/Male-71).

Oral glucose tolerance test (OGTT) and homeostasis model assessment (HOMA) for insulin resistance (IR)

OGTT was performed at three and ten months on overnight fasted rats by administering the glucose solution, at a dose of 2.0 g/kg body wt. Blood samples were collected at 0 min, (before OGTT), 30, 60 and 120 min after OGTT. Plasma glucose and insulin levels were estimated by the glucose oxidase-peroxidase kit (Ozone Biomedicals Pvt. Ltd., New Delhi, India) and RIA kit (BRIT-DAE, Mumbai, India) methods respectively. IGT and HOMA-IR was calculated as reported earlier [Patil et al. 2014].

Fasting and postprandial blood glucose:

Fasting and postprandial blood glucose levels in these experimental animals were monitored every month by glucometer (One Touch Horizon).

Estimation of triglycerides:

Plasma triglyceride was estimated enzymatically by using commercially available kit (Biosytems, Barcelona, Spain) following the instructions of the manufacturer.

Estimation of total cholesterol

Total cholesterol was estimated in the plasma by using commercially available kit (Biosystems, Barcelona, Spain).

Estimation plasma free fatty acids *(FFA)*

Free fatty acids estimated in plasma samples at the end of the experiment using a commercially available kit (BioAssay Systems, Hayward, USA).

Statistical analysis:

In this study, an analysis of variance (ANOVA) test and SPSS software version 19.0 software were used for comparing mean ± standard deviation (SD). Statistical significance was set at $p < 0.05$.

RESULTS:

Effect of HFHF diet on body weight:

Body weight measurement carried out after one month of feeding show that significantly higher in rats fed HFHF diet compared to rats fed AIN-93 diet (Fig-1). Energy balance determination indicates that body energy gain in rats fed HFHF fed diet.

Effect of HFHF diet on OGTT and insulin resistance:

Feeding HFHF diet to WNIN rats for 3 months resulted in the development of IGT (Fig-2). Insulin resistance is associated with PD, as the homeostasis model assessment for insulin resistance (HOMA-IR) indices were significantly ($p < 0.01$) higher in the HFHF groups than the controls (FIG 2C). Further, results from an OGTT conducted before terminating the experiment indicated that HFHF-fed animals maintained a PD state until the end of the experimental period (10 months), as demonstrated by significantly higher HOMA-IR ($p < 0.01$) HFHF rats than in the control rats (FIG-2D).

Effect of HFHF diet on lipid profile:

Rats fed HFHF diet exhibited significantly (0.01) higher plasma triglycerides and significantly (0.05) lowered HDL levels at the end of the experiment but total cholesterol levels marginally reduced and LDL levels slightly higher in HFHF fed rats compared to control.

DISCUSSION:

In this present paper, we find out that long term HFHF diet is able to induce changes in energy utilization and accumulated triglycerides leading to early phase obesity developed in a WNIN rat model. The increased plasma lipids (TG and LDL) are partly due to the lower cost of lipid deposition during high fat feeding (Hariri and Thibault (2010). However, the decrease in net energy found in rats fed HFHF diet suggests that other energy sparing mechanisms take place, with the skeletal muscle as a possible candidate site as it accounts for about 30 % of whole-body energy requirements in rats (Rolfe et al. 1997).

In this study, it was found HFHF led to glucose intolerance in rats. However, after 3 months and end of the experiment HOMA-IR significantly higher in HFHF fed rats compared to control rats. HFHF group rats shown higher plasma glucose concentrations than the control group after 3 months and 10 months of the experimental period. This indicates that HFHF fed diet led to glucose intolerance. However, it has been suggested in several studies that a high-fructose diet can certainly cause changes in message transfer after insulin action, resulting in insulin resistance (Bezerra et al. 2000), although this will not affect the number of receptors of muscle and hepatic insulin. Lavau et al. (Lavau et al. 1979) pointed out in their report that a high-fat diet could lower the activity of the intracellular enzymes associated with fatty acid synthesis and decrease the intracellular capacity to utilize glucose, which in turn resulted in a blunted glucose metabolism response to insulin. Van Amelsvoort et al. (Van Amelsvoort et al. 1986) has suggested that saturated fatty acids can cause decreased affinity of insulin in peripheral tissue.

Many experimental studies, using fasting insulin level as a marker, consistently have shown that subjects with higher fasting insulin levels have higher risks of developing diabetes (Saad et al. 1988; Charles et al. 1991; Haffner et al. 1995). Besides, Insulinogenic index correlates well with early phase insulin response to intravenous glucose tolerance test (Yoneda et al. 1992) and has been used as a parameter of early insulin response in several other studies (Kadowaki et al. 1977; Boyko et al 1996). Our results thus suggest that decreased early response of insulin secretion or b-cell dysfunction may reflect a primary defect of the high-fat diet group. The suggestion based on the results of that high-fat diet can cause more serious impairment of insulin response.

Animals maintained on the HFHF diet exhibited modest but significant hyperglycemia, hyperinsulinemia, and hypertriglyceridemia compared with the control group. The increased synthesis of triacylglycerol results primarily from both increases in the VLDL particle secretion rate by the liver and in VLDL particle size. The mechanisms potentially responsible for the overproduction of VLDL in the insulin-resistant state. They found evidence for enhanced lipoprotein assembly, reduced intracellular apolipoprotein B degradation, and increased expression of

microsomal triacylglycerol transfer protein. Together, these findings help to explain the increased assembly and secretion of apolipoprotein-B-containing lipoprotein particles in a fructose-fed, insulin-resistant animal model. Chronic high fructose exposure seems to indirectly cause hyperinsulinemia and obesity through other mechanisms. One proposed mechanism involves glucose transporter 5 (GLUT5), a fructose transporter that is found to have significantly higher expression levels in young Zucker obese rats compared to lean controls, implying a possible role of GLUT5 receptors in the pathology of metabolic syndrome associated with fructose feeding and insulin resistance. In rats fed 66% fructose for 2 weeks, insulin receptor mRNA, and subsequent insulin receptor numbers in skeletal muscle and liver were significantly lower compared to rats fed a standard chow diet. Insulin resistance has also been correlated with intracellular TG stores, which are involved in lipotoxicity and beta cell failure leading to diabetes.

CONCLUSION:

Feeding a HFHF to WNIN rats for a period of 10 months could impair glucose tolerance but not develop diabetes. Higher levels of fasting insulin and HOMA-IR were observed in the HFHF fed diet fed rats. It is possible HFHF diet may cause a promotion of triglycerides due to high-insulin levels in WNIN rats. The present research useful reference for related studies in the future research.

Conflicts of interest: The authors declare no conflict of interest.

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Table 1: Ingredients and composition of diet

S No.	Ingredient	AIN 93 Control (g/Kg)	HFHF (g/Kg)
1	Fructose	-	320.00
2	Fat (tallow oil)	-	240.00
3	Starch	560.00	-
4	Casein	140.00	140.00
5	Sucrose	100.00	100.00
6	Oil	40.00	40.00
7	Cellulose	50.00	50.00
8	AIN 93 G/M Mineral mix	35.00	35.00
9	AIN 93 G/M Vitamin mix	10.00	10.00
10	DL-Methionine	1.80	1.80
11	Choline chloride	2.50	2.50

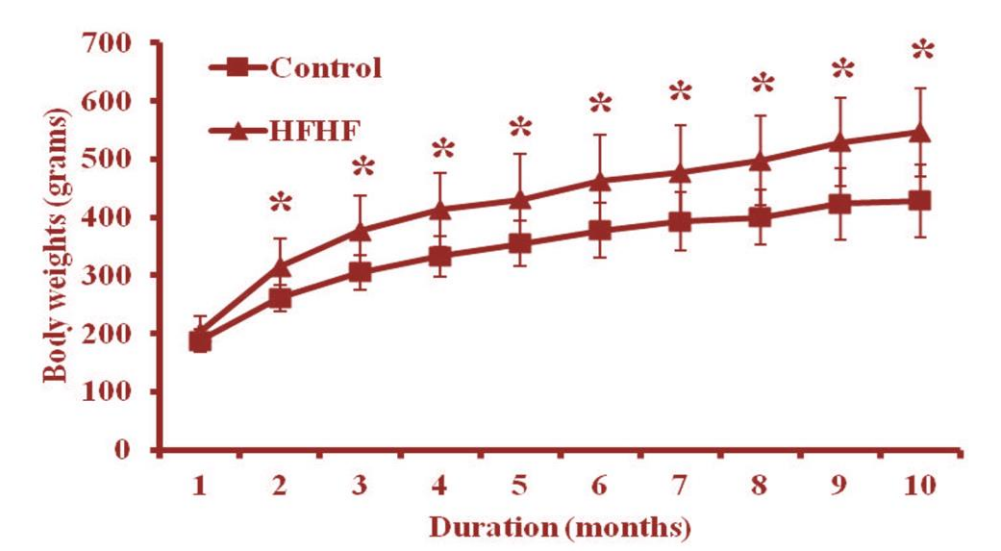

Figure1. Body weights of control and HFHF rats increased from 1months to 10 months Results are mean ± SD . (*, p<0.05vs HFHF).

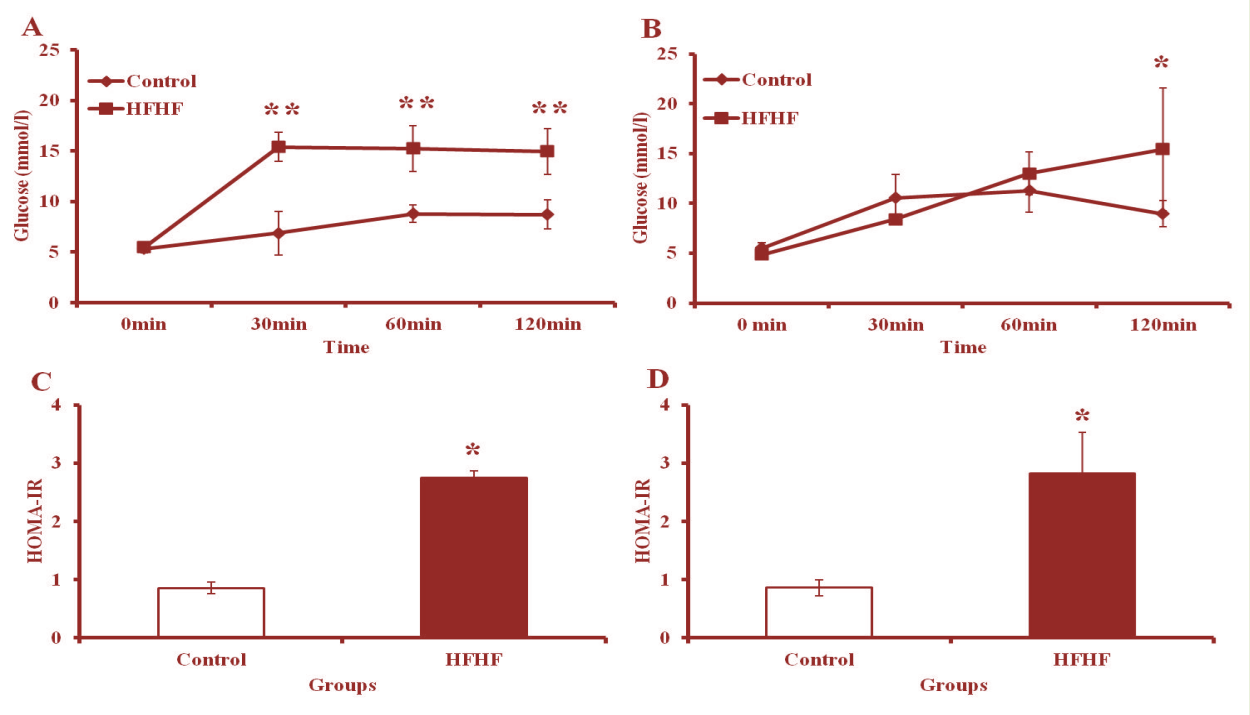

Figure 2. Glucose response during an oral glucose tolerance test (OGTT) at three (A), and ten months (b); HOMA-IR at three (C), and ten months (D) after feeding of their respective diets. Values are mean ± SD, n=69. *p<0.05, vs. control, ** p<0.01, vs. HFHF group. HFHF, high fructose high fat

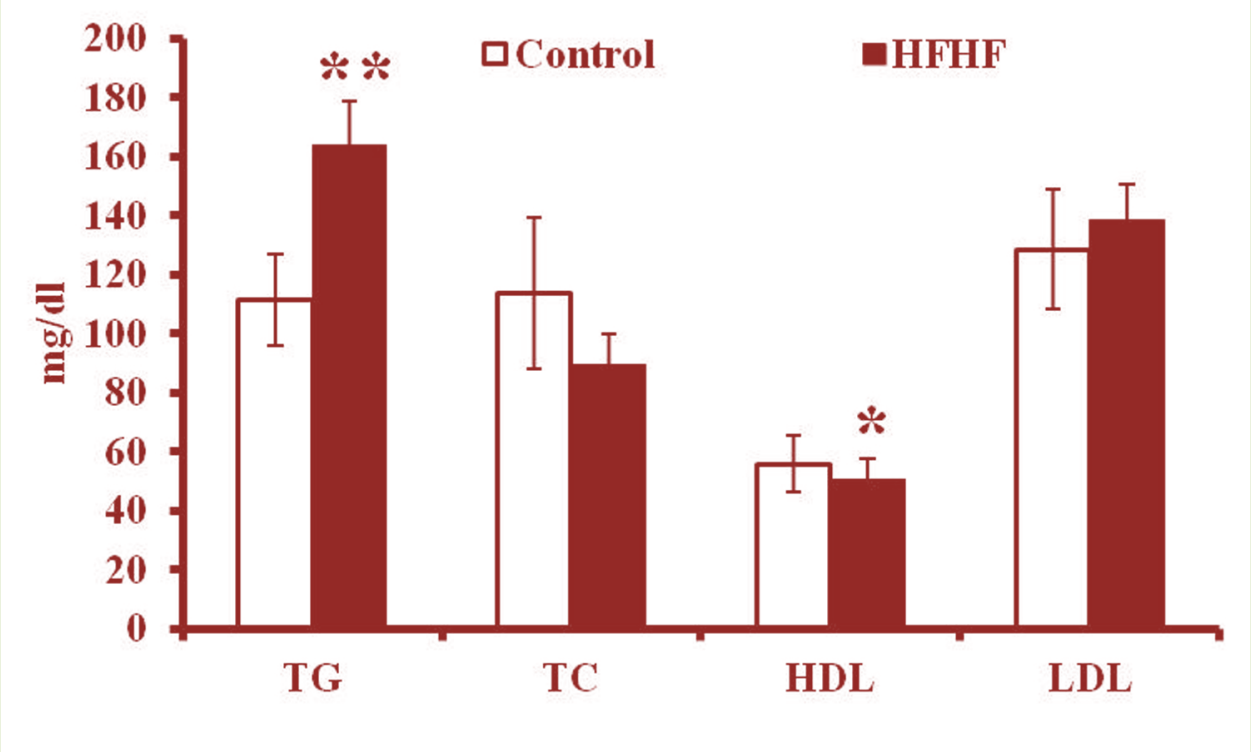

Figure 3. Lipid profiles of control and HFHF fed rats after 10 months of experimental period. Values are mean ± SD, n=6-9. *p<0.05 and ** p<0.01, vs. HFHF group. HFHF, high fructose high fat (TG, triglycerides; TC, total cholesterol; HDL, high density lipo proteions and LDL, lowdensity lipo proteins)