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Effect of Short-Term High Fat Diet Inducing Obesity on Hematological, Some Biochemical Parameters and Testicular Oxidative Stress in Male Rats

Sherif M. Shawky

Department of Physiology, Faculty of Veterinary Medicine, Sadat City University, Egypt

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ABSTRACT

Obesity constitutes a health problem due to its increasing worldwide prevalence. Among the health detriments caused by obesity, reproduction is disrupted. Some studies have shown a relationship between obesity and infertility, but until now it remains controversial. The objective of the current work was to examine the effect of diet-induced obesity on blood parameters, liver and kidney function tests, lipid profile and testicular oxidative stress. For that purpose, Male rats were fed *ad libitum* with a standard diet (control group; n.= 15) and high fat diet (HFD group; n.= 15) for 6 weeks. Hematological parameters, urea, creatinine, albumin were similar between the two groups. Intergroup testosterone levels were also comparable. The high fat diet induced significant increase in serum triglycerides, cholesterol, low density lipoprotein and very low density lipoprotein cholesterol concentrations. This diet also increases significantly alanine aminotransferase and aspartate aminotransferase activities and decreased total protein level and high-density lipoprotein cholesterol concentration. Furthermore, HFD showed a significant increasing in malondialdehyde contents in testes and decreasing in superoxide dismutase activity, the results of this study concluded that short-term high fat diet affect on liver enzymes and causing oxidative stress in testes.

Introduction

Genetic and environmental factors play a role in the development of obesity. Diet is one of the main environmental factors that contribute to obesity. Human studies have shown that increased fat intake is associated with body weight gain which can lead to obesity and other related metabolic diseases. Animal rodent models are useful tools for studying obesity as they will readily gain weight when fed high-fat diets (Buettner *et al*., 2007).

Experimental studies have reported that animals fed a high fat diet (HFD) for more than two months develop weight gain, dyslipidemia, hyperglycemia and oxidative stress (Messier *et al*., 2007; Arango *et al*., 2009). Besides, consumption of a calorie-rich diet results in lipid accumulation, excess production of inflammatory cytokines, and macrophage infiltration that favour the progression of liver disease (Wei *et al*., 2007). As well as, feeding HFD lead to a significant elevation in liver aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) activities and decreased total protein level (Uthandi and Ramasamy, 2011).

Mice fed HFD develop increased levels of oxidized low density lipoproteins, free fatty acids and triglycerides (Vincent and Hinder, 2009).

Obesity is as an important risk factor for male infertility (Du Plessis *et al*., 2010). Mice fed HFD have decreased in sperm motility, fertilization rate and pregnancy rate, as well as increases in sperm DNA damage and sperm intracellular reactive oxygen species have been reported (Ghanayem *et al*., 2010, Bakos *et al*., 2010). However, Tortoriello *et*

*Corresponding author: Sherif M. Shawky
E-mail address: shsh00076@yahoo.com

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al. (2004) found no impairment in the fertility of male mice after they were fed HFD. Sprague-Dawley rats fed HFD from weaning to 90 days had a reduction in testosterone levels (Vigueras-Villaseñor *et al*., 2011), and male mice fed HFD (for 9 weeks) showed a trend toward reduction in testosterone levels compared to the control group (Bakos *et al*., 2010). In addition, obesity increased oxidative stress in reproductive system that defined as stress induced by increased numbers of molecules containing free oxygen. High fat diet increased malondialdehyde (MDA) contents of testes (Galaly *et al*., 2014).

There is no available literature about the effect of fat inducing diet on hematological parameter, as well as relationship between obesity and infertility still controversial and not full studied Therefore, the aim of this study was to determine the effect of high-fat diet induced obesity on hematological, biochemical parameters and testicular oxidative stress in male rat.

Materials and methods

Animal model

Thirty male Wister rats (100-120 g) were used in this experiment. Animals were obtained from Al-Zyade experimental animal production center, Giza, Egypt. Animals were quarantined and allowed to acclimate for a week prior to the experiment. The animals were handled under standard laboratory conditions of a 12-hour light/dark cycle in a temperature and humidity-controlled room. Water and feed were supplied *ad libitum*. All animal-handling procedures were carried out following the regulations of Institutional Animal Ethics Committee and with their prior approval for using the animals.

Experimental protocol

Rats were randomly divided into two groups of fifteen animals each (n.=15). Group 1 served as control and received water and feed *ad libitum.* Group 2 (obese group) rats were fed on high fat diet for 6 weeks.

High fat diet (HFD)

Chemical analysis of HFD pellets and pellets ingredient were performed according to (Khalifa, 2010).

Chemical and ingredient compositions of high fat diet

Ingredient composition	(g, %)
Yellow corn	40.0
Wheat flour	30.0
Powder milk	10.0
Wheat bran	5.0
Corn oil	5.0
Berseem hay	8.5
Vitamins and minerals	1.0
Sodium chloride	0.5
Chemical composition	**(g, %)**
Total protein	11.2
Ether extract	10.6
Ash	5.81

Blood sampling

At the end of the experiment, blood samples collected from retro-orbital puncture under diethyl ether anesthesia. Blood samples were drawn into dry tubes (for obtaining serum) and heparinized tubes (for obtaining whole blood). Serum were separated by centrifugation and stored at -20°C for subsequent analysis

Hematological and Biochemical Investigations

Hematological parameters were determined by standard methods. Hemoglobin concentration was determined by the Cyanomethemoglobin Method (Pilaski, 1972). Packed cell volume (PCV) was determined by microhematocrit method as described by Feldman *et al*. (2000) using microhematocrit centrifuge (TGM12 capillary hematocrit centrifuge). The red cells (RBC) were counted under the high power of microscope (MSK-1 light microscope) by using double improved Neubauer counting chamber (Feldman *et al*., 2000) and white blood cells (WBC) were counted under the low power of microscope by using double improved Neubauer counting chamber (Wintrobe *et al*., 1967).

The activities of serum AST and ALT were estimated according to the method of Young (2001), Total protein was estimated by the method of Young (2001). Urea was estimated by the method of Patton and Croush (1977) and creatinine was estimated by the method of Young (2001) using Dia-

mond Diagnostic Kits obtained from Mid-Egypt Company.

The serum cholesterol concentration was determined according to method of Young (2001). Determination of high-density lipoproteins cholesterol (HDL-cholesterol) level was carried out according to the method of Grove (1979). Serum triglycerides level was determined according to the method of Young (2001) using Spinreact Diagnostic Kits obtained from Mid-Egypt Company.SQ4802 Spectrophotometer (Unico, USA) used for measuring the levels of different parameters.

Low density lipoprotein cholesterol (LDL-cholesterol) level and very low-density lipoprotein cholesterol (VLDL-cholesterol) were calculated as described by Friedewald (1972) as follows:

$$LDLcholestrol(mg/dl) = Totalcholesterol - (\frac{triglyceride}{5} + HDLcholesterol)$$

$$\text{VLDL cholesterol (mg/dl)} = \frac{\text{triglyceride}}{5}$$

The serum testosterone level was assayed by ELISA radio immunoassay (Wilson and Foster, 1992). Using kits of Hellabio biokits Company (USA) and following the manufacturer's instructions. The amount of testosterone was expressed as ng/ml.

Preparation of testicular tissue samples

The testis was removed and quickly excised, minced with ice cold saline, blotted on filter paper and homogenized in phosphate buffer (pH7.4), the supernatant were frozen at -20°C for further determination of antioxidant enzyme activities and MDA level. Tissue homogenate was prepared according to Combs *et al*. (1987).

Lipid Peroxidation and Antioxidant Enzyme

Measurement of testicular Malondialdehyde (MDA) Concentration

Malondialdehyde was determined by the method of Yashkochi and Masters (1979). MDA reacts with thiobarbituric acid (TBA) in an acid medium giving a colored TBA-complex measured colorimetrically at 520-535 nm against blank and MDA values were expressed as n moles MDA/gm tissue protein.

Measurement of testicular Superoxide Dismutase Activity

Superoxide dismutase (SOD) activity was estimated according to Giannopolitis and Ries (1977). The optical absorbance was measured at wave length 560 nm against blank reagent. SOD= Reading (absorbance) of (SOD)/ g tissue protein.

Protein determination

The total protein concentration of supernatant was determined by the method of Young (2001).

Statistical analysis

All data were expressed as mean ± standard error (SE). Paired t-test was used to compare control and fat inducing diet variables. Differences were considered significant when p values were less than 0.05. All analysis was performed using the statistical package (SPSS) version 16.0 (Chicago, IL, USA).

Results

Effect of high fat diet on body weight

The present data showed that there was no significant difference at the beginning of the experiment in initial weight of rats in two groups while the significant difference ($p < 0.05$) present at the end of the experiment in the body weight of HFD group compared to control group (Table 1).

Table 1. Effect of high fat diet on body weight

Parameters	Control Group	Obese group
Initial body weight (g)	116.60±2.66	112.60±3.78
Body weight after 6 weeks (g)	136.80±2.08 [b]	169±4.37 [a]

Different letters in the same raw show significant difference at the level of $p < 0.05$

Effect of high fat diet on hematological parameters

The results of hematological parameters have been summarized in Table 2. HFD rats exhibited no significant changes in Hb, PCV, WBCs and RBCs, in compare with control one.

Table 2. Effect of fat inducing diet on hematological parameters in rats

Parameters	Control Group	Obese group
Hemoglobin (g/dl)	15.64±1.14	16.49±0.30
PCV (%)	40.40±1.69	39.40±0.40
WBCs ($\times 10^3$ mm^3)	7.67±1.05	9.43±0.99
RBCs ($\times 10^6$ mm^3)	7.15±0.64	6.88±0.33

Effect of high fat diet on biochemical parameters

The results of biochemical parameters have been summarized in Tables 3, 4 and 5. High fat diet induced a significant increase ($P < 0.05$) in ALT, AST, cholesterol, triglyceride, LDL-cholesterol and VLDL-cholesterol. On the other hand caused a significant decrease in total protein and HDL-cholesterol and had no effect on albumin, urea and creatinine.

Table 3. Effect of fat inducing diet on Total protein, Albumin, AST and ALT in rats

Parameters	Control group	Obese group
Total protein (g/dl)	5.18±0.23^a	4.02±0.14^b
Albumin (g/dl)	2.77±0.22	2.43±0.09
AST (U/L)	114.30±2.86^b	167.02±15.3^a
ALT (U/L)	53.40±6.43^b	73.87±4.56^a

Different letters in the same raw show significant difference at the level of $p < 0.05$

Table 4. Effect of fat inducing diet on urea and creatinine in rats

Parameters	Control group	Obese group
Urea (mg/dl)	40.35±01.93	46.83±2.30
Creatinine (mg/dl)	0.73±0.07	0.53±0.03

Table 5. Effect of fat inducing diet on triglyceride, total cholesterol, HDL cholesterol, LDL cholesterol and VLDL cholesterol

Parameters	Control Group	Obese group
TG (mg/dl)	46.40±2.73^b	91.80±5.31^a
CHO (mg/dl)	77.60±2.50^b	94.80±3.54^a
HDL (mg/dl)	49.20±2.65^a	35.4±1.78^b
LDL (mg/dl)	19.12±4.45^b	41.04±2.85^a
VLDL (mg/dl)	9.28±0.55^b	18.36±1.06^a

Different letters in the same raw show significant difference at the level of $p < 0.05$

Effect of high fat diet on testosterone hormone and testicular oxidative markers

Data in Table 6 showed that there is no significant difference in serum testosterone levels between HFD animals and control animals while there is a significance increase in MDA level and significance decrease SOD in HFD group in compare with control one.

Table 6. Effect of fat inducing diet on serum testosterone, malondialdehyde and superoxide dismutase in testicular tissue in rats

Parameters	Control group	Obese group
Testosterone (ng/ml)	3.30±1.03	2.57±1.16
MDA (nmol/g tissue protein)	1.82±0.06^b	3.04±0.19^a
SOD (IU/g tissue protein)	46.33±1.0^a	33.33±2.0^b

Different letters in the same raw show significant difference at the level of $p < 0.05$

Discussion

The high-fat diet used in the present study was effective in promoting obesity, as demonstrated by a significant increased in body weight. Fat deposits, adiposity index Body weight, and serum leptin increased significantly in animals feeding with high fat diet (Fernandez *et al*., 2011).

The obtained data revealed that there was no significant difference in the blood picture of high fat fed rats in compare with control group this may be attributed to the change in hematological parameters as a result of high fat diet may need long term exposure not short term, no available literatures about the effect of high fat diet on blood picture, however this point need further investigation.

In the present investigation, the increased levels of ALT and AST have been observed in serum of high-fat fed rat compared to control groups (Table 3) indicating the harmful effect of HFD on the liver. An elevation in the levels of serum marker enzymes is generally regarded as one of the most sensitive index of the hepatic damage (Kapil *et al*., 1995). Observed elevated levels of ALT and AST in serum of HFD fed rat indicates that the elevation might be due to hepatocellular damage caused by HFD toxicity. The findings of the present study match the result of Uthandi and Ramasamy (2011) who reported that HFD caused a significant increase in the serum levels of AST, ALT and ALP. Similarly, Recknagel (1987) reported that the hepatic damage induced in HFD fed animal resulted

in elevation in the level of AST and ALT in the blood. Also, in the present study, serum protein levels were decreased in HFD fed rats. The depletion in the protein levels might be due to localized damage in the endoplasmic reticulum or hazard effect of energy which librate through the metabolism of HFD (Uthandi and Ramasamy, 2011).

The obtained data revealed that increased serum total cholesterol, triglyceride, LDL-cholesterol and VLDL-cholesterol in high fat diet group (Table 5). The results were in agreement with that obtained by Suliman (2008) who stated that HFD increase the level of cholesterol. The increase level of cholesterol might be due to excessive loads of cholesterol on the liver resulting to down regulation of LDL receptors which carry cholesterol lead to cholesterol being re-circulated in the blood (Mustad *et al*., 1997). Mice fed HFD develop increased levels of triglycerides, oxidized low density lipoproteins, free fatty acids and VLDL-cholesterol (Vincent *et al*., 2009)

Obesity is an important cause of adverse health outcomes, including male infertility (Hammoud *et al*., 2006). In the present study there is no significance difference in testosterone hormone levels between HFD group and control one. Contradictory to the results in the present study Vigueras-Villaseñor *et al*. (2011) reported that Sprague-Dawley rats fed HFD from weaning to 90 days had a reduction in testosterone levels. Male mice fed HFD (for 9 weeks) showed a trend toward reduction in testosterone levels compared to the control group (Bakos *et al*., 2010). In this study the adiposity gain seen in the animals was not sufficient to produce a significant diminution in the testosterone levels, perhaps because the obesity installed was not severe, rats exposed to relatively short-term high fat diet inducing obesity.

On the other hand HFD exhibited a significant increase ($P<.05$) in testes MDA and significant decrease in SOD activity These findings are in accordance with those of Galaly *et al*. (2014) who stated that male rats fed with a high fat diet had significantly higher levels of MDA and lower levels of GSH and SOD compared with the control diet male rats. Obesity may induce oxidative stress and increase testicular oxidative stress.

Conclusion

It should be stated that short-term high fat diet inducing obesity has hazard effects on liver function tests, lipid profile and induced testicular oxidative stress in male rats, no alteration occur in hematological parameters and kidney function test.

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