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Lipid Profile and Some Hormonal Disorders in Serum of High-Fat Diet Fed Rats

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Abstract

Background: Chronic consumption of high-fat diet (HFD) induces obesity. The purpose of the current study was to evaluate the impact of high-fat diet-induced obesity on lipid profile and levels of certain hormones in male albino rats.

Material and Methods: A total of forty two 12-week old male albino rats were divided into three groups: control group fed a normal diet, obese group I fed 25% HFD and obese group II fed 50% HFD. Each group was divided into two subgroups (seven rats for each) feeding on the corresponding diet for four and eight weeks. Serum total cholesterol (TC), triglycerides (TG), high density lipoprotein cholesterol (HDL-Chol), low density lipoprotein cholesterol (LDL-Chol), free Triiodothyronine (FT_3), free Thyroxine (FT_4), Insulin, Leptin and Adiponectin were assessed at the end of 4^{th} and 8^{th} weeks. Also LDL/HDL-cholesterol ratios of control and obese groups were calculated at the same time intervals.

Results: The obtained results indicated significant increase in all parameters of serum lipid profiles in addition to elevation of the LDL/HDL-cholesterol ratios of obese rats compared to those of the controls. In response to HFD, marked increase was recorded in the levels of insulin and leptin while values of FT_3 and adiponectin were reduced significantly. On the other hand, HFD did not change the levels of FT_4. Most of the recorded changes were more obvious by increasing either the percentage of fats or the feeding period.

Conclusion: In conclusion, HFD induces some hormonal disorders accompanied by disturbance of the lipid profile.

Keywords: High-fat diet; lipid profile; hormones; LDL/HDL ratio; rats.

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Introduction:

The rise in obesity rates at the end of the twentieth and the beginning of the twenty-first centuries remains one of the major public health issues in the developed world today **(1).** Recently fast-food consumption has received considerable attention in terms of its contribution to the rising prevalence of obesity. Fast-food consumption in particular has been associated with poor diet quality and adverse dietary factors related to obesity, including higher intakes of calories, fat, saturated fat, and sugar-sweetened drinks **(2&3).**

High-fat diets are known to lead to a positive fat balance and consequently to adipose mass accumulation **(4&5).** The increase in adipose tissue causes the clinical problems associated with obesity, due to either the weight of the extra fat mass or to the increased secretion of free fatty acids, many peptides, and other adipokines by hypertrophic adipocytes **(6).** Obesity is characterized by hyperlipidemia, hyperleptinemia and resistance of hypothalamic satiety center to anorectic effect of adipose tissue hormones **(7, 8&9).** Moreover, Obesity is associated with significant adverse effects on health including metabolic, endocrinologic and cardiovascular complications **(10, 11&12).**

Numerous studies have been done to seek the effect of dietary fat on hepatic lipid homeostasis. **Fungwe *et al.* (13&14)** investigated the effect of cholesterol on the accumulation of liver lipids and proposed that hepatic triglycerides accumulation was developed by the enhancement of hepatic triglycerides synthesis and the reduction of

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fatty acid beta-oxidation. **Liu *et al.* (15)** suggested the roles of increased lipogenesis, decreased oxidation of fatty acids and decreased secretion of VLDL as causes for the accumulation of triglycerides in the liver in the cholesterol-fed rats. **Xu *et al.* (16)** also reported that the impaired hepatic lipid homeostasis because of lipid accumulation attributed to the increasing activity of the enzymes involved in fatty acid biosynthesis by the dietary cholesterol.

Thyroid function was evaluated by measuring the levels of serum FT_3 and FT_4. The significance of thyroid hormones arises from their known reciprocal relationship to the total cholesterol and lipids in blood **(17).** According to **Iglesias *et al.* (18),** hyperthyroid patients showed a decrease in body weight associated with a significant lowering in the level of serum cholesterol**,** while patients with hypothyroidism showed elevated cholesterol and triglycerides levels when compared to normal subjects.

Insulin is an important regulator of energy homeostasis. It stimulates glucose, free fatty acids and amino acids uptake by tissues. Also, insulin plays an effective role in regulation of leptin gene expression since some studies have shown that hyperinsulinaemia increased plasma leptin concentration and gene expression **(19).**

Leptin, a hormone released from fat tissue, participates in mediating appetite and fuel utilization **(20).** Plasma leptin concentrations increased in animals with dietary-induced obesity and in vast majority of obese humans **(21&22).** Moreover, many hormonal factors and nutritional manipulations other than the extent of adiposity are known to affect circulating leptin values **(23).**

The main target tissue and the precise mechanism of adiponectin action are not fully understood. Negative correlation between obesity and circulating adiponectin has been well accepted **(24).** Studies of a possible relationship between adiponectin and lipid metabolism changes associated with thyroid dysfunction are scarce **(18, 25&26).**

The main purpose of the current study was to investigate the effect of diet-induced obesity on lipid profile and levels of certain hormones in male albino rats.

Material and Methods

Animals and dietary protocol

Forty two male albino rats (12-week old) were employed in this study. The rats were kept in controlled environmental conditions (22±2°C; 55±5% relative humidity and 12h light/dark cycle) and fed on a standard diet according to National Research Council **(27)** and water ad libitum. After one week of acclimatization, the rats were randomly divided into three groups (14 rats for each). The first group was fed a standard laboratory diet for four and eight weeks (7 rats in each interval) and served as control animal subgroups. The remaining groups were made obese by feeding them 25% fat diet (obese group I) and 50% fat diet (obese group II) for the same time intervals. Table 1 shows composition of the experimental diets based on the AIN-93 G diet **(28)** with slight modifications

Table 1. Experimental diet compositions (%).

Component	Control group	Obese group I	Obese group II
Starch	51.55	31.55	11.55
Soya bean	18.15	18.15	18.15
Sucrose	17.93	12.93	7.93
Corn oil	5.15	5.15	5.15
Cellulose	5.15	5.15	5.15
Vitamin and mineral mix*	2.07	2.07	2.07
Lard	0	25.00	50.00

* AIN-93 G Vitamin and mineral mixture.

Serum biochemical measurements

At the end of the experimental periods, rats were fasted overnight (12h). They were then anaesthetized with diethyl ether and blood samples were collected from the abdominal inferior vena cava. Serum was obtained by blood centrifugation at 4000 rpm for 10 min at 4°C and immediately stored at -80°C for further analyses. Serum TC, TG and HDL-Chol were determined by enzymatic colorimetric methods using commercial kits from Boehringer Mannheim (Mannheim, Germany), while LDL-Chol was calculated using Friedewald's equation **(29),** which is (in mg/dL):

[LDL-chol] = [TC]-[HDL-chol]-[TG/5].

Insulin level was measured by an enzyme immunoassay kit (SPI-Bio société de pharmacologie et d'lmmunoloie-Bio, France) while radioimmunoassay kits (Institute of Isotopes, Budapest, Hungry) were used to estimate levels of FT_3 and FT_4. Leptin level was measured by using Rat leptin assay kit (IBL, Gumma, Japan) and Adiponectin concentration was estimated by ELISA with a commercially available kit from APLCO Diagnostics (Salem, NH).

Statistical analysis:

Statistical analysis was carried out by a computer program (Costate) using ANOVA two ways analysis followed by Duncan's multiple range tests **(30).**

Results

The effects of high-fat feeding on the lipid profile of male albino rats are shown in figure 1. The sera of HF supplemented groups had significantly ($p<0.05$) high levels of TC, TG, HDL-chol and LDL-chol relative to the normal control rats. The recorded elevation in all indices of lipid profiles exhibited dose and time-dependent manner.

As represented in table 2, LDL/HDL-chol ratios were increased in response to feeding of rats on HFD. Obese group I showed an increase in the LDL/HDL-chol ratios by 3.1% and 5.3% after consumption of HFD over 4 wk and 8 wk respectively, while obese group II showed an elevation by 11.5% and 14.9% at the same time intervals.

The serum FT_4 level was unaffected by the HF intake either the low or high dose and for the two time intervals (fig. 2-B). Regarding FT_3 level, HF supplementation induced marked reduction ($p<0.05$) in this parameter compared to the rats fed normal diet (fig. 2-A). This decline became more obviously either by increasing the concentration of fats or the feeding period. The level of serum insulin did not change significantly subsequent to supplying of rats with 25% HFD for 4 weeks but the same parameter showed marked elevation ($p<0.05$) after 50% HF supplementation for the same time interval (fig. 2-C). In addition, greater increase in the values of serum insulin was recorded at 8 weeks than 4 weeks in both HFD groups. As shown in fig. 2-D, levels of leptin increased markedly ($p<0.05$) in both HFD groups after 4 and 8 weeks in comparison with the corresponding controls. On contract, adiponectin decreased in a significant manner ($p<0.05$) in response to HF supplementation. This decline became more obvious either by increasing the concentration of fats or the feeding period (fig. 2-E).

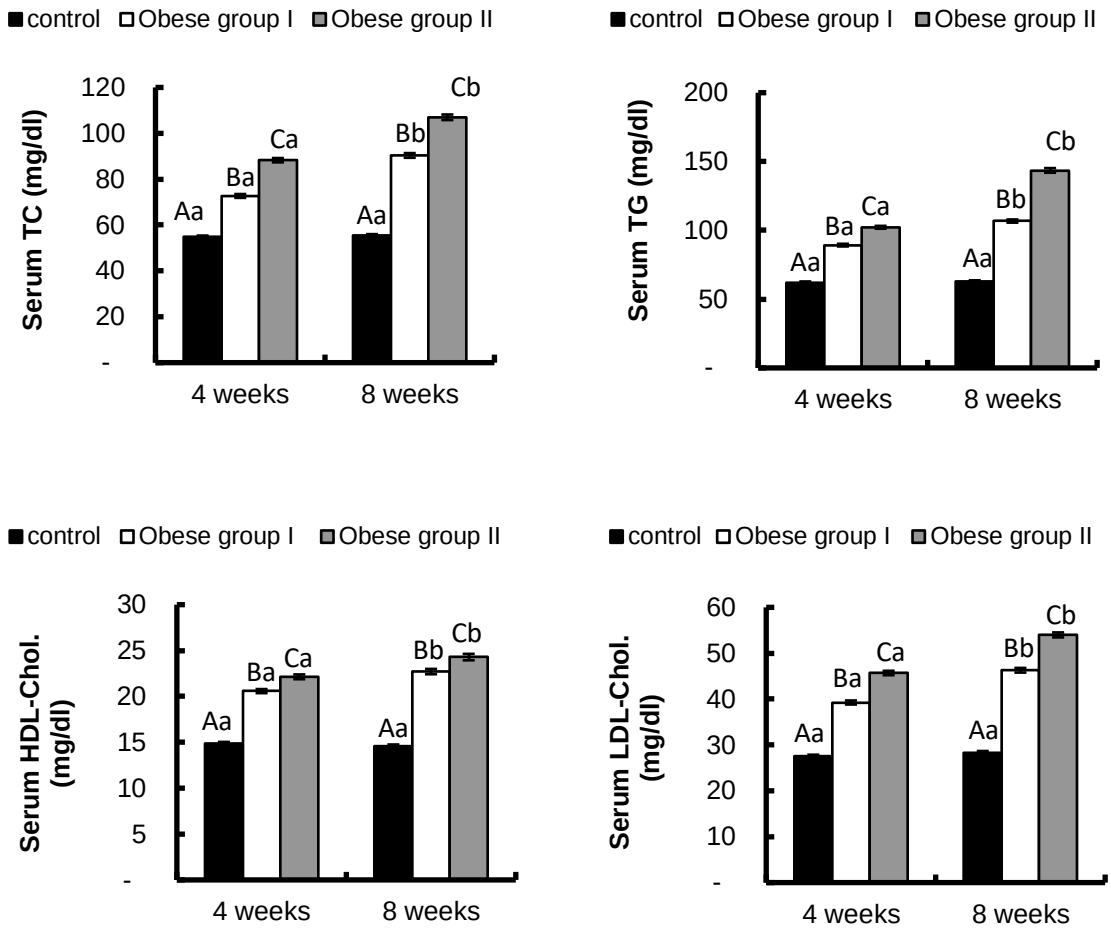

Fig.1: Impact of HFD on serum lipid profiles. The results are expressed as means±SE of 7 rats in each group. Values bearing different superscripts (A, B, C) within the same time interval are different significantly (P<0.05). Values bearing different superscripts (a, b) between the different time intervals are different significantly (P<0.05).

Table 2. LDL/HDL-chol ratio**s.**

	Control group	Obese group I	Obese group II
4 weeks	1.85	1.91	2.06
8 weeks	1.93	2.04	2.22

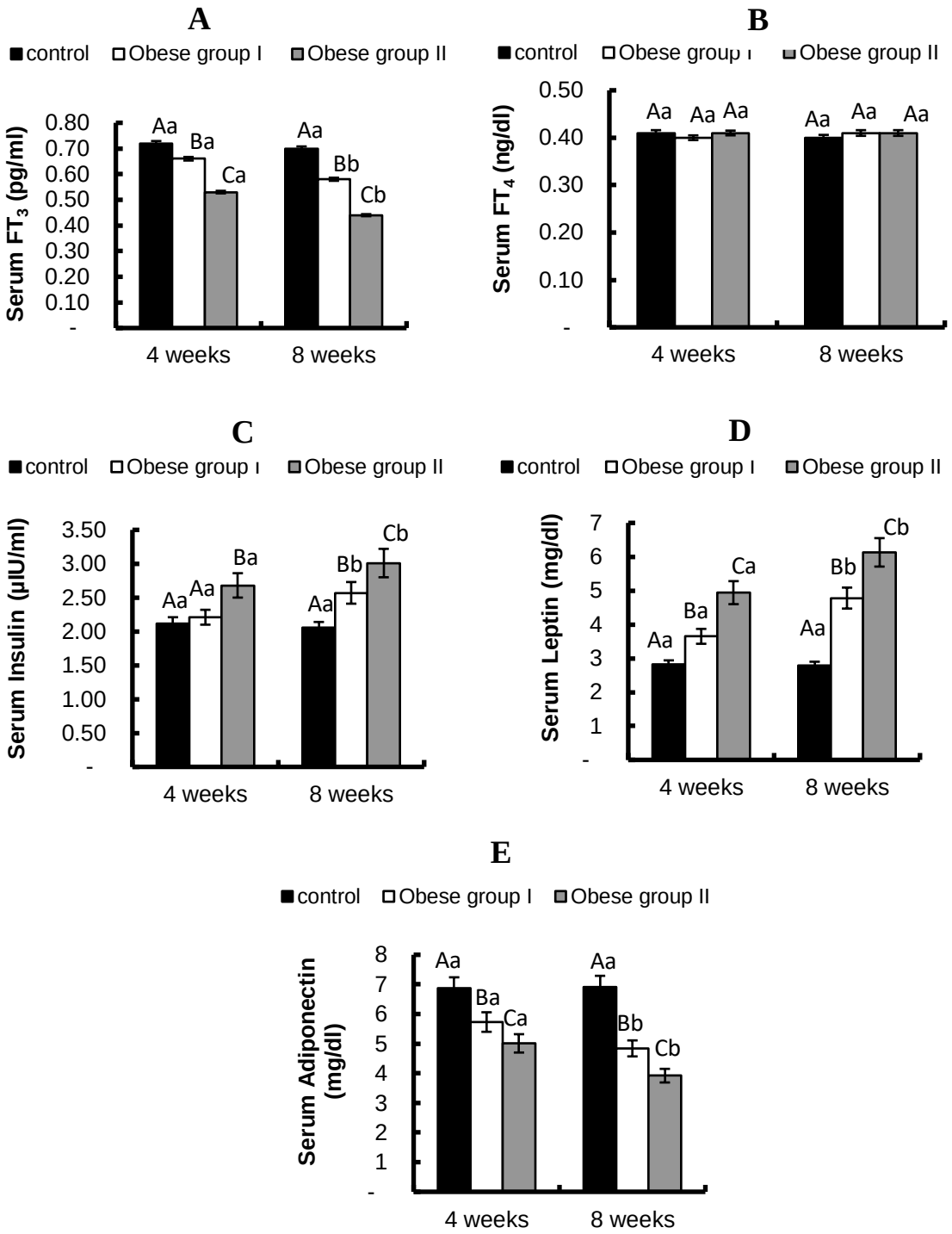

Fig.2: Impact of HFD on the level of certain hormones. The results are expressed as means±SE (n=7). Values bearing different superscripts (A, B, C) within the same time interval are different significantly ($P<0.05$). Values bearing different superscripts (a, b) between the different time intervals are different significantly ($P<0.05$).

Discussion

The prevalence of obesity has increased dramatically over recent decades, such that World Health Organization reported overweight and obesity to be worldwide and epidemic **(31).** It is a heterogeneous complex disorder with multiple etiologies characterized by excess body fat that threatens physical and mental health **(32).** Studying different responses to development of obesity has become a critical research field **(33).** The current study evaluates the impact of HFD, for short and long periods of time,

on indices of lipid profile and certain hormones of male albino rats.

Our data revealed hyperlipidemia marked by elevation of the levels of the measured lipid parameters in obese rats compared to controls. The association between obesity and hyperlipidemia is well established by many researchers **(34, 35&36).** Also, our results are consistent with previous work by **Pownall *et al.* (37)** and **Grundy and Vega (38)** who found that diets containing a high proportion of saturated fatty acids elevate plasma concentrations of TC and LDL-chol. Hyperlipidemia is a common disorder of lipid metabolism and it is the major cause for the manifestation and development of atherosclerosis and coronary heart diseases **(39).** According to **Momiyama *et al.* (40),** the LDL/HDL-chol ratio has become recognized as a stronger risk predictor of cardiovascular diseases than each lipid parameter. The current study revealed that LDL/HDL-chol ratios rose in response to feeding of rats on HFD. Moreover, it is well known that hyperlipidemia induced by HFD and high cholesterol diet may be responsible for development of hepatic steatosis **(41&42).**

Circulating thyroid hormones (FT_3 and FT_4) have been indicated to have a permissive role in adaptive thermogenesis by influencing several aspects of energy metabolism, such as substrate cycling, ion cycling and mitochondrial proton leaks **(43, 44&45).** Therefore, it is believed that thyroid disorders might contribute to the pathogenesis of obesity. In the present study, the reciprocal relationship between the concentration of thyroid hormones (FT_3 and FT_4) in serum and hyperlipidemia (induced by feeding fat) is evident and depends on the percent of fat content (Fig. 2). These results seemed to be in complete accordance with earlier studies made by **Long *et al.* (46) and Heibashy *et al.* (35).**

Fig. (2) shows a significane decrease in the level of FT_3 while only a numerical change occurred in the level of FT_4. These results may be due to disturbance in hypothalamus-pituitary axis, the conversion of FT_3 to FT_4 or/and conversion of reverse FT_3 to FT_4 as a result of feeding rats on HFD causing hypothyroidism. This result confirmed the statement said by **Liu *et al.* (22)** that "FT_3 is considered to be the major biologic mediator of the thyroid function test".

The present study revealed that serum insulin level increased significantly in the obese rats as compared with the corresponding controls. This finding is in accordance with that of **Kahn *et al.* (3); Van Guilder *et al.* (47) and Abdel-Nabi *et al.* (48).** Chronic excess energy consumption has been shown to contribute to hyperinsulinemia and insulin resistance. The liver plays a critical role in energy metabolism and is a major insulin target organ responsible for glucose homeostasis **(49).** Inside the liver, insulin acts through a complex signaling network and functions as an important regulator of lipid and carbohydrate homeostasis. Deficiency in insulin signaling may cause insulin resistance and subsequently lead to systemic insulin resistance and type 2 diabetes mellitus **(50&51).** Selective hepatic insulin resistance, a hallmark of obesity, is manifested by a failure to inhibit gluconeogenesis, but with continued lipogenesis in response to insulin **(52).** Hyperlipidemia that was recorded in the present study may be attributed to the hepatic insulin resistance induced by HFD. This interpretation is supported by **Laplante and Sabatini (53).** Several mechanisms could explain how obesity, especially visceral adiposity, leads to insulin resistance. For example, free fatty acids (FFA) released from fat deposits, especially visceral fat, can block the insulin signal pathways directly and thus interrupt insulin action, as well as insulin secretion **(54).** Moreover, increased amounts of FFA in the portal circulation may impair the metabolism and action of insulin and increase gluconeogenesis in the liver **(55).**

Leptin is produced mainly by adipose tissue. It is also synthesized in small amounts in several other sites like stomach, heart, placenta, mammary gland and ovarian follicles **(56).** It plays an important role in energy balance by inhibiting energy intake and by regulating body weight and energy homeostasis. Our results revealed that serum leptin level is greatly elevated in obese rats compared to the controls. This elevation was positively correlated with both the dose of fat supplementation and time of administration. This result is in agreement with that of **Venner *et al.* (57); Antunes *et al.* (58) and Abdel-Nabi *et al.* (48).** This finding indicated

that obesity is a state of leptin resistance. The mechanisms of leptin resistance are unknown, but may result from defects in leptin signal or transport through the blood-brain barrier **(59)** or could be due to receptor defects, post-receptor defects or disruption of any of the integrative neuronal circuits necessary for leptin action **(60).**

Adiponectin is an endocrine factor produced by adipocytes and is involved in regulation of energy metabolism. Clinical importance of adiponectin comes from its insulin-sensitizing, anti-inflamatory and anti-atherogenic properties. High adiponectin levels are inversely correlated with obesity, insulin resistance, risk for development of type II diabetes, dyslipidemia and cardiovascular diseases (**61&62).** In consistence with previous studies **(63, 64&65),** the current study revealed significant reduction in the level of adiponectin in obese rats. The factors contributing to lower the levels of adiponectin in obese cases are not clear.

On the basis of the present findings, we can conclude that high-fat diet-induced obesity could induce a disorder in the levels of vital hormones as well as disturbance of the lipid profile. So we should avoid the high fat food especially the fast meals that contain large amounts of saturated, trans and polyunsaturated fats and cholesterol.

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