

Effect of exogenous leptin on serum levels of lipids, glucose, renal and hepatic variables in both genders of obese and streptozotocin-induced diabetic rats

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ABSTRACT

Objective(s): Leptin exerts various effects on appetite and body weight. Disruption of the obesity gene is precedent to fatness. Insulin or glucose elevates leptin, but streptozotocin reduces it. However, controversial data exist for the effects of leptin on diabetes and leptin level in each gender. Leptin can damage the kidney function but little evidence exists for its hepatic effects. The aim of this study was to investigate the probable sex-dependent differences in blood sugar levels, lipid profile, and renal and hepatic biochemical factors in the obesity and streptozotocin-induced diabetic rats after leptin administration.

Materials and Methods: Wistar rats of both sexes were randomly divided into two groups, namely obese and diabetic rats. Each group was further divided into male and female subgroups. Extra fat and carbohydrate was added to the diet to induce obesity. Furthermore, streptozotocin (55 mg/kg, IP) was injected to induce diabetes. The treatment groups received leptin (0.1 mg/kg SC) for 10 days, and then, blood samples were taken from the orbital sinus for laboratory evaluations.

Results: Leptin resulted in a significant weight loss in both sexes ($P < 0.001$), food intake reduction in male rats ($P < 0.05$), LDL reduction in female rats (obese ($P < 0.05$) and diabetic ($P < 0.001$)), and glucose level decline in the female diabetic rats ($P < 0.001$). However, total protein concentration, LFT (liver function tests), urea and creatinin concentrations among different groups did not show any significant changes.

Conclusion: Leptin caused some discrepant results, especially regarding the LDL and glucose levels in diabetic female rats.

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Introduction

The obesity gene (*lep* or *ob*) produces leptin with a 167 amino acid sequence (1). The leptin gene is expressed in a variety of tissues, including the skeletal muscle, placenta, mammary gland, mucosa of the gastric fundus, hypothalamus, hippocampus, cerebral cortex, and the cerebellum (2). Various influencing factors including glucocorticoids, insulin and hypoxia modulate leptin gene expression in murine models. The expression of *lep* gene can be decreased by fasting and increased by refeeding. Adipocytes produce and secrete leptin and the metabolic status or the mass of the adipose tissue regulates its concentration (3). For example, insulin or glucose treatment increases the leptin level and insulin deficiency, induced by streptozotocin (STZ), reduces it (1, 4, 5). Reduction of

leptin level during energy restriction is also linked to alterations of insulin and glucose. In condition of energy restriction, the leptin concentration can be a good predictor (6). Leptin has profound effects on appetite and body weight and plasma leptin concentration enhances with increasing fat mass (6).

Some of the consequences of disruption of the *ob* gene, which inhibits leptin production are early onset obesity and overweight (7), and it is obvious that obesity increases the risk of diabetes mellitus type 2 (6). The *ob/ob* mouse is diabetic and insulin resistant. Furthermore, loss of body fat, low levels of leptin, insulin resistance, diabetes mellitus and proteinuria are clinical features in patients and rodents with lipotrophy (7, 8). Also, low leptin concentration has been suggested to increase the risk of diabetic renal

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disease (9). There are some clinical conditions associated with leptin deficiency, including hereditary leptin deficiency, lipodystrophy and weight loss (10).

Furthermore, membranoproliferative glomerulonephritis (MPGN) type 2 has been more frequently reported in a partial form of lipodystrophy (8) while in generalized lipodystrophic patients with proteinuria and diabetes, no diabetic nephropathy was observed (8).

It is known that many obese persons have high plasma leptin concentrations, and men with insulin resistance have even higher leptin level as compared to sensitive ones (7). However, when sex is taken into consideration, women show a higher serum concentration of leptin (11). Leptin levels in women are associated with insulinemia, independent of fat mass. Besides, comparison between the leptin level and the blood lipids in humans did not show a correlation in either sex (12,13) but Murer *et al* (14) have reported a correlation in their study. Although leptin regulates appetite and metabolism (15), data about the effect of leptin on serum glucose, insulin and body weight in the literature is contradictory (7). For instance, based on a human study, when leptin was decreased more, the insulin sensitivity and lipid profile were improved more; however, in another study the insulin sensitivity and lipid profiles recovered with leptin treatment (10). Because of controversial data on the effects of leptin in diabetes (7) and lipid concentration as well as its level in each sex, this study was conducted to investigate the leptin role on some blood metabolic factors, body weight, and potential diabetic nephropathy as well as hepatic enzyme alterations in the obese and diabetic rats in both sexes.

Materials and Methods

This study was approved by considering the guidelines of care and use of laboratory animals, 8th edition. Wistar rats of both sexes were purchased from the Faculty of Medicine, Animal house. The animals were maintained at 25 °C in a room with a 12/12 light/dark cycle and had free access to water and food. High fat and carbohydrate diet (40% fat and 10% sugar) was added to the daily diet in the obese group for 6 months (16); the energy percentage of food was calculated as follows: fat= 9.3 kcal/g, carbohydrate= 4.2 kcal/g and protein=4.3 kcal/g (17). The composition of diet is summarized in Table 1. A single intraperitoneal dose of streptozotocin 55 mg/kg (STZ) (Sigma Aldrich, Germany) was administered to induce diabetes as described previously (18). After 72 hr, diabetes was confirmed from a tail blood sample by a glucometer (Clever Chek, Taiwan). The inclusion criteria were a blood glucose level more than 250 mg/dl and a body weight of 400 g for male and 320 g for female rats. The

exclusion criteria were a blood glucose level lower than 250 mg/dl, a body weight below 350 g for male rats and below 260 g for female rats and severely ill rats.

Each major group had four subdivisions as summarized in Table 2. In diabetic group, mortality reduced the number to six. All rats were weighed at the beginning of the study. Food intake and weight of the obese rats were measured daily. The blood samples were taken from the orbital sinus on days zero and 14 and centrifuged at 2500 RPM (round per minute) for fifteen min. Then, serum samples were kept at -70 °C. Treated rats took leptin (rat leptin recombinant protein, Cell GS,UK), at a dose of 0.1 mg/kg (19) subcutaneously (SC) for ten days. The control animals received the same volume of distilled water. The site of injection was changed to alleviate the injection pain. At the end of the experiment, rats were deeply anesthetized with ether and sacrificed. The leptin level was measured by the leptin mouse/rat ELISA kit (Bio Vendor, Czech Republic, catalogue number RD291001200R) according to manufacturer's instruction and the enzymatic kits (Pars Azmun, Iran) were used to measure HDL (high density lipoprotein), LDL (low density lipoprotein), cholesterol, triglyceride, liver function tests (LFT) and glucose levels by a photometer (Convergys 100, Germany). The data were analyzed by the Graph pad Prism 5 and a $P < 0.05$ was considered significant. The repeated measures of ANOVA and the unpaired t-test were used if the distribution of data is Gaussian.

Table 1. Rodent high fat diet ingredients

Supplement materials for mouse food (500 Kg)	Amount (Kg)
Soy bean	100
Rapeseed	1
Cotton seed	3.5
Wheat bran	50
alfalfa	0
Barely	10
Corn	14
Wheat	60
Fish powder	22.5
Molasses	20
salt	1
Methionine	1.5
Lysine	1.5
Phosphate	2.5
Immunovet	0.25
Rovabio	0.25
Oil	5
fat	167
Sugar	35
Meat vitaminized supplement	2.5
Meat mineralized supplement	2.5

Table 2. Study groups

Obese				Diabetes			
Male		Female		Male		Female	
Control	Leptin	Control	Leptin	Control	Leptin	Control	Leptin
N=5	N=7	N=5	N=7	N=5	N=6	N=5	N=6

Results

The data are presented as mean±SEM. As illustrated in Figure 1, in spite of an enhancement in leptin levels in leptin treated groups; no statistically significant difference was noticed between male and female rats, male and control rats and female and control rats in the obese groups as well as the diabetic female rats. However, the leptin level in the diabetic male group illustrated significant increase as compared to the control group ($P<0.01$). Furthermore, a lower level of leptin was observed in the female groups, which was not statistically significant.

Using one way repeated measures of ANOVA, there was a significant difference of weight over time among different groups, $P<0.0001$ (Figure 2). The weight reduction in females was earlier than male rats.

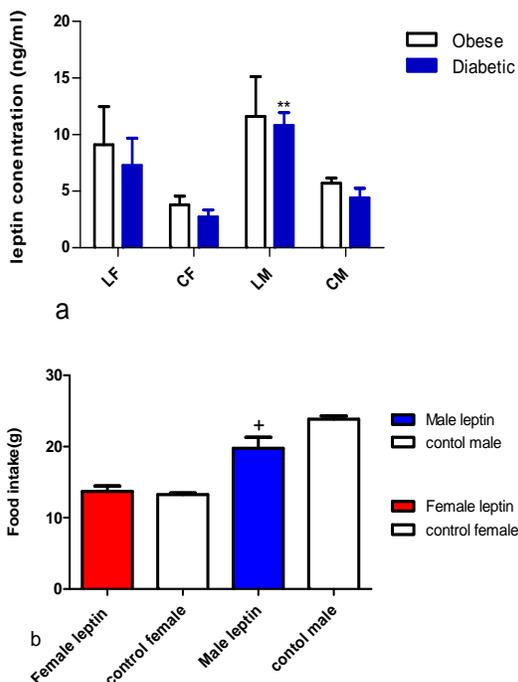


Figure 1. (a).The leptin level in obese and diabetic groups. Data analysis was done by unpaired t-test. ** $P<0.01$. LF=Leptin female group, CF=Control female group, LM=Leptin male group, CM=Control male group. (b) Comparison of food intake in the obese group during 10 days of leptin treatment. Data was analyzed by the unpaired t-test with Welch’s correct. $P<0.05$ was considered significant. + $P<0.05$

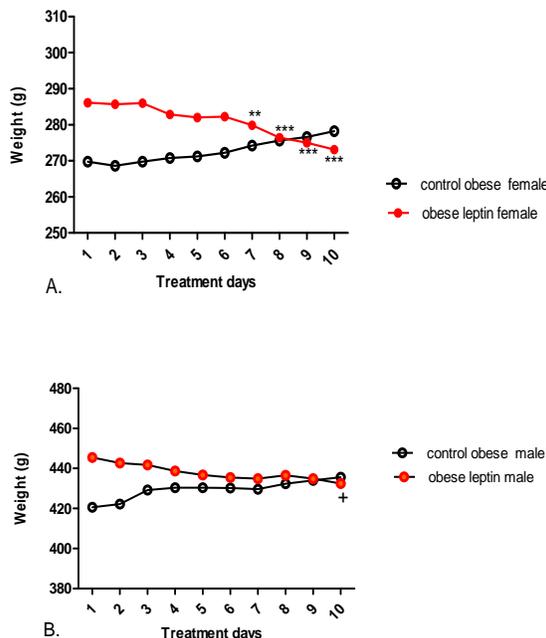


Figure 2. Weight loss in the obese groups during 10 days of leptin administration. Comparison of differences using Bonferroni test revealed significant differences especially in female leptin treated group. A.** $p<0.01$ day 1 vs. day 7, *** $p<0.001$ day 1 vs. days 8, 9 and 10 in female groups. B. + $p<0.05$ day 1 vs. day 10 in male group

However, the male leptin-treated rats consumed lesser food than their control animals in the obese groups ($P<0.05$, Figure 1 (b)).

Tables 3 and 4 illustrate the glucose level, lipid profile, liver enzymes and urea and creatinin concentrations of the obese and diabetic groups, respectively.

The urea and creatinin concentrations did not show any significant alteration among different groups in spite of creatinin level reduction in the obese and diabetic rats. The creatinin level in the obese groups was more than the diabetic counterparts. Meanwhile, in female diabetic rats, it decreased more than male rats, though it was not significant. Finally, total protein concentration and LFT including the AST (aspartate amino transferase) and ALT (alanine amino transferase) levels showed no statistically significant differences in treatment groups.

There was a negative correlation between the logarithm of weight and food intake in male leptin-treated rats in the obese group while the leptin level and food intake showed a negative correlation only in the female leptin-treated obese rats. There was also a positive correlation of LDL and triglyceride in the female rats both in the obese-control and diabetic leptin-treated rats; however, a negative correlation was observed in male rats in the diabetic-control group.

Table 3. Biochemical variables in the obese rats after 10 days treatment with leptin (values are expressed as mean±SEM)

Biochemical parameter mg/dl	Leptin/female ^a N=7	Leptin/male ^b N=7	Control/female N=5	Control/male N=5	Statistical significance
Glucose	95.45±7.01	104.7±5.27	104.6±6.40	108.5±4.57	NS
LDL	25.18±5.86 ⁺	49.57±14.37	91.57±18.81	109.0±24.92	+ P<0.05
Cholesterol	106.5±7.09 ⁺⁺⁺	143.8±13.25 [*]	197.5±14.89	226.9±27.04	+++ P<0.001 *P<0.05
HDL	58.76±4.76	67.99±7.75	71.05±11.07	78.75±8.33	NS
Triglyceride	112.6±10.88 ⁺	131.2±15.31 [*]	174.5±19.89	195.4±23.54	+P<0.05 *P<0.05
Cholesterol/ HDL	1.88±0.21	3.23±0.75	2.27±0.32	2.97±0.49	NS
LDL/HDL	0.48±0.15	1.63±0.57	0.85±0.27	1.48±0.41	NS
Total protein	6.35±0.26	6.40±0.29	6.35±0.25	6.26±0.29	NS
AST	37.27±1.81	41.02±1.78	33.67±2.13	41.08±2.49	NS
ALT	30.02±1.90	37.23±1.26	30.21±2.67	36.72±2.69	NS
Urea	37.5±2.23	35.13±1.68	34.82±1.67	36.98±0.80	NS
Creatinin	0.54±0.13	0.54±0.12	0.68±0.13	0.70±0.18	NS

^{a+} comparison between obese female rats treated with leptin and control groups, ^{b*} comparison between obese male rats treated with leptin and control groups, NS=Non significant ($P>0.05$). Data analysis was done by an unpaired t-test using the Graph Pad Prism 5. Significant level was set at $P<0.05$

Table 4. Biochemical variables in diabetic rats after 10 days treatment with leptin (values are expressed as mean±SEM)

Biochemical parameter mg/dl	leptin/female ^d N=6	leptin/male ^e N=6	Control female N=5	Control male N=5	Statistical significance
Glucose	187.3±8.27 ⁺⁺⁺	203.4±7.38	294.5±8.01	274±24.71	+++P<0.001
LDL	35.72±7.96 ⁺⁺⁺	26.44±6.17	88.14±7.03	54.97±15.78	+++P<0.001
Cholesterol	119.5±9.64 ⁺⁺	111.3±5.32 ^{***}	192.6±12.67	197.8±15.37	++P<0.01 ***P<0.001
HDL	66.48±5.05	67.42±8.97	71.39±3.45	102.1±12.99	NS
Triglyceride	86.77±8.59 ⁺⁺	87.74±4.33 [*]	164.1±17.82	203.1±25.25	++P<0.01 *P<0.05
Cholesterol/HDL	1.84±0.20 ⁺⁺	2.96±0.10	1.75±0.17	2.00±0.19	++P<0.01
LDL/HDL	0.57±0.16 ⁺⁺	1.23±0.07	0.47±0.14	0.59±0.21	++P<0.01
Total protein	6.34±0.31	6.29±0.32	6.35±0.25	6.26±0.29	NS
AST	36.37±1.86	40.70±2.07	33.67±2.13	41.08±2.49	NS
ALT	28.62±1.49	36.50±1.24	30.20±2.67	36.72±2.69	NS
Urea	37.46±1.03	37.91±1.19	37.64±1.19	37.43±1.50	NS
Creatinin	0.33±0.06	0.37±0.05	0.46±0.13	0.41±0.08	NS

^{d+} comparison between diabetic female rats treated with leptin and control groups, ^{e*} comparison between diabetic male rats treated with leptin and control groups, NS=Non significant ($P>0.05$). Data analysis was done by an unpaired t-test using the Graph Pad Prism 5. Significant level was set at $P<0.05$

Table 5. Correlation coefficients for the association between certain metabolic variables in obese and diabetic rats treated with leptin

Variables	r ²	Sex/group	P value
Food intake (g) and leptin	-0.83	Female/ leptin obese	P=0.01
Weight (g) and food intake	-0.75	Male/leptin obese	P<0.05
	0.89	Female/control obese	P<0.05
LDL and triglyceride (mg/dl)	-0.95	Male /control diabetes	P= 0.01
	0.86	Female /leptin diabetes	P<0.05

Discussion

In the present study, our results demonstrated that some biochemical variables were altered sex-dependently after leptin administration. For example, the LDL levels diminished significantly only in female rats in both obese and diabetic treated groups, and the significant glucose reduction was exclusively observed in female leptin-treated diabetic rats (Table 4).

In general, the obese animals showed greater leptin concentrations than diabetic groups, which agreed with studies indicated that lesser leptin levels are present in the STZ-induced diabetic rats (4, 5). Furthermore, obesity has been reported to be associated with renal function disturbance (20, 21) that in turn leads to increased leptin levels (6, 22, 23). Moreover, Kennedy *et al* (13) recently reported that the upper-body obesity and consequential insulin resistance was associated with higher leptin concentration in men. Other investigators argued that obesity leads to insulin resistance (24), and insulin stimulates leptin expression because an increase in serum leptin levels in the obesity and type 2 diabetes was also reported (25). In our study, it is likely that insignificant variations in leptin concentration in the obese groups are due to near-maximal secretion of leptin (26), and higher leptin concentrations in the male groups are due to the heavier body weight or probably the male hormone (i.e. androgen) plays a role here. These results are also in agreement with Haghshenas *et al* study (27), which reported no significant change of insulin and leptin level in male rats with high fat diet and endurance training. In another study, women showed higher leptin levels (12). Also, male control animals gained weight earlier than females while treated female rats lost their weights in advance of male rats. The baseline levels of leptin and weight loss showed inconsistent results from none to positive or negative effects in human and animals (7), in addition, variations in leptin levels are not always in parallel with the body fat extent (28). It has been reported that only male rats show a correlation between serum leptin and body weight (29). Perhaps smaller body weight, lower leptin values (14) or estrogen might explain why female rats lost weight earlier than their male counterparts in this study (30). It seems that a higher concentration of leptin is the reason of lower food intake in the male obese rats in the present study which is in agreement with the report of Kanoski *et al* (31) who showed that leptin injection into the hippocampus decreased food intake after 24 hr in rats.

In several reports the lipoprotein- lipid profile shows a sexual disparity; in fact hepatic lipase activity is higher while lipoprotein lipase activity is lower in male rats (32). Whether these hormones are regulated by leptin or not is beyond the aim of this study. But, it was shown that leptin increases lipoprotein lipase production while decreases hormone-sensitive lipase activity by macrophages (33). Moreover, it has been reported that high fat diet led to rapid leptin resistance

(34), and the leptin resistance might cover the effects of leptin on the obese conditions (10). The results of this study revealed a general improvement in metabolic status after leptin treatment in obese and diabetic rats of both sexes. Despite the report on a positive relationship between baseline leptin and triglyceride concentrations in men (14), such correlation was not present in the current study. These differences may be due to different factors of study design or species.

In the present study, the glucose level is in the standard limit in the obese groups, so it seems that leptin has no effect on the usual glucose range. As stated by Shetty *et al* (35), leptin plays a permissive role in endocrine effects and encountering energy-restricted conditions; therefore, it is useful in hypoleptinemic and sensitive states rather than in normal or hyperleptinemic conditions such as obesity which are resistant to leptin effects. It seems that the significant reduction of glucose in the leptin-treated female diabetic rats may be explained by the effects of estrogen; because, recombinant leptin has been reported to amplify estrogen levels in women. Estrogen, like leptin decreases food intake and obesity in humans and animals of both genders (30). However, the amount of the food-intake reduced in the male fat group in this study. It is noteworthy that the exogenous leptin regulates blood-glucose level before exerting the real effects on fatness or feeding in *ob/ob* mice. Furthermore, leptin alters the liver glucose transport in the wild type and *ob/ob* mice (26). According to Ceddia *et al* (7) administration of leptin shows controversial effects in diabetes. The results of this study confirmed the inhibition of diabetes. However, a genetic model of the obesity and type two diabetes suggests that lack of the leptin receptor may protect the mutant rats against type one diabetes (36).

It seems that the strongest effect of leptin in diabetic group was to decrease the glucose concentration and the beneficial effects on the kidney led to lower creatinin levels in this group. But, leptin has been reported to cause diabetic nephropathy, either at low or high levels (9, 37, 38) and glomerular sclerosis (39), while the normal leptin concentration has been reported to inhibit thickening of the extracellular matrix (21). However, plasma leptin levels and creatinin levels demonstrate a negative correlation in a human study (40).

It is likely that the short duration or small sample size of this study is the cause of the insignificant effects of leptin on the kidney function. Considering the alterations of liver enzymes, adiponectin levels show a negative correlation with liver enzymes while no significant change in liver enzymes following leptin treatment is obvious in this study (40).

Conclusion

It is likely that leptin is a potential candidate to adjust metabolic disorders in obese and diabetic rats especially in female diabetic animals. The leptin-

induced weight loss occurred faster in female than male obese rats, and without disturbing food intake in female animals, the hypoglycemic effect as well as the reduction of LDL was also more pronounced in female diabetic rats. In order to further differentiate gender-associated issues of leptin regarding the metabolism and blood biochemistry, long-term studies are required to be done.

Conflict of interest

The authors declare no conflict of interest.

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