LEPTIN EFFECTS ON , THYROID HORMONES AND SOME BIOCHEMICAL PARAMETERS OF FEMALE RABBITS (*LEPUS CUNICULUS*) SERUM.

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ABSTRACT

Thirty local female rabbits were divided randomly and equally into three groups. The 1st group used as control which injected with phosphate buffer salin. The 2nd group was injected daily with leptin (5µg/animal) S/c. The 3rd group was injected subcutaneously daily with (10µg/animal) S/c. All groups were treated for 60 days, and blood samples were collected every 15 days, serum was tested for T3, T4 hormones, total Cholesterol (TC), HDL (height density lipo-protein) , LDL (low density lipo- protein), TG (triglycerides) and Body weight was taken every 15 days during the experiment .

The result show significant ($P \leq 0.05$) decreased in body weight after administration of leptin in two doses. Significant increased in Thyroid hormone after leptin administration. While lipid profile (TC, TG, LDL, HDL,VLDL) show significant decreased with leptin treatment(5 and 10µg/animal) S/c.

INTRODUCTION

Leptin, is a peptide hormone that was initially reported to be synthesized and secreted exclusively from the adipocytes of white fat (1).

Leptin is the product of the obese (ob) gene. The research of leptin began in 1994 (2,3). Leptin is synthesized mainly by white adipose tissue, but other tissues such as placenta, skeletal muscle, stomach fundus, osteoblasts, also express and secrete it (4,5).

It is made up of 167 amino acids with an amino-terminal secretory signal sequence of 21 amino acids; It is a globular protein with a tertiary structure similar to a haemopoitic cytokine (6). It regulates food intake, body adiposity and reproductive competence, and plays a role in

fetal growth, gut derived satiety, immune or pro- inflammatory responses and angiogenesis and lipolysis(7).

Leptin concentration in blood is proportional to total body fat mass. After secreted into blood, leptin circulates as a 16 kD protein and is partially bound to plasma proteins. Leptin performs its function mainly via binding to specific receptors (4). Thus, leptin can act as a hormone. However, it also plays a role as a paracrine agent. For example, local leptin secreted by human osteoblasts may directly contribute to osteoblastic cell growth and bone mineralization (5). Leptin has a wide tissue distribution and the small intestine may have the highest concentration (8).

The aim of this study investigate the effects of chronic administration of leptin in two doses on body weight, thyroid hormones and and some biochemical parameters of female rabbits serum.

MATERIAL AND METHODS

Thirty female rabbits purchased from local markets, weighted 1400-1500 gram were divided equally and randomly into three groups , 1st group used as control was injected with phosphate buffer saline 2nd and 3rd groups were injected daily (5 and 10 µg/animal) s/c with leptin hormone respectively, leptin hormone was brought from(Biovision Co. U.S.A) and prepared by dissolved with 3mM acetic acid then diluents with phosphate buffer slain.

Animals weighed every 15 day till the end of treatment, after (8 weeks). Each two experimental animals were kept in separate cage. The animal were provided with green alfalfa (Medicago sativa) and tap water ad libitum. The animals were given a prophylaxis drug against coccidiosis (Amprollium 1g/L of drinking water) and maintained air-conditioned Temp.(24 Cº) under standard husbandry condition with alternate 12 hours light /dark period.

Blood samples were collected at the same time directly from the heart (cardiac puncture) in the day 0 and then every 15 days, blood collect in plastic tube without anticoagulant and then refrigeratored for 12 hour as maximum then centrifuged (5000rpm) for 15 min. to separate serum that used in estimation of hormonal (Leptin,T3,T4) and biochemical parameters , serum samples stored in eppendorff at -20Cº until used in analysis.

Statistical analysis

All the recorded data were analyzed for ANOVA using a complete Randomized design (CRD) with help of computer packaged program (SPSS) (Statistical Packages for the Social Science) (V.19). Least significant differences (LSD). Was calculated to compare the variations

between the treatments were ANOVA showed significant differences. The data were expressed as mean ± stander deviation (mean ±SD) level of significant was set at P≤ 0.05.

RESULTS

Results in table (1) indicated there were significant (P≤ 0.05) decreased in body weight in female rabbits treated with of leptin hormone at both doses (5 and 10 µg/animal) S/c. for 8weeks as compared with control group and in all studied periods while there was in significant effects to leptin dose on the body weight as compared between treatment groups of leptin .

Table (1) Effects of leptin administration on female rabbits body weight/g

Treatment N=10	periods				
	0 day	15 day	30 day	45 day	60 day
Control	1454 ±43.99 Aa	1487 ±95.57 Aa	1504 ±46.95 Aa	1465 ±62.29 Aa	1453 ±71.34 Aa
LEP 5ug/animal	1480.00 ±101.54 Aa	1290.00 ±81.78 Bb	1193.00 ±135.15 Bc	1087.00 ±154.27 Bd	1005.00 ±.98.46 Bd
LEP 10ug/animal	1458.30 ±96.45Aa	1261.70 ±75.46 Bb	1145.00 ±132.18 Bb	1062.00 ±79.97 Bb	975.00 ±110.57 Bc

- Capital letters denote differences between groups P≤0.05 Vs control.
- Small letters denote differences within groups P≤0.05 .

Table (2) indicated that the administration of leptin hormone to female rabbits in doses (5 and 10 µg/animal) S/c for 8 weeks caused significant (P≤ 0.05) increased in serum thyroid hormones concentration as compared with control group and in all studies periods.

Table (2) Effects of leptin administration on serum T3 and T4 level

parameters	treatment	periods				
		0 day	15 day	30 day	45 day	60 day
T3 (ng/ml)	Control	1.38 ±0.01Aa	1.37 ±0.05 Ca	1.41 ±0.01 Ca	1.40 ±0.02 Ca	1.41 ±0.03 Ca
	LEP 5ug/animal	1.42 ±0.05 Ae	1.81 ±0.07 Bd	2.20 ±0.06 Bc	2.78 ±0.07 Bb	3.08 ±0.11 Ba
	LEP 10ug/animal	1.47 ±0.04 Ae	2.10 ±0.13 Ad	2.64 ±0.24 Ac	3.16 ±0.31 Ab	3.46 ±0.26 Aa
T4 (µg/dl)	Control	4.92 ±0.36 Aa	4.99 ±0.08 Ba	5.08 ±0.11 Ca	5.00 ±0.07 Ca	5.12 ±0.11 Ca
	LEP 5ug/animal	5.06 ±0.09 Ad	5.43 ±0.13 Ac	5.80 ±0.09Bb	5.97 ±0.14 Ba	6.16 ±0.14 Ba
	LEP 10ug/animal	5.13 ±0.10Ae	5.61 ±0.16Ad	6.16 ±0.35Ac	6.34 ±0.49 Ab	6.68 ±0.49Aa

- Capital letters denote differences between groups P≤0.05 Vs control.
- Small letters denote differences within groups P≤0.05 .

Table (3) indicated that the serum total protein concentration decreased significant (P<0.05) in both doses 5 and 10 µg/animal of leptin treated groups compared with control and also in all periods of experience while serum glucose concentration increased significant (P<0.05) in both leptin treated groups compared with control group and also in all periods of experience .

Table (3) Effect s of leptin on serum T.protein and Glucose concentration

parameters	treatment	Periods				
		0 day	15 day	30 day	45 day	60 day
T. Protein (g/L)	Control	67.44 ±1.07 Ba	66.80 ±2.03 Ba	68.03 ±1.60 Ba	68.67 ±2.02 Ba	67.79 ±1.51 Ba
	LEP 5ug/animal	76.54 ±1.90 Aa	73.34 ±3.73 Ab	70.23 ±2.11 Ac	70.42 ±3.32 Ac	70.83 ±1.92 Ac
	LEP 10ug/animal	77.29 ±2.06 Aa	74.11 ±2.75 Ab	71.33 ±1.26 Ab	71.83 ±2.13 Ab	71.37 ±1.97 Ab
Glucose (mg/dl)	Control	129.14 ±2.13 Aa	132.50 ±1.63 Ca	132.71 ±1.22 Ca	134.34 ±2.79Ca	132.92 ±1.44 Ca
	LEP 5ug/animal	132.10 ±3.24 Ae	151.60 ±0.82 Bd	163.72 ±1.85 Bc	179.54 ±6.83 Bb	200.82 ±5.83 Ba
	LEP 10ug/animal	134.77 ±5.88 Ae	165.42 ±8.39 Ad	176.67 ±10.26 Ac	197.40 ±9.47 Ab	209.45 ±10.45Aa

- Capital letters denote differences between groups P≤0.05 Vs control.
- Small letters denote differences within groups P≤0.05 .

The results of serum total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL), low density lipoprotein (LDL) and very low density lipoprotein (VLDL) concentrations have been presented in the Table (4A& 4B).

the results Table (4A) indicated significant (P<0.05) decrease in serum concentrations of total cholesterol, TG, in female rabbits treated with leptin hormone in both doses 5 and 10 µg/animal compared with control group and within group.

Table (4A) Effects of leptin administration on serum Cholesterol and Triglyceride

parameters	treatment	periods				
		0 day	15 day	30 day	45 day	60 day
CHOLESTROL (mg/dl)	Control	81.36 ±0.78 Ba	82.30 ±1.28 Aa	82.58 ±1.20 Aa	82.12 ±1.66 Aa	82.97 ±1.72 Aa
	LEP 5ug/animal	90.17 ±0.80 Aa	81.22 ±0.90 Bb	72.10 ±0.55 Ac	60.80 ±0.62Bd	53.33 ±0.80Be
	LEP 10ug/animal	90.54 ±0.53 Aa	79.56 ±4.12 Bb	71.49 ±1.16 Bc	59.14 ±2.80 Bd	52.93 ±1.28Be
TRIGLYCERID (mg/dl)	Control	67.12 ±2.03 Aa	66.94 ±2.25 Aa	67.98 ±2.77 Aa	66.86 ±1.98 Aa	67.32 ±3.09 Aa
	LEP 5ug/animal	65.51 0.64 Ba	59.42 ±1.27 Bb	51.03 ±1.12 Bc	44.90 ±0.63 Bd	40.62 ±0.66 Be
	LEP 10ug/animal	64.13 1.12 Ba	58.74 ±1.58 Bb	49.72 ±1.62 Bc	43.88 ±1.78 Bd	39.65 ±1.05 Be

- Capital letters denote differences between groups P≤0.05 Vs control.
- Small letters denote differences within groups P≤0.05 .

The results table (4B) indicated that the serum Lipoprotein (LDL, HDL, VLDL) concentrations significant (P<0.05) decrease in female rabbits treated with leptin hormone in both doses (5 and 10 µg/animal compared with control group and in all periods of experience.

Table (4B) Effects of leptin administration on serum Lipo-protein (LDL,HDL,VLDL)

parameters	treatment	periods				
		0 day	15 day	30 day	45 day	60 day
LDL (mg/dl)	Control	10.14 ±0.11Ba	11.79 ±0.55Ba	10.36 ±0.28Ba	10.66 ±0.75Aa	11.58 ±0.71Aa
	LEP 5ug/animal	21.23 ±1.19Aa	15.79 ±1.29Ab	13.99 ±0.39Ab	7.49 ±0.95Bc	4.28 ±1.86Bd
	LEP 10ug/animal	21.69 ±2.52Aa	15.47 ±2.01Ab	14.33 ±1.04Ab	6.80 ±1.45Bc	5.32 ±1.80Bc
HDL (mg/dl)	Control	57.79 ±1.53Aa	57.11 ±1.64Aa	58.62 ±2.186Aa	58.08 ±2.22Aa	57.92 ±2.11Aa
	LEP 5ug/animal	55.23 ±1.45Ba	53.54 ±0.96Bc	47.89 ±1.02Bc	44.32 ±0.73Bd	40.93 ±1.47Be
	LEP 10ug/animal	57.33 ±1.85Aa	52.53 ±0.96Bc	46.67 ±1.42Bc	43.61 ±1.05Bd	39.67 ±0.98Be
VLDL (mg/dl)	Control	13.42 ±0.40Aa	13.38 ±0.45Aa	13.59 ±0.55Aa	13.37 ±0.39Aa	13.46 ±0.61Aa
	LEP 5ug/animal	13.12 ±0.22Ba	11.88 ±0.25Bb	10.20 ±0.23Bc	8.98 ±0.12Bd	8.12 ±0.41Be
	LEP 10ug/animal	13.02 ±0.23Ba	11.74 ±0.31Bb	9.94 ±0.35Bc	8.77 ±0.35Bd	7.93 ±0.21Be

- Capital letters denote differences between groups P≤0.05 Vs control.
- Small letters denote differences within groups P≤0.05 .
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DISCUSSION

The results in table (1) show significant decrease in body weight of female rabbits after administration of leptin (5 and 10 µg/animal).The recent result indicated that the Leptin, upon binding to specific receptors in different areas of the hypothalamus (9).

Leptin effects on body weight were mediated through effects on hypothalamic centers that control feeding behavior and hunger, body temperature and energy expenditure. (10) was involved in the control of satiety and energy metabolism through the regulation of several neurotransmitters decreased hunger and food consumption, mediated at least in part by inhibition of neuropeptide Y synthesis. Neuropeptide Y is a very potent stimulator of feeding behavior. (11).

Increased energy expenditure, measured as increased oxygen consumption, higher body temperature and loss of adipose tissue mass. (12).

This result agreement with (13) were show leptin acts within the hypothalamus to influence neuropeptides involved in the regulation of food intake and energy expenditure, including neuropeptide Y (NPY). and this agrrement with (14,15).

The results revealed a significant increase in concentration of thyroid gland hormones (T3& T4) due to administration of leptin hormone in the present study may be due to Leptin was exerts its effects on different endocrine axes and in particular on the hypothalamic–pituitary–thyroid axis , Leptin receptors were also found outside the central nervous system, supporting the view that leptin may act peripherally as well. (16).

The presence and function of leptin in the thyroid gland in vertebrates, other than mammals are scanty. High levels of leptin have been found in the brain of several teleosts (17)

(18). Show that the Prolonged leptin administration in Wistar rats is associated with growth of the thyroid gland; morphometry showed that the increase in the weight of the gland is coupled to a net increase in the epithelium/colloid ratio and increase in the levels of circulating T3 and T4

Compelling evidence indicates that the thyroid gland weight increase occurs as a result of a leptin-stimulated release of thyroid-stimulating hormone (TSH), at least in euthyroid rats (19, 20), this may due to enhancing the hypothalamic production of TRH (21).

The present results table (3) showed that there were significant increases of serum glucose and decreased in total protein concentration due to administration of leptin to females

rabbit in doses 5 µg/animal and10 µg/animal S/c as compared to control group and in all periods experience.

Electrophysiological characterization of the effects of leptin on pancreatic β -cells revealed that leptin hyperpolarized the cell membrane, which led to inhibition of insulin secretion. This hyperpolarization has been demonstrated to be due to an increase in membrane conductance caused by the opening (activation) of K-$_{ATP}$ channels. Thus, the K-$_{ATP}$ channel is a molecular target of leptin in pancreatic β -cells for inhibition of insulin secretion. (22).

Leptin may also influence pancreatic β -cell and function through its ability to activate the sympathetic nervous system (23). Such activation of the sympathetic nervous system results in inhibition of insulin secretion by pancreatic β-cells (24).

(25) Suggest that inhibitory action of leptin on preproinsulin gene expression may constitute an additional mechanism that is supposed to prevent sustained overproduction of insulin and hyperinsulinaemia. There is indeed further evidence that leptin can inhibit peripheral secretion of insulin (22,26).

Many hormones are present in circulation both as free hormone and bound to plasma protein. Binding proteins are thought to have a role in modulating the availability of free hormone (the generally metabolically active form) for interaction with target tissues (27). Several studies have demonstrated the presence of a bound fraction of leptin in serum/plasma (28,29). Although a few studies have identified a splice variant of the leptin receptor as responsible for a portion of the binding (particularly in pregnant mice) (30) and another study has identified binding of leptin to α2- macroglobulin (31), the variety and identities of the leptin-binding proteins of serum, so the decreased in total protein concentration may be the one of causes that protein in serum will be binding with the leptin hormone and exert its effects on target tissues in the body .

Table(4A & 4B) The results revealed a significant decrease in concentration of serum lipid profile (Total cholesterol, Triglyceride, HDL, LDL, VLDL) due to administration of leptin hormone in doses 5 µg/animal and 10 µg/animal S/c as compared to control group and within treatment groups .

Because the liver plays a role in lipid metabolism, leptin acts directly on the liver to exert some of its metabolic effects. Indeed, leptin receptors are found in the liver, (32)

leptin administration to ob/ob mice elicits many changes in the expression of genes involved with lipid metabolism in the liver. (33). The direct effects of leptin on the liver, Cohen et al., (34) knocked out leptin receptors specifically in hepatocytes.

Result in this study were agreement with Xavier, *et al*., (35) which show administration of leptin cause significant decrease in the concentration of serum cholesterol ,

triglycerides, LDL/HDL cholesterol profile and this data supported the idea that leptin can modulate intermediate metabolism via direct action upon peripheral tissue (36,37).

Yoshida et al., (38) were found that direct effect of T3 on fully differentiated adipocytes showed stimulation of leptin expression and secretion.

تاثير اعطاء هرمون اللبيتين على مستوى هرمونات الغدة الدرقية وبعض المعايير الكيميوحيوية لمصل دم اناث الارانب

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الخلاصة

استهدفت الدراسة تاثير اعطاء هرمون اللبيتن بجرعتين 5 و 10 مايكروغرام / حيوان على وزن الجسم وتركيز هرمونات الغدة الدرقية وتركيز الدهون ومستوى السكر والبروتين الكلي في مصل دم اناث الارانب

اجريت الدراسة على 30 انثى ارنب وضعت في اقفاص وقسمت الى ثلاثة مجاميع المجموعة الاولى، مجموعة السيطرة والتي حقنت (phosphate puffer saline) والمجموعة الثانية حقنت بهرمون اللبتين 5 مايكروغرام / الحيوان ، المجموعة الثالثة حقنت بهرمون اللبتين 10 مايكروغرام / الحيوان . لمدة 60 يوم تم جمع العينات الدم كل 15 يوم .

اظهرت النتائج انحفاض معنوي ($P\leq0.05$) في وزن الجسم بعد اعطاء هرمون اللبتين بجرعتين ، زيادة معنوية في تركيز هرمونات الغدة الدرقية (T3,T4) ويزادة تركيز كل من الكلوكوز وانخفاض البروتين الكلي ، اما بالنسبة الى دهون الدم (الكولسترول الكلي ، الجليسيرات الثلاثية ، VLDL LDL,HDL,) فقد اظهرت الدراسة انخفاض في تركيز الدهون الدم عند اعطاء البيتن في جرعتين (5 و10 مايكروغرام / الحيوان)

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