Effect of Gonadal Sex steriods On Serum Leptin Level of Both Adult Male and Femal Rats

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Abstract:

The present study was undertaken to evaluate the influence of gonadectomy and sex steriods (estradiol and testosterone) on serum leptin. A total number of 96 adult white albino rats of both sexes were used in this study. The animals were divided into three groups. The first group was served as control , The second group was the gonadectomized , while the third one was gonadectomized and treated with gonadal sex hormones (estradiol and testosterone).

Blood samples were collected at the beginning of the experiment (0 time), 2 weeks and 4 weeks there after. The results indicated that serum leptin were higher in adult female than in male rats of similar body weight , orchidectomy resulted in a significant elevation in serum leptin levels but ovariectomy resulted a significant decrease in serum leptin levels. Estrogen treatment caused a significant elevation in serum leptin level in female rats. While testosterone treatment caused a significant reduction in serum leptin in male rats.It is conclude that gonadal steroids may affect serum leptin concentration in rats.

Introduction :

Leptin, is an adipocyte – derived hormone that is essential for normal regulation of body weight (Schubring et al., 2000) and metabolism (Atcha et al., 2000) It regulates adipose tissue mass through hypothalamic effects on energy expenditure (Pelleymounter et al., 1995). It is detectable in fetal cord blood as early as 18 weeks of gestation and dramatically increases after 34 weeks. In newborn, the serum leptin concentration is positively corrlated with the body weight, fat mass and body mass index (BMI) (Thomas et al., 2000). The expression of leptin RNA is increased by insulin (Saladin et al.,1995), glucocorticoids (Slieker et al.,1996) and several cytokines such as tumor necrosis, factor alpha and interlukin –1 (Grunfeld et al.,1996). Also, serum leptin levels are elevated in some pathologic disorders acute sepsis (Bornstein et al., 1997). In contrast, fasting, solely reduces serum leptin

level (Boden et al.,1996). Leptin may regulate different and unexpected system in addation to its main function in energy homeostasis as gonadal – adrenal- somatotroph axis (Cheung et al., 1997).

Leptin is influenced by sex hormones and adiposity (Sudi et al., 2001). the sex based difference in leptin concentration observed between women and men. Thus, after adjusting for fat mass, females have higher leptin levels than males (Garcia – Mayor et al., 1997).Pinillia et al., (1999) reported that serum leptin concentration is increased significantly two weeks after ovariectomy in rats.

Spicer and Francisco, (1997) suggested that leptin produced inside the ovary as paracrine factor to effect steroid synthesis in the follicle and corpus luteun or may play an important role in follicle development and luteinization (Guo et al., 2001) . On the other hand, testosterone has a potent negative effect on serum leptin in boys, but not in girls (Wabitsch et al.,1997).

The present work was carried out to evaluate the influence of gonadectomy and sex steriods on serum leptin level of adult rats.

Materials and Methods:

Animals:

Ninty six healthy adult albino rats of both sexes weighing (180-220g) were used in this study. The animals were kept in separate steel wire cages and supplied with a normal commercial diet and water.

Experimental Design:

Animals were divided into three equal groups :

***Group 1* : (control group)**

16 animals of both sex were kept under normal laboratory conditions during the experiment.

***Group* 2 : (Gonadectomized group)**

16 male rats were orchidectomized (Byron,1978),and 16 femals were ovariectomized (Yallampalli et al., 1994).All gonadectomized rats were left until healing and acclimatization.

***Group* 3 : (Gonadectomized and treated group) .**

Orchidedtomized rats were injected intramusculary daily with testosterone as the oenanthate and propionate in oily solution (Chemical Industries Development . S.A.A., Pyramids, Giza) at a dose of 1mg/kg body weight for 4 weeks (Hoffmann et al., 1984)

Ovariectomized rats were injected intramuscularlly with estradiol (Estradiol benzoate in oily solution – Misr CO. Pharm. Ind. S.A.A. Materia Cairo A.R.E. C.C.R. 32048), In a dose of 0.19 mg/kg body weight twice weekly for 4 weeks (Nilsson and Carlsten, 1994).

Sampling :

Blood samples were obtained by decapitation of rats in clean centrifuge tubes at (0 time – 2 weeks – 4 weeks). Serum was separated by centerifugation of blood at 3000 rpm for 15 minutes and stored at -20ºC until used for analysis.

Hormonal assay :

Leptin hormone was assayed using radioimmunoassay technique with the aid of computerized gamma counter supplied from Bechman Scientific Indstrument Division. using commerical leptin RIA kit (Linco . Research, Inc. 14 Research Park, Drive . St. Charles, Missouri. 63304 U.S.A). The standard curve was calibrated by the method of Gettys et al., 1986) and serum leptin expressed as ng/ml.

Statistical analysis:

Statistical analysis for the results were carried out according to method of Snedicor and Cochran (1967) using student t-test.

Results and Discussion :

Leptin seems to have an essential role in the regulation of food intake and energy expenditure and thus in regulating energy homeostasis and secondarily, body weight (Campfield et al., 1995). Circulating leptin levels are closely related to the percentage of the body fat (Ostund et al., 1996) and correlete with body mass index (BMI) in patient with normal weight, obesity and diabetes (Haffner et al., 1996).

In the present study (Table 1and 2), it has been found that female rats have a higher serum leptin level compared to male rats with similar body weight.

Table (1) : Serum leptin concentration (ng/ml) in adult female rats (control, ovariectomized and ovariectomized treated with estradiol) at 0 time, 2 weeks , and 4 weeks.

Group / Time	Control group	Orchidectomized group	Orchidectomized treated group
0 time	1.90 ± 0.16	*0.75 ± 0.21	1.92 ± 0.09
After two weeks	1.85 ± 0.12	*0.69 ± 0.03	**2.08 ± 0.11
After four weeks	1.92 ± 0.20	*0.60 ± 0.44	**2.99 ± 0.32

± standard error of mean
* $p < 0.05$
** $p < 0.01$

Table (2) : Serum leptin concentration (ng/ml) in adult male rats (control, orchidectomized, and orchidectomized treated with testosterone) at 0 time, 2 weeks, 4 weeks.

Group / Time	Control group	Orchidectomized group	Orchidectomized treated group
0 time	1.12 ± 0.08	1.19 ± 0.33	*0.82 ± 0.05
After two weeks	1.19 ± 0.22	*2.32 ± 0.18	*0.94 ± 0.21
After four weeks	1.20 ± 0.09	*2.95 ± 0.11	*0.96 ± 0.19

* $p < 0.05$

These results are in agreement with Wu-Pengs et al., (1999), as (it has been) suggested that there is a sex difference in the regional distribution of neuropeptide Y mRNA containing cells in the hypothalami of mice (Ubran et al.,1993; Stephens et al., 1995). Furthermore, it could be due to stimulatory effect of estrogen on leptin secretion in females or due to the

inhibitary effect of androgen on leptin secretion in male In addition. Tome et al., (1997) showed that leptin concentration in infant boys were 40% lower than those in the girls of similar body weight.

In the present study, it has found that ovariectomy caused significant decrease in serum leptin concentration compared with the control group. The finding showed a stimulatory effect of estradiol on leptin secretion, which a greement with Walczewska et al., (1999) which may be attributed to increased expression of ob gene in certain site of adipose tissue Shimizu et al., (1997) or increase of ob mRNA expression in adipocytes Casabiell et al., (1998). These confirm the finding of Wade and Gray (1978) who demonstrated high affinity binding of 17- ß estradiol in the cytoplasmic fraction of various white adipose tissue in rats.

These results are in agreement with the report of Yoneda et al., (1998) who demonstrated that ovariectomy significantly reduced serum leptin level. Moreover, these findings support the report of Messinis et al., (1999) who demonstrated that a significant reduction in leptin value were seen in both phase of the cycle during the week following bilateral ovariectomy operation in women, however Nowicki et al., (2002) seen that gonadectomy did not influence plasma leptin concentrations in women if body fat mass is unchanged.

The significant increase caused by estradiol administration of ovariectomized rats in the present study are in agreement with the reports of Yoneda et al., (1998) who demonstrated that in rats ovariectomy reduced significantly serum leptin values, which reversed by estradiol supplement. In addition, Casabiell et al. (1998) observed the stimulatory role of estrogens on leptin secretion, since addition of estradiol to isolated adipocytes increased ob mRNA in vitro.

In the present study (Table 2), it has been found that orchidectomy caused significant increase in serum leptin concentration compared to the control group. While, testosterone administration to orchidectomized rats caused significant decrease compared to both orchidectomized and control group.

Similar results were reported elsewhere by Ogura et al.,(2000 a,b). The supperssive effect of testosterone on leptin level is confirmed by the results in vitro studies of cultured human adipocytes which revealed a direct effect of testosterone at the cell level (Wabitsch et al., 1997). Moreover,it has been reported. (Bhasin et al., 1996) that testosterone substitution lowered serum leptin levels independently of its effects on the proportion of body fat, suggesting a regulatory role of testosterone in leptin production.

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تأثير الهرمونات الجنسية على مستوى اللبتين في مصل كل من ذكـور وإناث الفئـران البالغة

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الدمام - المملكة العربية السعودية

الملخص :

اللبتين مادة تفرزها الأنسجة الدهنية في الدم وهي تقلل الشهية للطعام وتزيد من استهلاك الطاقة كما أنها تؤثر أيضاً على محاور النمو والتناسل بالإضافة إلى دورها في تنظيم الطاقة في الجسم.

وقد أجريت هذه الدراسة لمحاولة إيجاد تأثير كل من إزالة الأعضاء الجنسية وكذلك تأثير الهرمونات الجنسية على مستوى هرمون اللبتين في مصل كل من ذكور وإناث الفئران البالغة.

استخدم في هذه التجربة عدد ٩٦ من ذكور وإناث الفئران البيضاء البالغة حيث تم تقسيمهم إلى ثلاث مجموعات أساسية متساوية استخدمت المجموعة الأولى كمجموعة ضابطة ، المجموعة الثانية تم فيها استئصال المبيضين والخصيتين، أما المجموعة الثالثة تم فيها استئصال المبيضين والخصيتين ثم بعد ذلك تم حقنها بالهرمونات الجنسية (التستوستيرون والأستراديول) تم جمع عينات الدم عند بداية التجربة Time 0 وبعد أسبوعين وبعد أربعة أسابيع .

وقد أثبتت النتائج أن مستوى هرمون اللبتين في الإناث أكبر من الذكور. كما أن استئصال الخصيتين سبب حدوث زيادة معنوية في مستوى اللبتين . بينما أدى استئصال المبيضين إلى حدوث انخفاض معنوي في مستوى الهرمون كذلك وجد أن حقن التستوستيرون أدى إلى حدوث انخفاض معنوي في مستوى اللبتين أما حقن الأستراديول سبب ارتفاع معنوي في مستوى اللبتين . ومن هذه النتائج يتضح أن هرمونات الذكورة والأنوثة تلعب دوراً هاماً في تنظيم مستوى اللبتين في الدم.