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Disclosure Efficacy of Chronic Administration of Leptin Supplement on Spermatogenesis in Male Rats

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Abstract

This study is undertaken the explore effect of chronic leptin administration on body weight, reproductive organ weight, sperm count and male sex hormones. Rats were treated daily with a single intraperitoneal injection 15g/kg body weight of leptin for 45 days. Body weight, were measured every five days over the experimental periods. At the end of treatment, rats were anesthetized with ether and immediately killed by cervical dislocation. The right epididymis was removed and minced in 2 ml normal saline. The suspension was filtered and stained with 1 % eosin. Sperm count were conducted. Blood was collected from the heart puncture, clotted and centrifuged to obtain the serum. Serum testosterone, FSH, LH and leptin were measured using ELISA technique. The testis, epididymis and prostate were calculated their relative organ weights. In this study was significantly decrease body weight in leptin-treated group. However, Serum testosterone, FSH and LH levels were however increased significantly in leptin-treated group compared to controls. Sperm count was significant lower in leptin-treated groups when compared to age-matched controls.

Conclusion

It appeared leptin administration in daily doses for long term, significantly affecting body; seem to significantly increase weight, levels serum for testosterone FSH and LH levels in male rats. In addition, it also decreases sperm counts. Subsequently, leptin exposure is causing impairment of spermatogenesis.

Key words : Leptin , Spermatogenesis Status , Mal Rat , LH , FSH

Introduction

Leptin expression and circulating levels increase in parallel with the amount of adipose tissue during the fed state (Lonnqvist *et al.,* 1995) and the relationship between leptin levels and fat mass is curvilinear, rather than linear, with a wide range of individual leptin values at a specific level of body fat (Considine *et al.,* 1996). There is a higher positive correlation between serum leptin levels and total mass of adipose tissue rather than body mass index (BMI) (Maffei *et al.,* 1995). In addition to total tissue fat mass and the size of adipocytes, the pattern of adipose tissue distribution may also influence leptin levels (Tritos & Mantzoros, 1997). Leptin mRNA expression is higher in subcutaneous than in visceral fat depots (Hube *et al.,* 1996). Omental adipocytes express more β-1, 2 and 3 adrenergic receptors than subcutaneous adipocytes (Lonnqvist et al., 1995). The different receptor profile makes the former more responsive to the lypolytic actions of catecholamines and less responsive to the antilipolytic actions of insulin (Lonnqvist *et al.,* 1997).

Serum leptin levels are also affected by nutritional status, and fasting reduces leptin levels by approximately 30%, while excessive food consumption leads to an increase in the secretion of leptin by 50%. Leptin levels increase more when food rich in fat is taken (Houseknecht & Portocarrero, 1998). Leptin secretion however, declines during aging. This reduction is higher in women than in men, and is independent of BMI and other age-related endocrine changes (Isidori *et al.,* 2000).

For the ensuing it is evident that a number of factors influence leptin secretion. Although serum leptin levels in the main correlate well to fat mass, there nevertheless also appears that leptin is not only a static index of fat mass but it also acts as a sensor of energy balance. Once secreted into the circulation, leptin circulates in the plasma either in the free form or bound to the soluble leptin receptor (sOB-R or LEPRe) (Houseknecht *et al.,* 1996, Lammert *et al.,* 2001). In human and animal, increased leptin levels with adiposity are due to augmented ob gene expression and increased leptin production (Considine *et al.,* 1995, Hamilton *et al.,* 1995, Maffei et al., 1995, Ogawa *et al.,* 1995). The evident positive correlation between gonadotrophins and leptin, particularly during puberty in both the sexes, suggests that leptin has a significant role in reproduction and might exert its influence on reproductive activity via the hypothalamic-pituitary-gonadal axis. The presence of leptin proteins in corticotropes, somatotrophes, gonadotropes and thyrotropes of humans further supports this contention (Jin *et al.,* 1999, Vidal *et al.,* 2000). More support comes from observations that leptin expression in the anterior pituitary changes during the different reproductive states in the rat. For example, anterior pituitary leptin mRNA has been shown to have a 2-fold increase from metestrus to diestrus followed by an 86% decrease at proestrus (Akhter *et al.,* 2007). Besides, decreased fertility has been found to be an inherent part of the phenotypes of ob/ob, db/db and fa/fa rodent (Lane & Dickie, 1954, Coleman, 1978, Mounzih *et al.,* 1997). The infertility of the leptin-deficient ob/ob mouse can be corrected by the administration of leptin (Ahima *et al.,* 1996, Chehab *et al.,* 1996). In addition, animals homozygous for a leptin receptor defect show losses in both growth and reproductive system axes, where puberty is delayed and fertility severely impaired (Popovic *et al,* 2001, Urbanski, 2001). In addition to its centrally mediated effects, leptin also exerts its effects on a number of peripheral organs as evidenced by the presence of leptin receptors. Unlike the central nervous system leptin receptor, the expression of leptin receptor in ovarian granulosa cells is not

essential for fertility (Zamorano *et al.*, 1997). Ovaries from normally an ovulatory ob/ob and db/db mouse managed to ovulate when transplanted into non-mutant female mice (Friedman *et al.*, 1991, Spicer & Francisco, 1997). Thus, it seems the infertility of the ob and db female mice is a result of hypothalamic, rather than primary ovarian dysfunction. Administration of leptin to starved mice reverses the starvation-associated diminution in circulating gonadotrophins and gonadal steroids, and restores ovulatory function in starved females (Ahima *et al.*, 1996). In addition, incubation in vitro of bovine granulosa cells with physiological concentrations of leptin attenuated insulin-induced estradiol and progesterone release, suggesting that leptin may affect gonadal steroid synthesis by direct effects on granulosa cells, as well as by its influence on the gonadal axis at the hypothalamic-pituitary level.

Methodology

Twenty four, ten-week old, male rats with a mean body weight of 200 -250 g were found animal house, Science College, Thi-qar University. Animals were housed in plastic cages and kept in the animal lab. All animals were maintained under standard laboratory conditions and had ad lbitum excess to animal and water. Rats were equally divided into control and leptin-treated groups. Control rats were given 0.1 ml of 0.9 % normal saline daily for 45 days. Rats in the leptin-treated groups were given 15 µg/kg body weight of leptin intra-peritoneally daily for 45 days. Body weight was measured every five days at the end the experimental periods. After that, all animals were weighed and anesthetized with ether. Semen was squeezed out of the epididymis to use for sperm count were transferred into vials containing Bouin's fluid. The testis, epididymis, and seminal vesicles were removed and then cleared from fat; all organs were weighed to calculate Relative weight of organs. Blood samples were collected from the heart puncture for hormone assays.

Measuring of Serum hormones

Serum testosterone, FSH and LH levels were measured using (ELISA) kit.

Sperm count :For sperm count, tepididymis was minced with scissors in 3 ml of normal saline, and mixd then filtered with 80-µm nylon mesh. Eosin was added to stain for 30 minutes. An epididymal suspension was aliquot after that dilution was done in normal saline. After mixing, suspension was put onto a Neubauer's chamber.

Statistical analyses

Results for Body weight, serum hormones, organs weight, sperm account were analyzed using multivariate analysis of variance (ANOVA) with post hoc test. significance differences was at $p<0.05$.

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Results

Body weight was significant decrease in leptin-treated groups in comparing to controls as shown in table(1) .

Table(1): Comparison Body weight between two study groups.

Control group	leptin group
361±0.15	302±0.28
P value= 0.001	

Relative weight of genital organs

1. Testis weight

Mean relative testicular weight in the leptin treated group appeared significantly lower as compare to control as shown in table (2).

2. Epididymis weigh Mean relative epididymis weight was evidently higher in the control rats comparing to leptin group as shown in table (2).

Table (2): Relative Weight of genital organs: comparison between two study groups.

Relative Weight organs	Control group	leptin group	P value
Testis weight	0.87 ± 0.01	0.79± 0.05	< 0.05
Epididymis weight	0.36 ± 0.03	0.29± 0.01	< 0.05

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Evaluate hormone assay

1. Testosterone

Statistically, significant difference was evident between treated leptin group and control group as in table (3)..

2. FSH level

Mean levels of FSH in serum were significantly increase in rats treated with leptin when compared to control group as shown in table (3).

3. LH level

Mean LH level in rats treated with leptin was significantly higher than control as shown in table (3).

Table (3):Comparison reproductive hormones level in serum between two study groups.

Assessment hormone	Control group	leptin group	P value
Testosterone	2.05 ± 0.02	0.92 ± 0.02	< 0.001
FSH	0.12 ± 0.07	0.28 ± 0.03	< 0.05
LH	0.34 ± 0. 18	0.48 ± 0.05	< 0.05

Measuring of Sperm count

Sperm count was significantly decrease in leptin treated rats in comparing to control group as shown in table (4).

Table (4): Measuring of Sperm count

Control Group	Leptin Group
25.14 ± 0.15	12.21 ± 0.23
P Value= < 0.001	

Discussion

Leptin is an essential component for long-term body weight regulation. Since, leptin is predominantly expressed by adipocytes, and associates with a body fat ratio, the idea that body weight refers to total body fat mass. There by, an increase in the concentration of leptin in the blood, result in increase of fat mass, suppresses food intake is a powerful role in the regulation of body weight. So that it is reasonable to expect that any exogenous administration of leptin would also have a similar effect in normal rats. Previous report points out that a crucial role of leptin, possibly through the melanocortin pathway, in body weight regulation (Oswal & Yeo, 2007).

our finding was in consist with former study reported recently that leptin treatment effects body weight when given twice daily for nine days caused reduction in body weight (wang *et al.,* 2018). However, one study explored chronic duration of administration that it is also possible, there is a resistance to leptin in obese individuals resulted in weight increase as not predicted (Heymsfield *et al.,* 1999). It might Indicate that a chronic supplementation of leptin require significant response to elevation of body weight. The amount of adipose tissue that an individual will defend is a complex interaction between genes and environment. In addition, it is well- established that different approaches of control are largely linked to environmental factors. On other hand, it is explained that there is an interaction between environment and genes(Ravussin & Bogardus, 2000 and Seeley *et al.,* 1996).

Interestingly, our result referred that long period of leptin administration appeared significant affects testicular weight that is an interesting consideration, because previous studies indicated that Hyperleptinaemia in adult rats revealed no alteration in testis weight (Barash *et al.,* 1996 and Franca *et al.,* 2006). In related to relative epididymal weight, there was several studies reported similar findings to our results in leptin affected epididymal weight (Popović *et al.,* 2019; Bilal *et al.,* 2016 and Kölsch *e*2007).

Serum FSH level was significantly higher in leptin treated group as compare to control group. As corresponding to our study, it has been documented before in vitro, in male rat, it could be stimulate FSH release from pituitary gland by leptin exposure for long time (McCann et al., 1998). it has been suggested that leptin increases serum FSH levels in pigs model, GnRH secretion has been trigged by leptin induction (Parent *et al.,* 2000, Woller *et al.,* 2001)

In same context of FSH, LH levels in serum were notably higher in rats treated with leptin in comparing to control. Previous studies have also pointed to the action of leptin in stimulating LH secretion whether in vitro or in vivo (McCann et al., 1998 , Gonzalez et al., 1999 and Henry et al., 2001). It might be attributed to maturity age of animal as well as maturity is will stead to stabilization status in concern sexual age. Some Studies also reveal that leptin has ability in stimulating LH secretion throughout hypothalamus trigger in both male and female rats. Furthermore, Leptin could stimulate LH release via influence on the pituitary therefore; it could be determine Leptin receptors in pituitary glands. (Dearth et al., 2000, Chan & Mantzoros, 2001 and Tezuka et al., 2002).

In term of testosterone concentration, our findings referred that administration of leptin for chronic period acts on reducing levels of testosterone secretion. In corresponding to other study, it

has been mentioned formerly that leptin has negative correlation with testosterone levels in serum (Hanafy et al., 2007).

In generally, there was correlation between testosterone level and leptin level in males (Behre et al., 1997). The action of Leptin is direct inhibition in testicular steroidogenesis signal , resulting in hyperleptin is in obese men due to low testosterone secretion in Blood (Tena-Sembere et al., 2001). that was confirmed by Tena-Sempere et al., (2000), in vitro, that testosterone secretion inhibited by leptin acting at testicular level . In addition, there is an evident reveals that expression of leptin receptor mRNA is regulated by leptin, invivo (Tena-Sempere *et al.* , 2001). Eventually, Spermatogenesis is activated by testosterone, so that Low levels in testosterone secreation in testicular lead to hypo-spermatogenesis.

In this study, Sperm counts was significantly decrease in leptin treated groups that was matching with other investigations in rats (Chitra *et al.*, 2003, Ghosh *et al.*, 2002 and Latchoumycandane *et al.*, 2002). Essentially, testosterone and FSH have a synergistic action in spermatogenesis progression (Russell *et al.*, 1998). Production of sperm is stimulated by increasing testosterone and FSH in blood serum. It was evident decrease in sperm numbers due to long durations of leptin administration which lead to increase leptin concentration level in blood. The reason of this spermatozoa reduction may returned to delay spermatogenesis development due to disorder action of testosterone, FSH, LH which were affected by leptin as explain in this study.

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