Role of Testosterone Replacement Therapy on Plasma Ghrelin Level before and after Orchiectomy in Male Albino Rats

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ABSTRACT

The aim of the present study is to investigate whether testosterone replacement therapy play a role on plasma ghrelin level before and after orchiectomy in male albino rats. Four groups of animals were studied, control group, control group received testosterone propionate (T.P) 300 µg/Kg subcutaneously (s.c) daily for 4 weeks, orchiectomized group and lastly orchiectomized group receiving T.P by the same regimen as group two. Blood samples were taken at the end of the experimental period for carrying out the hormonal investigations; also body weight was measured from all studied animals. ***The results of this work s****howed that subcutaneous administration of T.P to sham operated and orchiectomy rats produces significant decrease in FSH and LH, however it produces significant increase in total testosterone, free testosterone, ghrelin, insulin-like growth factor-I (IGF-I) and total body weight when these results compared to group one and three respectively.* ***Conclusions:*** *We can conclude that plasma ghrelin level significantly increase after testosterone supplementation in both sham operated and orchiectomy rats, also there are +ve correlation between ghrelin level and testosterone level (both total and free), IGF-1 and body weight & negative correlation between ghrelin and FSH & LH.*

INTRODUCTION

Ghrelin is a noval polypeptide hormone predominantly synthesized by the stomach and involved in food intake regulation.[1] Ghrelin was identified as an endogenous ligand for an orphan receptor termed GH-secretagogue receptor possessing potent GH releasing activity.[2]

Previous studies conducted in healthy children and adolescents revealed that ghrelin levels reach a peak in early post natal life and then gradually decrease during childhood and adolescence.[3]

The precise mechanisms underlying these changes have not been fully elucidated.[4]

Further studies have suggested that androgen and ghrelin may reciprocally cross-talk, Gonadal tissues have in fact been proposed as a target for ghrelin action based on a higher number of binding sites in both ovary and testis.[5]

In addition to this first descriptive evidence that ghrelin might play a role in regulation of reproductive physiology, one study has shown that ghrelin affects the chorionic gonodotropin and cAMP induced

testosterone secretion in rat testis by inhibiting key enzymes of steroidogenic pathway,[6] Moreover, it has been demonstrated that gonads are not only target tissues for ghrelin, but also relevant sites of ghrelin production, in both testis and ovary, ghrelin is co-expressed with sex hormones in androgen producing cells like leydig and hilus interstitial cells.[7]

The hypthalmo-pituitary mechanism by which testosterone promotes GH secretion and there by elevates insulin-like growth factor-I (IGF-I) concentration in human are unknown.[8]

The mechanisms by which sex hormones, particularly androgen may regulate ghrelin expression and secretion are still poorly defined, moreover, it is still undefined whether their regulatory capacity could be influenced by the interference of other hormones and factors.[5]

So the aim of the present work is to study the role of testosterone replacement therapy on plasma ghrelin level before and after orchiectomy in male albino rats.

MATERIAL & METHODS

45 male albino rats weighing between (250-350 g) were used in this study, they were kept under constant environmental and nutritional conditions through out the experiment. Rats were randomly divided into four groups

Group I: Control animals underwent a sham orchiectomy and received no medications for 4 weeks (n= 8).

Group II: Control animals underwent a sham orchiectomy and received testosterone propionate (T.P) 300 µg/Kg subcutaneously (s.c) daily for 4 weeks.[9] (n= 10)

Group III: The animals underwent an orchiectomy at the start of the experimental protocol and received no medications for the length of the protocol 4 weeks (n= 12).

Group IV: The animals underwent a surgical orchiectomy and received s.c (T.P) 300 µg/Kg daily for 4 weeks (n= 15).

At the end of experimental periods, blood samples withdrawn into EDTA containing tubes for plasma and serum samples were taken in the morning after an over night fast. Body weight was measured from all studied animals at the end of experimental periods.

Methods:

- Determination of serum level of total and free testosterone by enzyme immunoassay in rat serum.[10]
- Determination of follicle stimulating hormone (FSH) and luitinizing hormone (LH) concentrations by using enzyme linked immunosorbent assay.[11]
- Insulin-like growth factor-I was determined by enzyme linked immunosorbent assay. [12]

Quantitative determination for rat ghrelin:

Plasma levels of ghrelin were assayed using reagent kits and methods provided by Kamiya BioMedical Company. Samples were collected in tubes containing EDTA. 2Na (1.25 mg/ ml blood) and aprotinin (500 units/ml blood). Plasma ghrelin determined by 2 sites sandwich enzyme linked immunosorbent assay (ELISA), measure the absorbance at 450 nm.[13]

Surgical procedure:

Weigh and anesthetize the animal. Bilateral orchiectomy was performed as described by **Svensson et al.**[14] a small surgical incision was made in the center of the scrotum, each testicle was exposed through the surgical orifice. The ductus deferens and main arteries and veins were isolated and ligated. Subsequently the duct and blood vessels were severed allowing the testicle and epididymis to be removed. The incision was then closed, sutured and swabbed with iodine solution. The sham operation involved the exposure of the testis without excision.

The post operative procedure was implemented by proper antibiotics, finally the rats were housed in separate cages and allowed free access to food and water.

Statistical analysis:

Data analyses were performed with the soft ware package SPSS program version 11, the results of continuous variables are presented as mean ±SD and statistically analyzed using the unpaired "t" test.

Pearson's and spear man's correlation were applied to examine the relationships among ghrelin and the other studied parameters.

RESULTS

Five rats died during the experimental period two from group III and three from group IV

Subcutaneous administration of T.P to sham operated rats produces significant increase in serum level of total T and free T when compared to normal control ($P<0.05$) (table1 & fig 1,2), also it produces significant reduction in FSH and LH ($P<0.05$), when compared with control (table 1& fig 3,4).

As regard the effect of T.P supplementation on plasma ghrelin level it produces significant increase ($P<0.05$), this values corresponding to significant increase in IGF-1 ($P<0.05$). Table (1) & Fig (5, 6) as compared to control values.

After orchiectomy the serum level of total T and free T show significant decrease ($P<0.05$), but orchiectomy produces significant increase in serum level FSH and LH ($P<0.05$) when compared with sham operated rats (table 1& fig 1, 2, 3, 4). Also orchiectomy produces significant reduction in plasma level ghrelin ($P<0.05$) and IGF-I ($P<0.05$) when compared with sham operated rats (table 1 & fig 5,6)

T.P supplementation to orchiectomized rats produce significant increase in total T and free T when compared to orchiectomy group, but it produces significant decrease in FSH and LH ($P<0.05$) {table1 & fig 1,2,3,4}.

Ghrelin level was significantly higher after T.P supplementation, also this corresponding with significant increase in IGF-I ($P<0.05$) when compared with orchiectomy group (table 1 & fig 5,6).

As regards the effect of T.P supplementation in sham operated and orchiectomy in rats it produces significant weight gain in both groups ($P<0.05$) when compared the results with either sham operated orchiectomy or orchiectomy respectively (table1 & fig7).

There are +ve correlation between ghrelin level and testosterone level (both total and free), IGF-1 and body weight (fig 8, 9, 12, 13) & negative correlation between ghrelin and FSH & L H (fig 10, 11).

Table 1: Effect of T.P supplementation (300 µg/Kg) before and after orchiectomy on hormonal level and body weight in male albino rats (values are mean ±SD)

Groups	No.	Total testosterone ng/ml		Free testosterone ng/ml	FSH µIU/ml	LH µIU/ml	IGF-1 ng/ml	Ghrelin Pg/ml	Total body weight (g)
GpI Sham operated (control)	8	1.9 ± 0.1		0.12 ± 0.02	2.39 ± 0.16	2.55 ±0.32	44.65 ±2.98	127.35 ± 5.78	294.5 ±10.31
GpII Sham operated+T.P	10	2.30 ± 0.35		0.16 ± 0.02	1.49 ±0.33	1.39 ±0.47	51.45 ± 4.92	191.07 ±34.07	314.8 ±10.11
		t_1	3.64 *	4. 44*	6.92*	6.11*	3.62*	5.53*	4.19*
GpIII Orchiectomy	10	1.18 ± 0.23		0.08 ± 0.02	3.61 ± 0.41	3.19 ±0.19	34.12 ± 2.69	75.05 ±16.13	254.9 ± 14.81
		t_2	9.00 *	4.00*	8.71*	4.92*	7.8*	9.53*	6.68*
GpIV Orchiectomy +T.P	12	1.69 ± 0.21		0.11 ±0.02	2.48 ± 0.07	2.65 ±0.12	40.68 ± 0.55	121.2 ± 2.70	273 ± 9.32
		t_3	5.67 *	5.00*	8.69*	7.71*	7.63*	8.92*	3.35*

* Denotes signifiant (P<0.05). (T.P) = Testosterone propionate

t_1= Gp II vs GpI t_2= Gp III vs GpI t_3= Gp IV vs Gp III

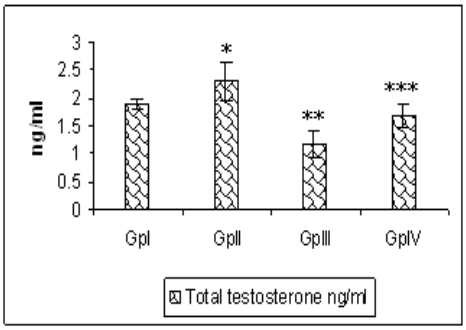

Fig (1) Total testosterone levels in all studied groups *GpII vs GpI **GpIII vs GpI ***GpIV vs GpIII

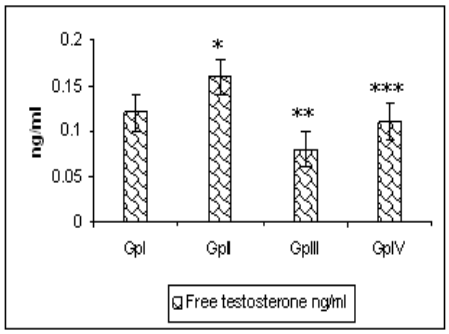

Fig (2) Free testosterone levels in all studied groups*GpII vs GpI **GpIII vs GpI ***GpIV vs GpIII

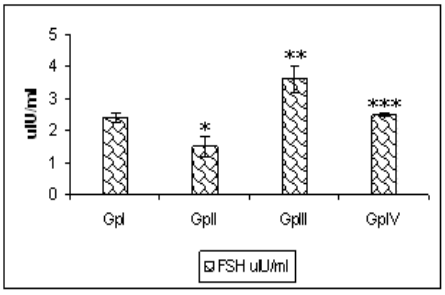

Fig (3) FSH levels in all studied groups *GpII vs GpI **GpIII vs GpI ***GpIV vs GpIII

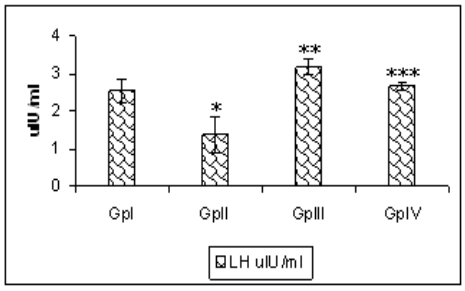

Fig (4) LH levels in all studied groups *GpII vs GpI **GpIII vs GpI ***GpIV vs GpIII

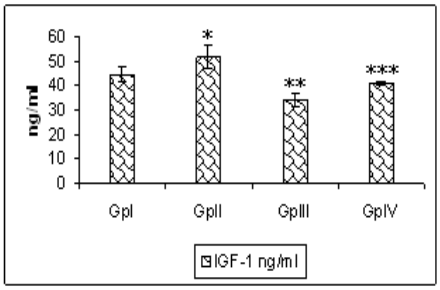

Fig (5) IGF-1 level in all studied groups*GpII vs GpI **GpIII vs GpI ***GpIV vs GpIII

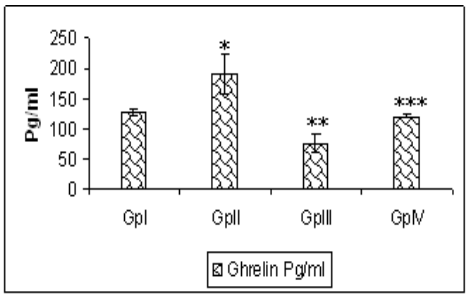

Fig (6) Ghrelin levels in all studied groups *GpII vs GpI **GpIII vs GpI ***GpIV vs GpIII

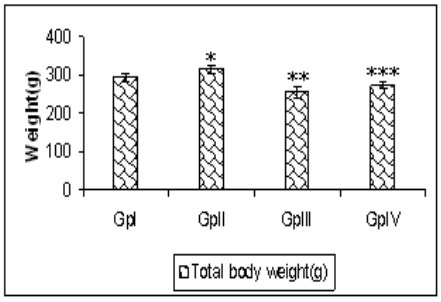

*Fig (7) TBW level in all studied groups*GpII vs GpI **GpIII vs GpI ***GpIV vs GpIII*

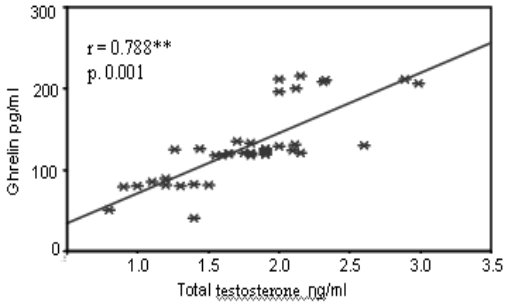

Fig (8) Correlation between ghrelin and total testosterone among the studied groups

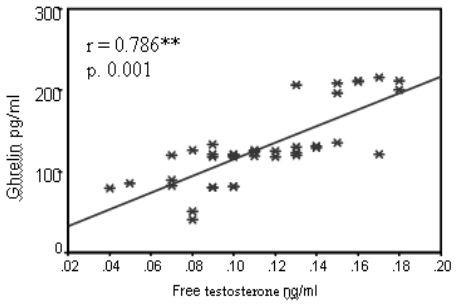

Fig (9) Correlation between ghrelin and free testosterone among the studied groups

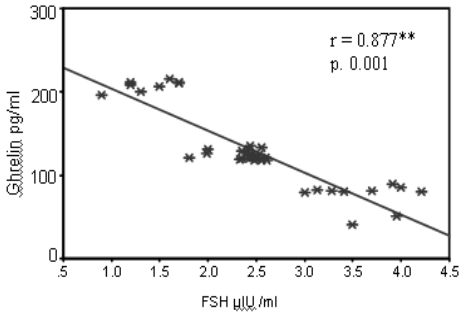

Fig (10) Correlation between ghrelin and FSH among the studied groups

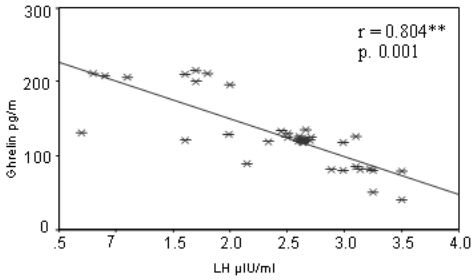

Fig (11) Correlation between ghrelin and LH among the studied groups

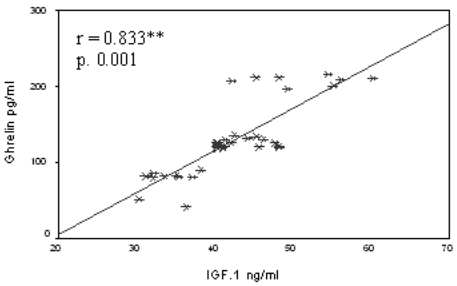

Fig (12) Correlation between ghrelin and IGF-1 among the studied groups

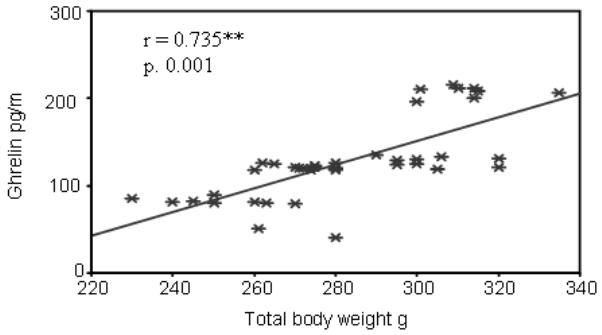

Fig (13) Correlation between ghrelin and total body weight among the studied groups

DISCUSSION

In our present study, we have attempted to examine the possible role and mechanism of action of testosterone replacement therapy on plasma ghrelin level before and after orchiectomy in male albino rats. It is clear from the present results that s.c administration of T.P to sham operated and orchiectomy rats produces significant decrease in FSH and LH, however it produces significant increase in total testosterone, free testosterone, ghrelin, IGF-I and total body weight.

Our results can be partly explained by previous research finding, that androgen play an important regulatory role regarding ghrelin secretion or catabolism. Also the relationship between androgen and ghrelin is positive in male individuals.[15]

Tena Sempere.[6] proved a good link between energy homeostasis and fertility, and the clear cut reproductive effect of other regulator of energy homeostasis and growth, the potential role of ghrelin in the control of gonadal function has so far received little attention[16].

Pagotto et al.[16] demonstrated that total and free testosterone and plasma ghrelin levels were significantly lower in hypogondal men. [17]

Ishikawa et al.[17] reported that ghrelin levels were positively correlated with both total and free testosterone concentration.[18]

Also **Le benthal et al.**[18] suggested that testosterone replacement therapy significantly increase plasma ghrelin.[19]

The normalization of the androgen status rather than an unnatural increase or decrease may recover suppressed ghrelin secretion and may possibly reestablish a balanced energy homeostasis. [20]

The mechanism by which testosterone level alter ghrelin concentration may be explained by that testosterone may act directly on both peptide expression and synthesis as well as on ghrelin metabolic pathway. [16]

Another explanation is that testosterone substitution normalize serum leptin secretion in hypogonadal patient, we can therefore hypothesize that leptin normalization may be involved in elevation of ghrelin levels.[21]

Testosterone supplementation significantly increase in body weight in sham operated and orchiectomy rats (tab1 & fig7).

Testosterone induces an increase in free fatty acid (FFA) release from the visceral fat depot.[22]

The link between FFA and ghrelin has been demonstrated by **Broglio et al.**[23] who reported that FFA infusion increase the ability of ghrelin to induce growth hormone (GH) secretion from the pituitary, [23]

Additionally it has been shown that a fat rich diet known to increase circulating FFA, was able to increase circulating ghrelin levels in experimental rats.[24]

Barreiro et al.[5] observed that ghrelin peptide was selectively detected in rat leydig cells at advanced stages of maturation,[5] also **Tena-Semper et al.**[6] demonstrated that ghrelin expression became undetectable after selective elimination of mature leydig cells by administration of cytotoxic compound.[6]

The significant decrease in FSH and LH observed in this work in sham operated and orchiectomy rats treated by T.P could be explained by increase in ghrelin level in this work (table1) may participate in the regulation of gonadotropin secretion. [25]

Zigman et al., proposed that the role of ghrelin as a peripheral signal for energy insufficiency and its role in reproductive effect, it appears feasible that circulating ghrelin might contribute to functional control of the reproductive axis and its integration with energy balance. [26]

Ghrelin inhibit pulsatile LH secretion in overiectomized female rats **Furuta et al.**[27]

In vivo ghrelin inhibit GnRH from the hypothalamus, but direct stimulatory action on basal L H & FSH in vitro in the pituitary. [28]

Fernandez et al.[25] indicated that marked impairment in gonadal function and fertility in the ob/ob mice can be reversed when crossed with ghrelin Ko mice, imply the major role of ghrelin in gonadal function. [29]

It is clear from the present work that testosterone supplementation in Gp II and Gp IV produces significant increase in IGF-I (table 1& fig 6).

These results were in agreement with the previous reports **Thomas et al.**[30] who reported that supra-physiological testosterone concentration augment GH and IGF-1 production in elderly male. [30]

In animals pulsatile GH secretion is controlled jointly via feed forward by GH releasing hormone and ghrelin (a GH releasing peptide), **Low et al.**[8] reported that the hypothalamo-pituitary mechanism by which testosterone promotes GH secretion and therapy elevates IGF-1 in animals could be due to that hormone.[8] In this work the elevated ghrelin level after testosterone supplementation (table1 & fig 6) could be one of the mechanisms to increase IGF-1 via increasing level of GH.[31]

We can conclude that plasma ghrelin level significantly increase after testosterone supplementation in

both sham operated and orchiectomy rats, also there are +ve correlations between ghrelin level and testosterone level (both total and free), IGF-1 and body weight & negative correlation between ghrelin and FSH & L H.

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دراسة دور التستوستيرون على هرمون الجيرلين قبل وبعد استئصا ل الخصية فى ذكور الفئران

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كلية الطب-جامعة طنطا

الهدف من هذه الدراسة هو بحث دور التستوستيرون على هرمون الجيرلين قبل وبعد استئصا ل الخصية فى ذكور الفئران .

أجريت هذه الدراسة على أربع مجموعات من الحيوانات : مجموعة ضابطة ، مجموعة ضابطة تم حقنها بالتستوستيرون بروبيونيت ٣٠٠ميكروجرام/كجم كل يوم لمدة أربعة أسابيع،مجموعة تم فيها استئصال الخصيتين،وأخيرا مجموعة تم حقنها بالتستوستيرون بروبيونيت بعد استئصال الخصيتين بنفس الجرعة والمد ة مثل المجموعة الثانية.

في نهاية مدة البحث تم أخذ عينات الدم لتحليل الهرمونات وتم أيضا وزن جميع الحيوانات التى استخدمت فى الدراسة.

وقد أظهرت نتائج هذا البحث أن التستوستيرون بروبيونيت أدى الى انخفاض مستوى كل من LH& FSH وزيادة فى هرمون التستوستيرون (الكلى والحر)،الجيرلين، IGF-1 ووزن الحيوانات فى كل من المجموعة الثانية والرابعة وكانت هذه النتائج ذات دلالة احصائية اذا ماقورنت بالمجموعة الاولى والثالثة على التوالى.

ونستنتج من هذا البحث انه يوجد علاقة طردية بين هرمون الجيرلين و التستوستيرون (الكلى والحر) و IGF-1 ووزن جسم الفئران وعلاقة عكسية بين هرمون الجيرلين و FSH & LH .