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Original Article

Gender differences of preptin levels in rat models of hypogonadism

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ABSTRACT:

Background: Preptin is a peptide hormone co-secreted with insulin and amylin from pancreatic β cells. Preptin has an in vitro and in vivo osteogenic effect.

Aim: we hypothesized that preptin levels will be different in both sexes and these sexual differences might play a potential role in bone thickness differences.

Material and methods: 80 albino rats were divided into 8 groups, Male Control (MC), Male Hypogonadised (MH), Male Hypogonadised with testosterone replacement (MHT), Female Control (FC), Female Hypogonadised (FH) and Female Hypogonadised with estradiol replacement (FHE), Female Hypogonadised with progesterone replacement (FHP) and Female Hypogonadised with estradiol and progesterone replacement (FHEP) groups.

Results: preptin levels were decreased significantly ($p \leq 0.001$) from 41.58±10.5 in MC group to 21.58±9.6 in MH group. Preptin level was increased significantly ($p \leq 0.001$) with testosterone replacement from 21.58±9.6 to 40.53±10.4. No significant differences ($p=0.8$) between MC group and MHT group. Preptin level was also decreased significantly ($p \leq 0.001$) from 31.21±8.3 in FC group to 13.30±7.6 in FH group and 17.18±7.5 in FHP group. Insignificant differences ($p=0.2$) were found in preptin level in FHE and FHEP group compared with FC group. Preptin levels in males were found to be significantly ($p=0.02$) higher in MC group (41.58±10.5) than FC (31.21±8.3). Also, there are significant differences ($p=0.04$) between preptin levels in MH group (21.58±9.6) and FH group (13.30±7.6) although both of them are reduced significantly in comparison with the control groups. In addition, preptin levels are significantly ($p=0.02$) lower in FHEP (30.21±8.1) compared with male hypogonadised with testosterone (40.53±10.4).

Conclusion: preptin levels are different in both sexes and these sexual differences might play a potential role in bone thickness differences and these levels are also different within the same sex according to presence or absence of sex hormones.

Key words: Preptin, gender differences, bone thickness, testosterone, estradiol.

INTRODUCTION:

Preptin, a 34–amino acid peptide *[Buchanan et al., 2001, and Liu et al., 2010]* is a physiologic amplifier of glucose mediated insulin secretion *[Yang et al., 2009]*. Preptin participates in bone anabolism and bone mass preservation observed in hyperinsulinaemic states *[Cornish et al., 2007]*. The serum preptin level was lower in osteoporosis and correlates positively with bone mineral densities (BMD) *(Li et al., 2013).* Significant positive correlation was found between serum preptin and bone formation markers *(Li et al., 2013).* It was indicated that preptin may be a potential therapeutic target for the treatment of osteoporosis *(Xiao et al, 2019).* All these studies confirm the role of preptin in bone formation.

Gender differences are documented in different conditions whether normal or disease states. One of these differences is the difference in bone thickness and heaviness in both animals and humans. We hypothesized that preptin levels will be different in both sexes and these sexual differences might play a potential role in bone thickness differences and these levels are also different within the same sex according to presence or absence of sex hormones. So, this study is designated to investigate the sexual differences in preptin levels in rats and demonstrate the effect of gonadectomy on these levels.

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MATERIAL& METHODS:

Eighty albino rats (30 males and 50 females) were divided into eight groups (each group 10 rats);

1. Male Control (MC)
2. Male Hypogonadised (MH)
3. Male Hypogonadised with testosterone replacement (MHT)
4. Female Control (FC)
5. Female Hypogonadised (FH)
6. Female Hypogonadised with estradiol replacement (FHE)
7. Female Hypogonadised with progesterone replacement (FHP).
8. Female Hypogonadised with estradiol and progesterone replacement (FHEP).

All rats were selected at 3 months of age (weight, 170-200 gm.) and obtained from the Zagazig University Animal Center. All rats were kept under conditions of controlled temperature (24 ± 1°C), relative humidity (50%–60%), and a light/dark cycle of 12 h light/12 h dark. Standard rat diet and drinking water were available all the time. All surgical procedures and postoperative care were approved by research ethics committee of Zagazig University (approval number ZU-IACUC/7/F/60/2021).

Gonadectomy in male rats:
The rats were anesthetized with a mixture of ketamine (100 mg/kg, i.p.) and xylazine (10 mg/kg, i.p.) then gonadectomy was performed through a ventral incision in the scrotum and bilateral removal of both testicles was done. The incision site is closed. The same incision was done without removal of testicles in the male control group as sham operated. Directly following surgeries, topical antibiotic was applied to the surgical site. After surgery, animals were allowed to recover for 14 days *(Moghadami et al., 2016).*

Testosterone replacement:
3-weeks after orchiectomy, the hypogonadised group with testosterone replacement received a single dose of testosterone undecanoate (100 mg/kg i.m., Nebido, Bayer Schering Pharma, Berlin, Germany) this treatment is effective in achieving physiological testosterone levels in orchiectomized rats for 4 weeks at least. The control group was treated with a single injection of vehicle (*Cheng C and de Groat 2016).*

Evaluation of testosterone concentrations:
Blood was collected from all rats after 4 weeks of castration and testosterone concentrations were measured in the same day of blood collection, using a chemiluminescence assay *(Zhang et al., 2017).*

Method of ovariectomy:
The female rats were put under general anesthesia with sodium pentobarbital (40 mg/kg body weight). Under complete aseptic technique, a short dorsal midline skin incision was made midway between the caudal edge of the ribcage and the base of the tail. Abdominal muscle wall incisions were made bilaterally to access to the peritoneal cavity, the ovary and the oviduct were taken out through the muscle wall incision. A sterile ligature is placed around the oviduct; the ovary was then excised through the oviduct near the ovary. The remaining tissue was moved back into the peritoneal cavity. The other ovary was excised by the same way. At last, the incisions were closed *(Huang et al., 2016).* In the shame operated group (female control), the same procedures were done without ligation of oviduct or removal of ovary, just the incision of the abdominal wall and closure.

Hormonal replacement:
2 weeks after ovariectomy, the rats in the FHE group were injected with β-estradiol 5 µgm/kg body weight and the rats in FHP group were injected with progesterone 10 mg/kg *(Kanazawa, 2019).* FHEP group was injected by both estradiol and progesterone.

Hormonal measurements:
Serum preptin was measured by Rat preptin ELISA Kits with detection range; 15.625-1000pg/ml purchased from MyBiosource.com. Estradiol and progesterone levels were measured by ELISA using commercial reagents (Sigma Co. Cairo, Egypt).

Statistical analysis:
Data were expressed as mean ± standard deviation (SD). Statistical analyses were performed using independent t-test to compare groups**.** All tests were performed by Graphpad Quickcalcs software.

RESULT:

Table 1; Testosterone and preptin levels in male rat groups (values presented as $\bar{x}$± SD): Preptin level was decreased significantly ($p \leq 0.001$) from 41.58±10.5 in male control sham operated group to 21.58±9.6 in male hypogonadised group. Preptin level was increased significantly ($p \leq 0.001$) with testosterone replacement from 21.58±9.6 to 40.53±10.4. No significant differences ($p=0.8$) between male control and hypogonadised group with testosterone replacement. A strong positive correlation was found between preptin levels and testosterone levels ($r=0.989$, $p=0.0001$).

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	MC	MH	MHT
1. Testosterone ng/ml	2.4±0.3	1.6± 0.2*[a]	2.5±0.3*[b]
2. **Preptin(pg/mL)**	41.58±10.5	21.58±9.6*[a]	40.53±10.4*[b]
R	**0.989 ***		

MC= Male Control
MH= Male Hypogonadised
MHT= Male Hypogonadised with testosterone replacement
*[a]= significant p≤0.05 compared with the control group
*[b] = significant p≤0.05compared with the hypogonadised group

Chart 1: Testosterone and preptin levels in male rat groups (values presented as x̄):

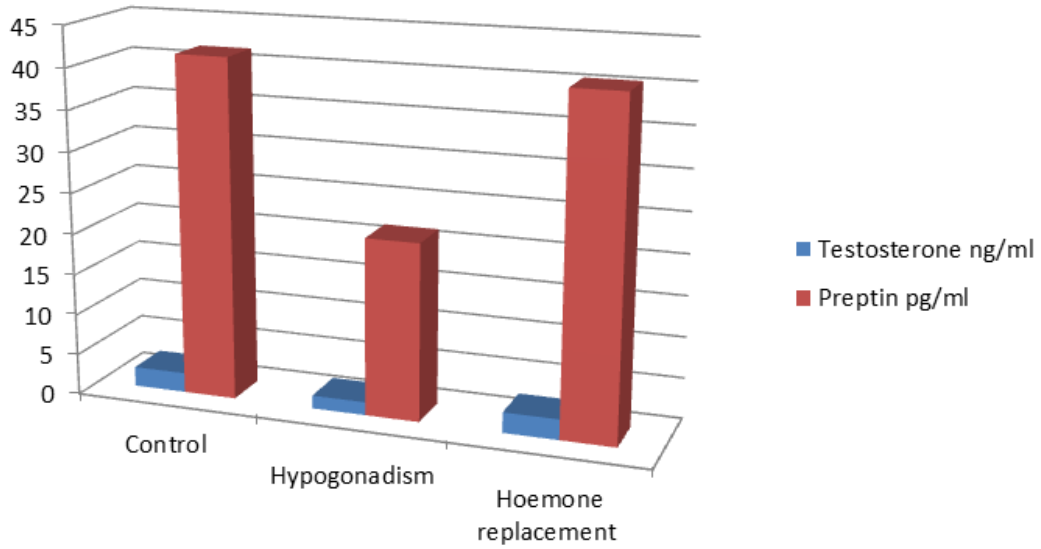

Table 2; Preptin levels in Female rat groups (values presented as x̄± SD):

	FC	FH	FHE	FHP	FHEP
Estradiol p.g/ml	73.5±4.5	33.5±2.5*[a]	73.4±4.4*[b]	34.5±2.3*[a]	82.4±3.7*[b]
Progesterone ng/ml	32.0±2.5	11.0±1.5*[a]	10.0±1.1*[a]	33±2.3*[b]	31±2.4*[b]
Preptin (pg/mL)	31.21±8.3	13.30±7.6*[a]	27.21±8.4*[b]	17.18±7.5*[a]	30.21±8.1*[b]
r with estradiol	0.967*				
r with progesterone	0.349 (insignificant)				

FC= Female Control
FH= Female Hypogonadised
FHE= Female Hypogonadised with estradiol replacement
FHP= Female Hypogonadised with progesterone replacement
FHEP= Female Hypogonadised with estradiol and progesterone replacement
*[a]= significant p≤0.05 compared with the control group
*[b] = significant p≤0.05compared with the hypogonadised group

Table 2 shows: Preptin level was decreased significantly (p≤0.001) from 31.21±8.3 in control group to 13.30±7.6 in hypogonadised group. Preptin level was decreased significantly (p≤0.001) from 31.21±8.3 in control group to 17.18±7.5 in FHP group. Insignificant differences (p=0.2) were found in preptin level in hypogonadised group with estradiol and control group. Insignificant differences (p=0.2) were also found in preptin levels in hypogonadised group with estradiol and progesterone compared with control group. A strong positive correlation was found between estradiol levels and testosterone levels (r=0.967, p=0.0001). A weak positive correlation was found between preptin levels and progesterone levels (r=0.349, p>0.05).

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Chart 2: Preptin levels in Female rat groups (values presented as x̄):

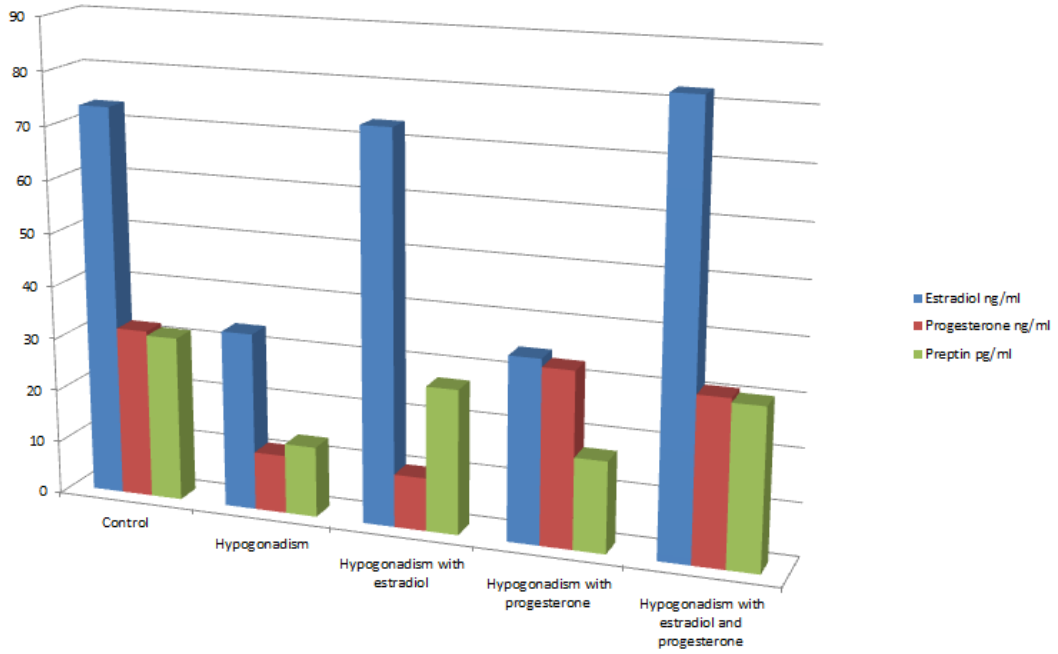

Table 3: Comparison between preptin levels in male and female rats:

	Male	**Female**
Control group	41.58±10.5	31.21±8.3* (p=0.02)
Hypogonadised group (orchiectomized or ovariectomized)	21.58±9.6	13.30±7.6* (p=0.04)
Hypogonadised with hormone replacement (Testosterone in male or estradiol+ progesterone in female)	40.53±10.4	30.21±8.1* (p=0.02)

*= significant p≤0.05 compared with the male groups

Table 3 shows that preptin levels in males were found to be significantly (p=0.02) higher in male control group (41.58±10.5) than female control (31.21±8.3). Also, there are significant differences (p=0.04) between preptin levels in male hypogonadised group (21.58±9.6) and female hypogonadised group (13.30±7.6) although both of them are reduced significantly in comparison with the control groups. In addition, preptin levels are significantly (p=0.02) lower in female hypogonadised with hormone replacement (estradiol+ progesterone in female) (30.21±8.1) compared with male hypogonadised with testosterone (40.53±10.4).

DISCUSSION:

Preptin is a 34–amino acid peptide which increases bone area and mineralization in mice *[Cornish et al., 2007 and Li et al., 2013]*. Many studies confirmed the role of preptin in bone formation. Gender differences are documented in different conditions whether normal or disease states. One of these differences is the difference in bone thickness and heaviness in both animals and humans. We hypothesized that preptin levels will be different in both sexes and these sexual differences might play a potential role in bone thickness differences and these levels are also different within the same sex according to presence or absence of sex hormones. So, this study is designated to investigate the sexual differences in preptin levels in rats and demonstrate the effect of gonadectomy on these levels.

In the present study, preptin levels were decreased significantly (p≤0.001) in MH group compared with MC group. Preptin level was increased significantly (p≤0.001) with testosterone replacement. No significant differences (p=0.8) between male control and hypogonadised group with testosterone replacement. In the present study, testosterone replacement restores preptin levels to nearby normal values which indicate a significant role of male sex hormones in the process of bone formation and differences in bone thickness in both sexes. Our results and explanations are consistent with ***Vutthasathien and Wattanapermpool, (2015)*** who reported favorable outcomes for testosterone replacement in cardiovascular diseases.

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The findings of the present study were also consistent with ***Cheng and de Groat, (2016)*** who reported the effect of orchiectomy on the active and passive properties of the bladder and urethra and testosterone replacement alleviated many of these changes. The results of the present study were also in agreement with ***Nickolenko et al., (2014)*** who found that testosterone deficiency may contribute to the severity of hepatic steatosis and testosterone replacement may play a protective role in hepatic steatosis and nonalcoholic fatty liver disease development.

In this study, preptin levels were significantly lower ($p \leq 0.001$) in FH group compared with FC group. These findings are consistent with ***Aahmad et al., (2018)*** who found that serum preptin and estradiol levels were significantly lower in the postmenopausal women than the premenopausal subjects. In addition, in the current study, a significant reduction was found in preptin levels in FHP group ($p \leq 0.001$) in comparison with FC group and insignificant differences ($p=0.2$) were found in preptin level in hypogonadised group with estradiol and control group. Also, insignificant differences ($p=0.2$) were also found in preptin levels in FHEP compared with FC group. These findings are in line with ***Aahmad et al., (2018)*** who found a positive correlation between serum preptin levels with estradiol and femur neck BMD and they reported the reduction of preptin levels in postmenopausal women and a positive correlation with estradiol and bone mineral densities**.** Our findings are also consistent with the finding of ***Kanazawa., (2019)*** who found that ovariectomy suppressed hepatic autophagy and the latter is improved by progesterone.

The present study showed that preptin levels in males were found to be significantly ($p=0.02$) higher in male control group (41.58±10.5) than female control (31.21±8.3). These findings suggest that preptin might have a role in sexual differences in bone thickness and this suggestion is supported by ***Van Doorn (2020)*** who reported that the action of preptin contributes to the regulation of bone mass, both in rodents and humans. Also, in our study, there are significant differences ($p=0.04$) between preptin levels in male hypogonadised group (21.58±9.6) and female hypogonadised group (13.30±7.6) although both of them are reduced significantly in comparison with the control groups. In addition, preptin levels are significantly ($p=0.02$) lower in female hypogonadised with hormone replacement (estradiol+ progesterone in female) (30.21±8.1) compared with male hypogonadised with testosterone (40.53±10.4). Our finding is not in agreement with ***G. Yang et al., (2009)*** who found that fasting preptin levels were significantly higher in women compared to men. Species differences and differences in study design may contribute to this disparity. We suggested that the higher levels of preptin in males more than females might contribute to the sexual differences in bone thickness in males and females. Our suggestion is supported by ***Bebars et al., (2019)*** who found that serum preptin is significantly reduced in rachitic children. They also reported that preptin in milk of rachitic children′s lactating mothers was significantly decreased and they suggested a role for preptin in the etiology of rickets. Our findings and suggestion were also supported by ***Li et al., (2013)*** who reported the osteogenic effect of preptin in vitro and in vivo.

The mechanism of osteogenic action of preptin is previously explained by ***Cornish et al., (2007)*** who reported the stimulatory effect of preptin on primary fetal rat osteoblasts′ proliferation and the inhibitory effect on osteoblast′s apoptosis. They suggested that preptin may signal osteoblast proliferation through a G protein-coupled receptor that activates Gi-dependent phosphorylation of p42/44 MAPK **(*Cornish et al., 2007)***. A confirmed role of preptin is explained in the same study by preptin′s injection which promoted bone formation in adult male mice. Other studies demonstrated the osteogenic role of preptin like study conducted by ***Xiao et al., (2019*)** who found that preptin, dose-independently, promoted the cell proliferative activity and osteoblastic differentiation, as evidenced by an increased expression of several osteoblast-specific genes, including alkaline phosphatase. Preptin also stimulated the proliferation and differentiation of human osteoblast cells, in this case being mediated by the ERK/MAPK/connective tissue growth factor (CTGF) pathway ***(Liu et al., 2010)***.

In the present study, a strong positive correlation was found between preptin levels and testosterone levels ($r=0.989$, $p=0.0001$). These results are consistent with ***Celik et al., (2011)*** who found that serum preptin levels were significantly higher in patients with PCOS than in healthy controls. If we took in consideration the study of ***Şentürk et al., (2018)*** who found that PCOS patients had significantly higher free and total testosterone levels compared to the healthy women. We will conclude that testosterone and preptin levels are higher in PCOS than healthy and this will confirm the positive correlation between preptin and testosterone levels.

In addition, a strong positive correlation was found between estradiol levels and testosterone levels ($r=0.967$, $p=0.0001$). A weak positive correlation was found between preptin levels and progesterone levels ($r=0.349$, $p>0.05$). These findings are in line with ***Aahmad et al., (2018)*** who found a positive correlation between serum preptin levels with estradiol ($P = 0.036$) and they suggested that serum preptin levels in women decrease after menopause and have a positive correlation with estradiol**.**

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CONCLUSION:

preptin levels are different in both sexes and these sexual differences might play a potential role in bone thickness differences and these levels are also different within the same sex according to presence or absence of sex hormones.

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Declaration: I declare that this article has not been published elsewhere.

Conflict of interest: None

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