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ORIGINAL ARTICLE

The effects of zinc deficiency and testosterone supplementation on leptin levels in castrated rats and their relation with LH, FSH and testosterone

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Abstract

AIM: The aim of this study was to investigate how zinc-deficiency and testosterone supplementation, both in combination and individually, affect plasma LH, FSH and leptin levels in castrated rats.
DESIGN: Group 1, Control Group. Group 2, Castration Group. Group 3, Testosterone Group. Group 4, Zinc-deficient Group. Group 5, Testosterone, Zinc-deficient Group. Group 6, Zinc-deficient, Castration Group. Group 7, Testosterone, Castration Group. Group 8, Zinc-deficient, Testosterone, Castration Group.
MEASUREMENTS: Plasma zinc, leptin, LH, FSH, free and total testosterone levels were measured.
RESULTS: Group 2 had the highest levels of leptin and LH, besides having the highest FSH levels together with Group 6 ($p<0.01$). Groups 5 and 8 had the lowest leptin levels ($p<0.01$). Leptin levels in Groups 4 and 7 were higher than those in Groups 5 and 8, but lower than those in all other groups ($p<0.01$). LH levels in Group 4 were not different than those in Groups 3, 5 and 8, but significantly lower than those in all other groups ($p<0.01$). Free and total testosterone levels were higher in Group 4 than in castration groups that were not supplemented testosterone, but were lower in the former than in all others ($p<0.01$).
CONCLUSION: Plasma LH may be more effective than testosterone on plasma leptin and zinc can be an important mediator of the effect LH exercises on leptin.

Introduction

Leptin hormone is a signal molecule originating from the adipose tissue. Although it is mainly released from the white adipose tissue, it is also secreted from the brown adipose tissue in very small amounts [1]. Leptin interacts with quite a few systems ranging from gastrointestinal and hemopoietic systems to controlling hypertension and obesity [2]. Its biological effects are inhibition of food intake and increasing energy consumption. Leptin hormone is important in weight control of the body due to these effects. It is effective on the satiety center located in the ventro-hypothalamus

[3, 4]. Leptin is necessary to regulate neuroendocrine functions and energy use. It also plays an essential role in fetal development, commencement and development of puberty in children and making of blood [5–7].

Identification of leptin receptors in rats testes and leydig cells is evidence of a possible relation between this hormone and male reproductive system [8]. It is noted that leptin stimulates GnRH, LH and FSH secretion, thereby forming strong signals which start puberty in both males and females [9]. However, leptin levels differ between sexes. In males, leptin levels begin to increase in childhood, peaking in early stages of puberty, and decreases thereafter. Thus, leptin levels in females are three-four folds higher than those in males [10,11]. Serum testosterone level and testes measure are in inverse proportion to leptin level after puberty [10]. Whereas it is reported that exogenous testosterone supplementation in young males brings about a significant decrease in leptin levels [12], another study asserts that leptin inhibited testosterone secretion in healthy males, but leptin was not a determining factor on serum leptin levels [13].

Zinc, an important trace element, is the only metal that is found in almost all enzyme classes [14]. The fact that there are high concentrations of zinc in testes and accessory sex glands shows that it plays important roles in the reproductive system [15]. It is known that zinc ensures structural wholeness of the sperm membrane, increases sperm motility and regulates helicoidal movement of the sperm tail [16].

It is stated that zinc is related to fat metabolism, insulin resistance and obesity [17] and it is claimed that zinc deficiency in animals results in anorexia, weight loss, poor nutrient efficiency and growth disorders [18,19]. Zinc, which is an essential trace element, has a part in regulation of appetite [20]. The fact that obese individuals have high zinc and low leptin levels [20] indicates a relation between zinc and nutrition and thus zinc and leptin.

Results of the studies investigating the relation between zinc and leptin are inconsistent. It was reported that zinc administration did not alter leptin secretion [20]. Similarly, Olusi et al.[22] reported that there was not a significant relation between zinc and leptin. However, Chen et al.[23] noted that zinc might be a mediator of leptin production.

When the information above is evaluated in its totality, it can be said that there are complex relations between reproductive system and leptin, reproductive system and zinc and leptin and zinc. The aim of the present study is to investigate how zinc-deficiency and testosterone supplementation affected plasma LH, FSH and leptin levels, both in combination and individually, in castrated rats.

Materials and Methods

This study was conducted at Selçuk University Experimental Medicine Research and Application Center (SUDAM) on 80 adult, male, Spraque-Dawley rats, which were provided by the concerned center. SUDAM Ethics Committee approved the study protocol.

Experimental animals were divided into 8 groups as follows:

Group 1 (n=10), Control Group: The group which was not subjected to any procedure and which was fed on a normal diet (including approximately 97 mg of zinc per kilogram).

Group 2 (n=10), Castration Group: The group which was fed on a normal diet (including approximately 97 mg of zinc per kilogram) after being castrated under general anesthesia.

Group 3 (n=10), Testosterone Group: The group which was fed on a normal diet and to which 5 mg/kg/day intramuscular testosterone propionate was administered for 4 weeks.

Group 4 (n=10), Zinc-deficient Group: The group which was fed on a zinc-deficient diet (0.65 ppm zinc/g diet) for 4 weeks [24].

Group 5 (n=10), Testosterone, Zinc-deficient group: The group to which 5 mg/kg/day intramuscular testosterone propionate was administered for 4 weeks and which was fed on a zinc-deficient diet (0.65 ppm zinc/g diet) in the same period [24].

Group 6 (n=10), Zinc-deficient, Castration Group: The group which was fed on a zinc-deficient diet (0.65 ppm zinc/g diet) for 4 weeks after being castrated under general anesthesia [24].

Group 7 (n=10), Castration and Testosterone Group: The group which was fed on a normal diet and to which 5 mg/kg/day intramuscular testosterone propionate was administered for 4 weeks after being castrated under general anesthesia.

Group 8 (n=10), Zinc-deficient, Castration and Testosterone Group: The group which was fed on a zinc deficient-diet (0.65 ppm zinc/g diet) and to which 5 mg/kg/day intramuscular testosterone propionate was administered for 4 weeks after being castrated under general anesthesia [24].

Experimental animals were given 10 g rat food per 100 g of body weight daily.

Procedures

Castration procedure: Castration procedures were carried out surgically after the animals were put under general anesthesia using rompun (5 mg/kg) and ketamine (60 mg/kg) combination. After the scrotum was incised and spermatic cord was ligated, the testes were taken out and the scrotum skin was sutured.

Testosterone administration: Testosterone hormone was dissolved in sesame oil. Testosterone propionate administrations were made in the form of intramuscular injections that contained 5 mg/kg testosterone propionate in 0.1 ml sesame oil.

Biochemical analyses

At the end of the study blood samples (5 ml) were collected from all experimental animals by decapitation method in order to determine plasma leptin, LH, FSH and zinc levels and put into heparinized tubes. Plasma

was separated by centrifugation and stored in plastic-capped tubes at –80°C until analyses.

Plasma leptin measurements: Plasma leptin was analyzed using Rat Leptin RIA (Radioimmunoassay) test kit (Linco brand catalogue number RL-83K) and by the help of a Gamma Counter (DPC Gambyt CR). Results were presented as ng/ml.

Plasma LH determinations: Plasma LH analysis was made with Biocode brand rat LH kit (catalogue number AH R002) according to RIA method, using a Gamma Counter (DPC Gambyt CR). Results were expressed as ng/ml.

Plasma FSH determinations: Analyses of plasma FSH were carried out using Biocode brand (catalogue number AH R004) rat FSH kit according to IRMA (Immunoradiometric assay) method and by the help of a Gamma Counter (DPC Gambyt CR). Results were given as ng/ml.

Plasma free testosterone determinations: Plasma free testosterone was analyzed using Coat-A-Count Free Testosterone test kit (catalogue number TKTF1) according to RIA method and by the help of a Gamma Counter (DPC Gambyt CR). Results were presented as pg/ml.

Plasma total testosterone determinations: Plasma total testosterone levels were determined using Immulite brand commercial test (catalogue no: L2KTT2) by competitive immunoassay method in Immulite 2000 autoanalyzer. Results were given as ng/dl.

Plasma zinc analyses: Plasma zinc levels were analyzed by Schimatzu ASC-600 model Atomic Absorption Spectrophotometer. Measurements were repeated twice for each sample using flame atomization technique with light at 213.9 nm wavelength. Zinc levels were determined as μg/dl.

Statistical evaluation

Minitab for Windows release 13.0 computer software was employed for statistical evaluation of data. Arithmetic means and standard mean errors were calculated for all parameters. Variance analyses were used to find out the differences among groups. Values for which $p<0.01$ were considered significant.

Results

When experimental animals are evaluated in terms of weight, it is seen that while weights of groups were not different before the experiment, groups fed on zinc-deficient diet (groups 4, 5, 6 and 8) had significant weight loss in comparison to other groups (groups 1, 2, 3 and 7) at the end of the experiment ($p<0.01$). Weights of the rats in groups 1, 2, 3 and 7 were not different from each other (*Table 1*).

Comparison of leptin levels found in the study groups demonstrated that Group 2 (castration) had the highest leptin levels ($p<0.01$). Plasma leptin levels were higher in groups 1 (control) and 6 (castration, zinc-deficient) than in groups 3, 4, 5, 7 and 8 ($p<0.01$). Leptin levels in groups 1 and 6 were not different. Plasma leptin levels in groups 3 (testosterone), 4 (zinc-deficient) and 7 (castration, testosterone) were higher than those in groups 5 and 8 ($p<0.01$). Group 5 (testosterone, zinc-deficient) and group 8 (castration, testosterone, zinc-deficient) had the lowest leptin levels among all groups ($p<0.01$, *Table 2*).

Table 1: Mean Body Weight of Groups Before and After Experiments

Groups	Before of Experiments (g)	After Experiments (g)
1 Controls	180.56 ± 15.92	205.56 ± 16.40 [a]
2 Castration	182.40 ± 16.14	203.95 ± 14.28 [a]
3 Testosterone	182.85 ± 14.75	206.75 ± 15.50 [a]
4 Zinc deficiency	183.75 ± 15.41	168.50 ± 15.60 [b]
5 Testosterone, Zinc Deficiency	181.50 ± 14.93	165.86 ± 16.20 [b]
6 Castration, Zinc Deficiency	184.25 ± 15.22	165.35 ± 15.90 [b]
7 Castration,Testosterone	180.16 ± 16.24	205.41 ± 13.95 [a]
8 Castration,Testosterone, Zinc Deficiency	183.20 ± 15.30	164.80 ± 14.25 [b]
P		0.01

* a>b

Table 2: Plazma Leptin, LH and FSH Levels in Groups

Groups	Leptin (ng/ml)	LH (ng/ml)	FSH (ng/ml)
1 Controls	2.42 ± 0.22 [b]	4.36 ± 0.01 [c]	9.65 ± 1.04 [d]
2 Castration	8.35 ± 0.48 [a]	11.27 ± 0.31 [a]	84.27 ± 7.59 [a]
3 Testosterone	1.11 ± 0.16 [c]	2.98 ± 1.54 [d]	20.50 ± 2.80 [b]
4 Zinc deficiency	1.63 ± 0.44 [c]	3.26 ± 0.20 [d]	11.71 ± 0.87 [c]
5 Testosterone, Zinc Deficiency	0.45 ± 0.31 [d]	3.15 ± 0.28 [d]	7.19 ± 1.06 [e]
6 Castration, Zinc Deficiency	2.58 ± 0.52 [b]	6.12 ± 1.17 [b]	81.75 ± 13.08 [a]
7Castration,Testosterone	1.28 ± 0.18 [c]	4.38 ± 0.36 [c]	23.37 ± 5.75 [b]
8Castration,Testosterone, Zinc Deficiency	0.43 ± 0.15 [d]	3.39 ± 0.26 [d]	21.98 ± 2.83 [b]
P	0.01	0.01	0.01

* a>b>c>d for leptin, LH and FSH

Table 3: Plasma Free and Total Testosterone and Zinc Levels in Groups

Groups	Free Testosterone (pg/ml)	Total Testosterone (ng/dl)	Zinc (µg/dl)
1 Controls	4.86 ± 0.62 [d]	217.17 ± 17.10 [b]	94.50 ± 4.63 [a]
2 Castration	0.02 ± 0.00 [f]	20.00 ± 0.00 [d]	92.83 ± 6.55 [a]
3 Testosterone	13.63 ± 1.72 [a]	342.00 ± 15.65 [a]	98.25 ± 9.75 [a]
4 Zinc deficiency	1.96 ± 0.50 [e]	56.17 ± 7.73 [c]	58.12 ± 5.93 [b]
5 Testosterone, Zinc Deficiency	7.16 ± 1.97 [c]	323.17 ± 26.77 [a]	59.00 ± 5.72 [b]
6 Castration, Zinc Deficiency	0.02 ± 0.00 [f]	20.00 ± 0.00 [d]	60.00 ± 4.60 [b]
7Castration,Testosterone	10.68 ± 1.08 [b]	325.25 ± 17.26 [a]	95.52 ± 6.60 [a]
8Castration,Testosterone, Zinc Deficiency	7.05 ± 0.98 [c]	320.00 ± 25.83 [a]	59.33 ± 7.03 [b]
P	0.01	0.01	0.01

* a>b>c>d>e>f for free testosterone
* a>b>c>d for total testosterone
* a>b for zinc

Group 2 (castration) had the highest plasma LH levels ($p<0.01$). Plasma LH levels in group 6 (castration, zinc-deficient) were lower than those in group 2 ($p<0.01$), but higher than those in all other groups (groups 1, 3, 4, 5, 7 and 8) ($p<0.01$). Plasma LH levels in groups 1 (control) and 7 (castration, testosterone) were significantly lower than those in groups 2 and 6 ($p<0.01$), but significantly higher than those in groups 3, 4, 5 and 8 ($p<0.01$). While groups 3 (testosterone), 4 (zinc-deficient), 5 (testosterone, zinc-deficient) and 8 (castration, testosterone, zinc-deficient) had similar plasma LH levels, these were lower than those in all other groups (groups 1, 2, 6 and 7) ($p<0.01$, *Table 2*).

The highest plasma FSH levels were obtained in groups 2 (castration) and 6 (castration, zinc-deficient) ($p<0.01$). Plasma FSH levels in groups 3 (testosterone), 7 (castration, testosterone) and 8 (castration, testosterone, zinc-deficient) were found higher than those in groups 1, 4 and 5 ($p<0.01$). Plasma FSH levels in group 4 (zinc-deficient) were higher than those in groups 1 and 5 ($p<0.01$), but lower than those in groups 2, 3, 6, 7 and 8 ($p<0.01$). Group 1 (control) had plasma FSH levels higher than the levels in group 5 ($p<0.01$), but lower than those in all other groups ($p<0.01$). Group 5 (testosterone, zinc-deficient) had the lowest plasma FSH levels ($p<0.01$, *Table 2*).

Group 3 (testosterone) had the highest plasma free testosterone levels ($p<0.01$). Plasma free testosterone levels in group 7 (castration, testosterone) were lower than those in group 3 ($p<0.01$), but higher than those in all other groups ($p<0.01$). Groups 5 (testosterone, zinc-deficient) and 8 (castration, testosterone, zinc-deficient) had plasma free testosterone levels higher than those in groups 1, 2, 4 and 6 ($p<0.01$). Plasma free testosterone levels in group 1 (control) were higher than those in groups 2, 4 and 6 ($p<0.01$), but lower than those in groups 3, 5, 7 and 8 ($p<0.01$). Group 4 (zinc-deficient) had plasma free testosterone levels higher than those in groups 2 and 6 ($p<0.01$), but significantly lower than those in all other groups ($p<0.01$). Group 2 (castration) and 6 (castration, zinc-deficient) had the lowest plasma free testosterone levels ($p<0.01$, *Table 3*).

Plasma total testosterone levels in group 3 (testosterone), 5 (testosterone, zinc-deficient), 7 (castration, testosterone) and 8 (castration, testosterone, zinc-deficient) were not different from each other, but significantly higher than those in groups 1, 2, 4 and 6 ($p<0.01$). Group 1 (control) had plasma total testosterone levels significantly lower than groups 3, 5, 7 and 8 ($p<0.01$), but higher than groups 2, 4 and 6 ($p<0.01$). Plasma total testosterone levels in group 4 (zinc-deficient) were higher than those in groups 2 and 6 ($p<0.01$), but lower than those in all other groups ($p<0.01$). Groups 2 (castration) and 6 (castration, zinc-deficient) had similar plasma total testosterone levels, but these were significantly lower than those in all other groups ($p<0.01$, *Table 3*).

Plasma zinc levels in groups 1 (control), 2 (castration), 3 (testosterone) and 7 (castration, testosterone) were not different from each other, but higher than those in groups 4, 5, 6 and 8 ($p<0.01$). Plasma zinc levels in groups 4, 5, 6 and 8 were not different (*Table 3*).

Discussion

It was observed that although there was no difference between weights of groups at the beginning of the study, all groups fed on zinc-deficient diet (groups 4, 5, 6 and 8) had significant weight loss at the end of the study. It can be said that the weight loss observed in the groups fed on zinc-deficient diet is an expected result, since it was shown in a number of studies that zinc-deficiency led to weight loss [18,19,25,26]. Moreover, it is a widely accepted view that the most evident indicator of zinc-deficiency is insufficient food intake, that is loss of appetite and a decrease in body weight [27–29]. Studies reporting that zinc-deficiency in animals caused anorexia, weight loss, poor nutrient efficiency and retardation of growth are consistent with the weight loss we found in zinc-deficient groups in this study.

In the present study, the highest leptin levels were found in group 2, in which testosterone deficiency was induced by castration procedure. Results of the studies investigating the relation between leptin and testosterone are contradictory [10,12]. It is noted in a study carried out on male rats that leptin levels increase together with the increase in testosterone levels and thus the inhibiting role of testosterone on leptin is questionable [30]. Ahim et al. [31] reported that leptin treatment given to male rats with food deprivation significantly reduced the

decrease in LH and testosterone. However, other studies conducted on rats show that leptin does not affect testicular steroiogenesis [32,33]. A study conducted on old, hypogonadal males reported that increased leptin levels in circulation were not related to advanced age or decreased testosterone [34]. Similarly, Bray and York [35] claimed that there was not a significant correlation between serum testosterone and leptin. The present study shows that 4-week testosterone deficiency brought about a significant increase in plasma leptin levels. In other words, the decrease in testosterone levels results in an increase in leptin levels. In a study including hypogonadal patients who were not subjected to any procedure, Behre et al. [36] found low serum testosterone and increased leptin levels. In the same study some of the hypogonadal patients were given exogenous androgen and following androgen administration, increased testosterone and decreased leptin levels were obtained. The concerned study concluded that there was a significant negative relation between serum leptin and testosterone. Luukkaa et al.[12] also reported a similar result. In the study including 269 non-diabetic males, Luukkaa et al. [12] found a negative correlation between testosterone and leptin. They showed in the mentioned study that exogenous testosterone supplemented to 10 voluntary healthy males for a period of 12 months decreased leptin levels significantly and when testosterone treatment was interrupted in the testosterone group, leptin concentration returned to the levels before treatment. High leptin levels we found in group 2 where we induced testosterone deficiency by castration are consistent with the findings of Behre et al.[36] and Luukkaa et al. [12]. In this study we established the inverse relation between testosterone and leptin in group 3 which we supplemented testosterone and group 7 which we supplemented testosterone following castration. Groups 3 and 7 had high testosterone levels and decreased leptin levels in comparison to groups 1 (control) and 2 (castration). These findings point to the inverse relation between testosterone and leptin from a different aspect. However, it is seen that studies investigating the relation between testosterone and leptin [12,36] did not examine LH and FSH levels. In the present study we aimed to investigate not only the relation between leptin and testosterone, but also the interaction of this hormone with LH and FSH. Group 2, in which we obtained the highest leptin levels, also had the highest plasma LH levels as well as the highest FSH levels together with group 6 (castration, zinc-deficient). It is seen that studies investigating the relation between leptin, and LH and FSH generally focus on how this hormone affects LH and FSH. It is accepted that leptin is affected by steroid hormones and can be an important signal in the development of puberty as it stimulates LH secretion [37]. It is reported that leptin directly stimulates LH release and stimulates, though to a small extent, FSH release via NO activation in gonadotrophes [9]. It was reported in a study investigating the effects of leptin on gonadotropin secretion in starved male monkeys that leptin prevented the plasma LH and FSH inhibition that was stimulated by hunger [38]. In this study high LH and FSH levels we found in group 2 were expected due to testosterone deficiency following castration. However, the point that needs o be addressed here is whether the main factor affecting leptin secretion is testosterone or LH and FSH. We are going to try to answer this question in the following part of discussion when addressing findings of other groups. Nonetheless, it can be emphasized that the relation between leptin and LH and FSH does not seem unidirectional. It can be speculated that if leptin influences LH and FSH levels, than LH and FSH influence leptin in turn. The fact that in the present study the increase in LH and FSH levels brought about by induced testosterone deficiency in rats was accompanied by an increase in leptin concentration is an indicator that the relation between them is bidirectional. The decrease observed in LH and FSH levels parallel to the decrease in leptin levels in group 3 where only testosterone supplementation was made and group 7 where testosterone supplementation was made following castration supports our view that the relation between leptin, and LH and FSH is not unidirectional.

In the present study, plasma leptin levels were significantly lower in only the group fed on a zinc-deficient diet (group 4) than in the control group (group 1), which was not subjected to any procedure, together with groups 2 and 6. Results of the studies investigating the relation between zinc and leptin are contradictory. Bribiescas [21] showed that 50 mg zinc gluconate supplementation for 10 days did not have any effect on plasma leptin concentration. Likewise, Olusi et al. [22] reported that there was not a significant relation between serum zinc and leptin in healthy individuals. However, Chen and Lin [39] observed high leptin and low zinc levels in obese rats stimulated with sucrose and found that serum leptin levels further increased after zinc administration and that obesity could be reversed. The fact that obese individuals are reported to have low zinc and high leptin levels [20] can be pointed to as evidence of a possible relation zinc and leptin. Findings of Mangian et al. [28] who stated that plasma leptin levels were significantly inhibited in rats fed on zinc deficient diet reinforce decreased leptin levels we obtained in the zinc-deficient group in this study. The rats in the concerned group (group 4) where decreased plasma leptin levels were found also had significant weight losses. As decreased body weight causes a reduction in fat tissue, low leptin levels found in this group can be seen as a natural result of the decrease in fat tissue. However, Ott and Shay [40] demonstrated in their study that the decrease in serum leptin concentration in zinc-deficient rats resulted from not only the reduction in fatty tissue of the body, but also the decrease in leptin secretion from each gram of fatty tissue. In the zinc deficient group (group 4) we established significant decreases in plasma LH and total testosterone levels. Interestingly, there was no decrease in FSH levels when compared to controls. Zinc, an important trace element, is the only metal that is found in almost all enzyme classes [14] . Presence of high concentrations of zinc in testes and accessory sex glands demonstrates that it plays essential

roles in the reproductive system [15]. It was reported that zinc-deficient diet alone, led to hypogonadism [29] and that there was a positive relation between zinc and testosterone [41]. Prasad [42] also reported a similar finding. It was shown that borderline zinc deficiency for 6 weeks in rats reduced testosterone levels, but did not affect LH and FSH levels [43]. It was put forth that LH and FSH production was significantly suppressed in female rats fed on zinc-deficient diet [44]. In addition, Om and Chung [45] established that zinc deficiency in male rats significantly inhibited both testosterone and LH. A similar finding was reported by Martin et al.[46]. In the present study, zinc deficiency significantly inhibited plasma free and total testosterone only in rats fed on zinc-deficient diet (group 4) while significantly suppressing plasma LH levels despite decreased testosterone levels. However, this effect was not observed on plasma FSH levels. We think that these findings we obtained in group 4 are quite interesting and can provide a different interpretation of zinc-leptin and zinc-testosterone-leptin relations. That is because decreased testosterone levels we observed in this group resulted in increased leptin levels, as opposed to what is expected. The decrease in leptin levels here seems to stem from the decrease in LH levels. Several researchers showed that leptin affected LH release directly, when compared to FSH release [9,31,37]. In consideration of the information above, we can put forward as a suggestion that LH may be more effective on leptin release than testosterone. We attained the findings supporting this view of ours from group 3 to which we administered testosterone and group 5 which had testosterone supplementation and zinc-deficiency together. In both group 3 and group 5, plasma LH levels significantly decreased parallel to the significant decrease in leptin levels. In addition, testosterone levels were significantly high as opposed to group 4 that was fed on zinc-deficient diet. In other words, decreased testosterone in group 4 or increased testosterone levels in groups 3 and 5 go together with decreased plasma leptin and decreased LH levels. Therefore, it appears that LH levels are more determining than testosterone on plasma leptin.

Plasma leptin levels in group 6 where castration and zinc deficiency were applied together were lower only than those in group 2 where only castration was applied and were not different from those in the control group (group 1) which was not subjected to any procedure. What is noteworthy here is that despite induction of testosterone deficiency, zinc deficient diet prevents an increase in leptin levels caused by castration. It was observed in the same group (group 6) that plasma LH levels were significantly lower when compared to castrated group (group 2). However, FSH levels in this group were not different than those in group 2. The conclusion that can be stressed here is that zinc deficiency brought about a decrease in plasma LH levels, despite castration, and decreased plasma LH levels, in turn, prevented a possible increase in leptin, despite castration.

Group 8 that had castration, testosterone supplementation and zinc deficiency in combination had the lowest plasma leptin levels together with group 5 (testosterone supplementation, zinc-deficient diet). These groups also had reduced LH levels, which findings demonstrate that LH might comprise a significant control mechanism on plasma leptin and that zinc might be a mediator in this mechanism. The med-line scans we made did not reveal a study with which we can directly compare ours. Thus, we can say that ours is the first study using castration, testosterone supplementation and zinc deficiency, individually and in combination.

In conclusion of this study,

1. Castration procedure significantly increases plasma leptin in rats.
2. Testosterone supplementation results in decreased leptin levels.
3. Only zinc-deficient diet reduces testosterone and LH levels and significantly inhibits plasma leptin in rats.
4. The increase in leptin levels brought about by castration is prevented by zinc-deficiency.
5. The increase in leptin levels caused by castration is inhibited by testosterone.
6. Testosterone supplementation following castration and zinc deficiency in combination significantly inhibits plasma leptin and LH.

Conclusion:

Plasma LH levels may be more influential than testosterone on plasma leptin and zinc may be an important mediator in the effect LH exercises on leptin.

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