Al-Azhar Med. J. *Vol. 46(3), July, 2017, 627-641*
DOI: 10.12816/0040350

EFFECT OF TESTOSTERONE ON SERUM LIPOCALIN-2 IN ORCHIDECTOMIZED ADULT MALE ALBINO RATS

By

Maha Abdelhamid and Nanees F. El-Malkey

Department of Medical Physiology, Faculty of Medicine, Zagazig University

ABSTRACT

Background: Lipocalin-2 (Lcn2) was initially identified as a protein secreted from human neutrophils. It has been implicated in many functions as inflammation, cell survival, innate immunity and biology of the genitourinary system as a developmental and a protective factor. The effect of testosterone on the circulating lipocalin-2 level in adult male rats is still unknown.

Objective: To explore the effect of testosterone on serum lipocalin-2 levels in orchidectomized adult male albino rats.

Material and Methods: The present study was conducted on 32 adult male albino Wistar rats divided into 4 equal groups: group (I): Sham operated control group; group (II): Orchidectomized group; group (III): Orchidectomized with subcutaneous physiologic testosterone replacement therapy; group (IV): Orchidectomized with subcutaneous supra-physiologic dose of testosterone.

Results: In group (II) and group (III), serum lipocalin-2 level significantly decreased compared to control group. In group (IV), there was further significant reduction in serum lipocalin-2 when compared with control group, group II and group III with a significant negative correlation with serum testosterone level.

Conclusion: Normal physiologic levels of testosterone are needed for normal lipocalin-2 expression. Disturbance of testosterone level (either by decrease or increase) significantly decreased lipocalin-2 level.

Key words: Lipocalin-2, orchidectomy, testosterone, lipid profile.

INTRODUCTION

Lipocalin-2 (Lcn2), also known as neutrophil gelatinase associated Lcn2 **(Kjeldsen et al., 1993)**, was initially identified as a protein secreted from human neutrophils **(Kjeldsen et al., 1994).** In mice, multiple tissues can express Lcn2 including uterus, bone marrow, immune cells, liver, spleen, and kidney **(Aigner et al., 2007)**.

Moreover, Lcn2 was identified as a new adipokine that is expressed and secreted by adipocytes, and it was reported to have a role in metabolism **(Yan et al., 2007 and Zhang et al., 2008).** It is also known to play a vital role in innate immunity and protect against bacterial infection **(Kang et al., 2017).**

Interestingly, testosterone is an anabolic hormone that affects body composition, body fat distribution **(Marin et al., 1992),** triglyceride and cholesterol levels **(Phillips et al., 2003).** Furthermore, low testosterone concentration may be considered a risk factor for the development of the metabolic syndrome in men as it may lead to central obesity and dyslipidemia **(Laaksonen et al., 2004 and Muller et al., 2005).**

However, the effect of orchidectomy on body fat distribution and development of metabolic syndrome in rats showed conflicting results **(Varlamov et al., 2012).**

Luque-Ramʔrez et al. (2013) and Martʔnez-Garcʔa et al. (2013) have suggested that there are inter-connections between androgens and Lcn2. In rat studies, **Nantermet et al. (2004)** reported the presence of putative androgen response elements in the promoter regions of lipocalin encoding genes. Furthermore, Lcn2 influences the aromatase enzyme activity, which is considered a key enzyme of conversion of androgens to estrogen in adipose tissue and granulosa cells **(Fried & Greenberg, 2012 and Guo et al., 2012).**

To the best of our knowledge, no previous researches have studied the association between Lcn2 and serum testosterone level in adult male orchi-dectomized rats. However, several studies were done on the relation between Lcn2 and androgen in patients with polycystic ovary syndrome (PCOs) with clinical and/ or biochemical signs of hyper-androgenism and their results were inconsistent.

As **Diamanti-Kandarakis et al. (2008) and Gencer et al. (2014)** found that lipocalin-2 concentrations were significantly lower in women with PCOS. On the other hand, **Cakal et al. (2011) and Yilmazet al. (2017)** found high Lcn2 levels in PCOS with no association with body fat percentage and were associated only with free testosterone.

So, the aim of this study was to clarify the possible effect of orchidectomy and testosterone replacement therapy on serum Lcn2 levels and lipid profile in orchidectomized adult male albino Wistar rats**.**

MATERIALS AND METHODS

Thirty two healthy adult male albino Wistar rats were used. Their weight ranged from 200-250 g. The rats were derived from the animal house, Faculty of Veterinary Medicine, Zagazig University. The rats were kept in steel wire cages (50×30×20 cm), 4 rats per cage. They were housed at standard conditions (25-30°C, natural dark/light cycle), and received food and water ad libitum. The animals were left to acclimatize for one week, then the animals were divided into 4 equal subgroups: **control group (I):** Rats were sham operated. Each rat received a daily dose of 0.2 ml/ 100g body weight sesame oil subcutaneously (sc); **Group (II) (Orchi group):** Rats were bilaterally orchidectomized then received daily dose of 0.2 ml/ 100g sesame oil subcutaneously (sc)**; Group (III) (Orchi +hormonal replacement):** Rats were bilaterally orchidectomized then each rat received s.c. injection of physiologic dose of testosterone (0.3 mg/ 100g body weight dissolved in 0.2 ml sesame oil/ injection) given every other day **(Staprans et al., 1999); Group (IV) (Orchi +supra-T):** Rats were bilaterally orchidectomized then each rat received daily supra-physiologic doses of testosterone (3 mg/ 100g body weight dissolves in 0.2 ml sesame oil/injection) **(Jezek et al., 1993)**. Administration of drugs started one week after surgical procedures and continued for 14 days in all treatment groups.

Surgeries were performed after anesthe-tizing the rats with pentobarbital sodium (40 mg/kg) **(Irahara et al., 2001).** The

rats were placed in supine position. After shaving and sterilization of the skin of the scrotal area, the skin was incised, the tunica vaginalis was opened, and the testis and epididymis were removed after ligation of vas deference and scrotal blood vessels. Remaining tissues were returned to scrotal sac and incision was closed by non- absorbable sutures. The procedure was repeated on the other side. The same procedure was done in sham-operated rats without removal of the testis or epididymis **(Foley, 2005)**.

Body mass index (BMI) was estimated according to the equation: body weight (g) /lengh^2 (cm) = BMI (g/cm^2) **(Novelli et al., 2007)**.

Blood collection: At the end of experimental period, animals were sacrificed, under light ether anesthesia after an over-night fasting, and blood samples (6-8 ml / rat) were obtained between 9-11 a.m. The serum was stored at -20℃ until assayed.

Biochemical Analysis:

1. **Serum Lcn2 levels** according to **Goetz et al. (2002)** using rat Lcn2 enzyme-linked immunosorbent assay (ELISA) kit (Catalog Number: 201-11-5109, shanghai sunred biological technology, China).
2. **Serum total cholesterol (TC) levels** according to **Allain et al. (1974)** using rat cholesterol ELISA kit (Catalog Number: 2011-11-0198, shanghai sunred biological technology, China).
3. **Serum triglycerides (TG) levels** according to **Naito (1989)** using rat triglycerides ELISA kit (Catalog Number: 2011-11-0250, shanghai sunred biological technology, China).
4. **Serum high density lipoproteins (HDL) levels** according to **Warnick et al. (1983)** using rat HDL-cholesterol ELISA kit (Catalog Number: 2011-11-0255, shanghai sunred biological technology, China).
5. **Serum low density lipoproteins (LDL) levels** according to **Friedwald et al. (1972)** LDL was calculated as follows: LDL=TC-HDL-TG/5.
6. **Serum testosterone levels** according to **Tietz (1998)** using rat testosterone ELISA kit (Catalog Number: 2011-11-5126, shanghai sun red biological technology, China).
7. **Serum follicular stimulating hormone (FSH) levels** according to **Rebar et al. (1982)** using rat FSH ELISA kit (Catalog Number: 2011-11-0183, shanghai sunred biological technology, China).
8. **Serum Luteinizing hormone (LH) levels** according to **Tietz (1985)** using rat LH ELISA kit (Catalog Number: 2011-11-0180, shanghai sunred biological technology, China).
9. **Serum tumor necrosis- α (TNF-α)** according to **Engelberts et al. (1991)** by rat TNF–α ELISA kits (Elabscience Biotechnology, USA. Cat: EEL-H0109).
10. **Serum interleukin- 1β (IL-1β)** according to **Yasuoka et al. (2003)** using the rat IL-1β Elisa kit (Bender Med System GmbH, Vienna, Austria).

Statistical analysis: The data obtained in the present study were expressed as mean ± SE for quantitative variables, one way ANOVA with LSD was done to compare means between groups. Pearson correlation was done (P value less than

0.05 was considered significant). The statistical analysis was done by using SPSS program (version 18 for windows) (SPSS Inc. Chicago, IL, USA.

RESULTS

Our results showed no significant difference in BMI among all groups (p>0.05). In group (II) and (III), there was a significant decrease in serum Lcn2 versus control group (p<0.01; p<0.05 respectively), while there was a non-significant change in serum Lcn2 between group (II) and group (III) (p>0.05). In group (IV), serum Lcn2 showed a significant decrease versus control group (p<0.001), group (II) (p<0.01) and group (III) (p<0.001); [Table 1, Fig 1].

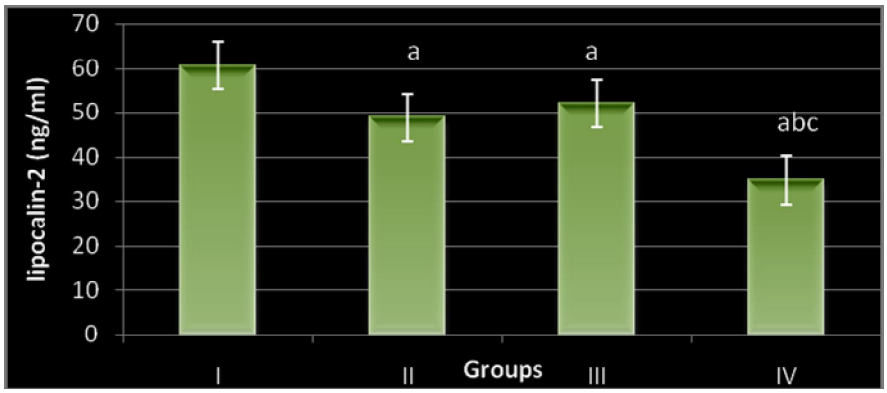


Figure (1): Serum Lcn2 in all groups.
a: sig versus group I,; b: sig versus group II; c: sig versus group III

Furthermore, serum testosterone significantly decreased in group (II) accompanied with significant increase in serum LH and FSH when compared with control group (p<0.001). In group (III), serum testosterone, LH and FSH showed insignificant difference compared to control (P>0.05). In group (IV), serum testosterone significantly increased, while serum LH and FSH significantly decreased. These changes were significant when compared to all groups (p<0.001) [Table 1].

Additionally, in group (II), there was no significant change in serum TG or HDL levels (p>0.05), while a significant increase was found in TC and LDL levels versus control (p<0.01; p<0.05; respectively). In contrast, group (III) showed a non-significant difference in TC, TG, LDL or HDL levels versus control (p>0.05). However, in group (IV), there was a significant increase in TC (p<0.001), TG (p<0.05), LDL (p<0.001) and a significant decrease in HDL (p>0.001) versus control [Table 2].

No significant difference was found in serum level of IL-1β and TNFα between group (I), (II) and (III) (p>0.05), but there was a significant decrease in serum levels of IL-1β and TNFα in group IV in comparison to other groups (p< 0.001) [Table 2].

Table (1): Serum hormonal levels in all studied groups.

Parameters \ Groups	I	II	III	IV
Lipocalin-2(ng/ml)	60.6±3.82	48.78±2.52^{*a}	52.01±2.38^{*a}	34.71±2.46^{*a,b,c}
Testosterone(ng/ml)	8.13±0.51	2.05±0.34^{*a}	8.56±0.44^{*b}	13.02±0.72^{*a,b,c}
LH (mIU/ml)	14.22±0.79	22.52±1.06^{*a}	13.6 ±0.71^{*b}	8.63 ±0.6^{*a,b,c}
FSH (mIU/ml)	12.28± 0.56	17.33 ±0.54^{*a}	13.26± 0.58^{*b}	8.76± 0.3^{*a,b,c}

Table (2): Serum lipid profile and BMI in all studied groups

Parameters \ Groups	I	II	III	IV
TC (mg/dl)	74.84± 3.8	88.2± 1.8^{*a}	77.48 ±1.8^{*b}	95.52 ±3.1^{*a,c}
TG (mg/dl)	104.81± 6.2	116.26 ±7.8	101.55 ±4.5	129.1 ±8.05^{*a,c}
LDL (mg/dl)	20.52± 1.6	28.53± 2^{*a}	20.53 ±1.2^{*b}	46.94 ±4.1^{*a,b,c}
HDL (mg/dl)	37.49± 1.8	36.41 ±1.6	36.71± 1.9	22.76 ±3.5^{*a,b,c}
BMI(kg/m^2)	0.61± 0.24	0.58 ±0.022	0.63 ±0.023	0.64 ±0.021
TNF-α (pg/ml)	1.33±0.67	1.51±0.10	1.41±0.40	0.52±0.6^{*a,b,c}
Il-1β(pg/ml)	4.05±0.26	5.03±0.20	4.82±0.17	1.48±0.16^{*a,b,c}

*: significant ($p<0.05$); a: versus (I); b: versus (II); c: versus (III)

No significant correlation was found between serum Lcn2 and BMI, serum TC, TG, LDL and HDL levels in groups (I, II, III) ($p>0.05$). However, there was a significant negative correlation between Lcn2 and TC, TG, LDL in group (IV) ($P< 0.01$, $p<0.05$, $p<0.05$; respectively) [Table 3, Fig 7A,B,C], and there was a significant positive correlation between its level and HDL ($p<0.05$) in the same group [Table 3, Fig 7D].

A significant negative correlation was found between serum Lcn2 and serum testosterone in group (IV) ($r=-0.781$, $p<0.05$)[Table 3, Fig 4], while a positive correlation was found between serum Lcn2 and serum LH ($r=0.713$, $p<0.05$) and serum FSH in the same group ($r=0.832$, $p<0.05$) [table 3]. A non-significant correlation was found between serum Lcn2 and testosterone in group (II) ($r=0.379$, $p>0.05$)[Fig 2] and (III), ($r= -0.229$, $p>0.05$) [Fig3].

Serum levels of Lcn2 correlated positively with IL-1β ($p<0.01$) and TNFα ($p<0.001$) in group IV ($r=0.868$, $r= 0.958$; respectively) [Fig 5&6].However, a non-significant correlation was found betweenLcn2 and IL-1β in group (I), (II) and (III) ($r=0.143$, 0.528 and 0.670; respectively). Moreover, a non-significant correlation was found betweenLcn2 and TNF-α in group (I), (II) and (III) ($r=0.632$, 0.337 and 0.616; respectively) [Table 3].

Table (3): Correlation between lipocalin-2 and measured biological parameters

Parameters \ Groups	I	II	III	IV
BMI(kg/m2)	0.224	0.019	0.095	0.287
Testosterone(ng/ml)	0.206	0.379	-0.229	-0.781*
LH (mIU/ml)	0.131	-0.109	0.223	0.713*
FSH (mIU/ml)	0.554	-0.135	0.104	0.832*
TC (mg/dl)	-0.683	-0.035	0.206	-0.847**
TG (mg/dl)	0.632	0.093	0.454	-0.736*
LDL (mg/dl)	-0.462	-0.318	0.469	-0.783*
HDL (mg/dl)	-0.187	0.255	-0.328	0.736*
IL-1β (pg/ml)	0.143	0.528	0.670	0.868**
TNFα (pg/ml)	0.632	0.337	0.616	0.958***

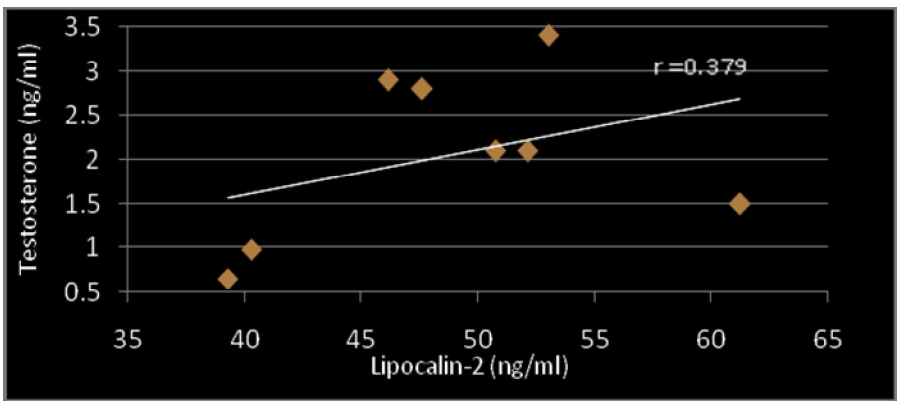


Figure (2): Correlation between serum lipocalin-2 and serum testosterone in group II.

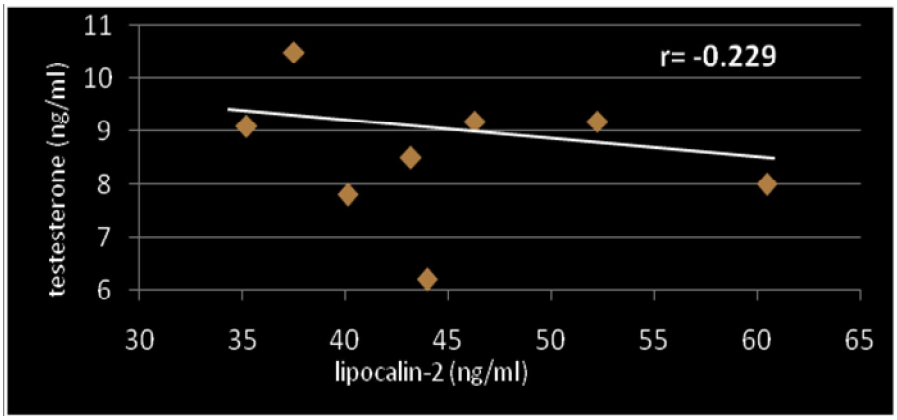


Figure (3): Correlation between serum lipocalin-2 and serum testosterone in group III.

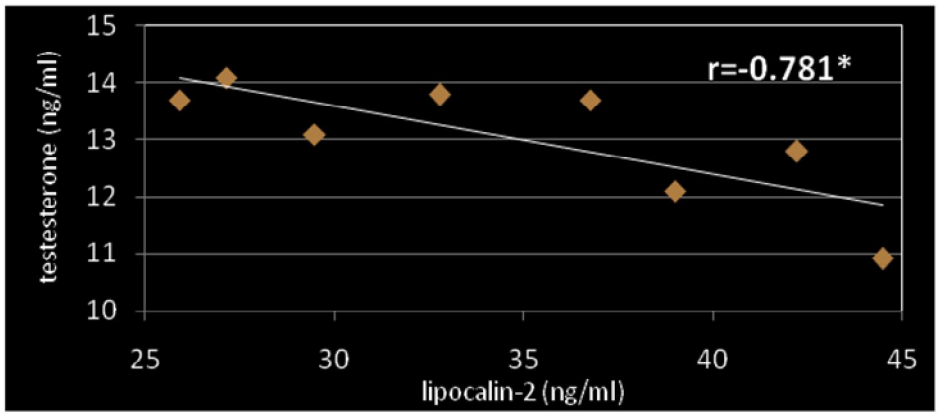
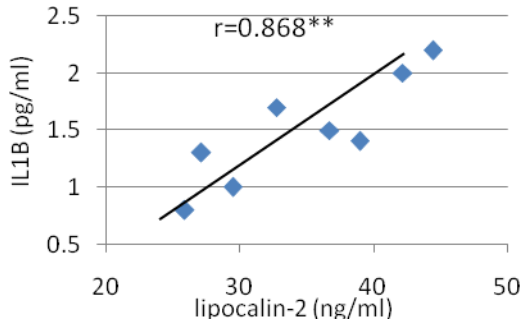


Figure (4): Correlation between serum lipocalin-2 and serum testosterone in group IV.



Figue (5): Correlation between serum lipocalin-2 and IL-1β in group IV.

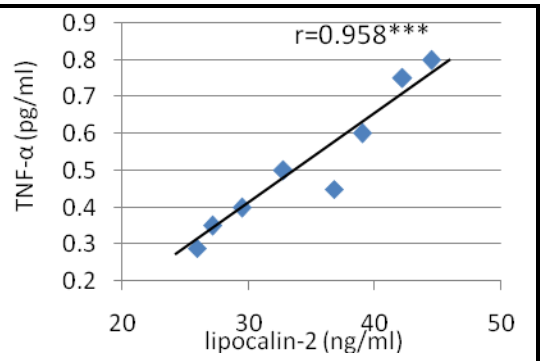
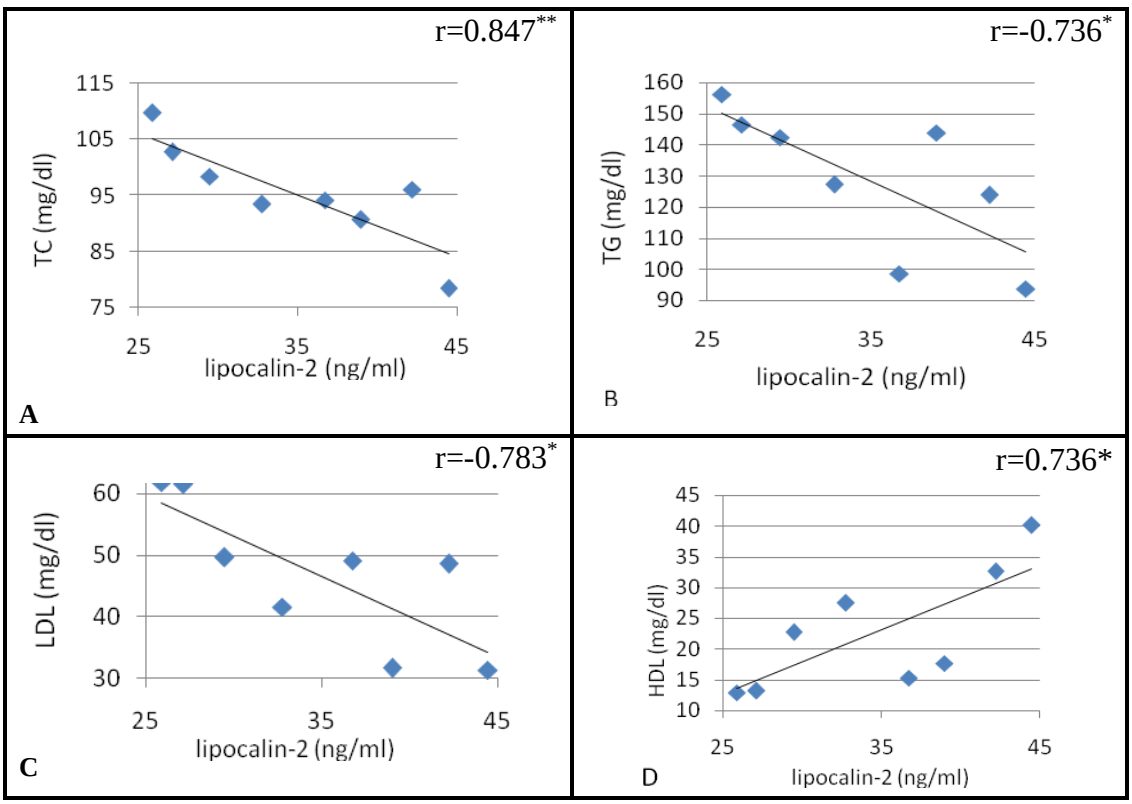


Figure (6): Correlation between serum lipocalin2 and TNF-α in group IV



Figures (7 A, B, C, D): Correlation between serum lipocalin-2 and lipid profile in group IV.
*significant: ($p<0.05$); **: ($p<0.01$); ***: ($p<0.001$).

DISCUSSION

Serum level of Lcn2 showed a significant reduction after orchidectomy. We expected an increase in serum Lcn2 level after orchidectomy because in human testosterone deficiency resulted in a state that includes certain criteria of metabolic syndrome **(Laaksonen et al., 2004),** like central obesity, dyslipidaemia, impaired glucose tolerance and insulin resistance **(Muller et al., 2005 and Brand et al., 2014).** These effects were attributed to an increase in body fat especially visceral fat **(Clegg et al., 2003 and Christoffersen et al., 2006)** which is one of the main sources of Lcn2 **(Yan et al., 2007).** However, in this study, there was no significant change in BMI of orchidectomized group versus control. This was in accordance with previous studies in rats which showed that orchidectomy in rats differs from human as it led to a decrease in food intake, percentage of lean body mass and an increase in fat percentage mainly in subcutaneous, but not visceral fat mass **(Clegg et al., 2003 and Christoffersen et al., 2006).** These reports about the change in body fat distribution following orchidectomy in rats might be partially responsible for the reduction in serum Lcn2.

Moreover, the reduction in Lcn2 level can also be explained by removal of the testis and epididymis during orchidectomy. Lcn2 protein was found to be expressed in male reproductive system as **Lee et al. (2003)** reported that Lcn2 mRNA is produced in both spermatogonial cells and Sertoli cells in the testis. In addition, Lcn2 is expressed in significant amounts in the caput of the epididymis bound to spermatozoa, and this expression was reported to be under androgen control **(Suzuki et al., 2004 and Plant & Zeleznik, 2014).** Interestingly, epididymal Lcn2 level increases by inflammatory stimuli that indicate a role for Lcn2 as a part of the innate immune response against bacterial infection in the epididymis **(Flo et al., 2004 and Zhang et al., 2008)**. Furthermore, Lcn2 was reported to play a role in normal function of spermatozoa. Mouse Lcn2 delivers ferric iron to spermatozoa by internalization **(Elangovan et al., 2004)**. It also enhances sperm motility by increasing pH inside the cell and elevating intracellular cAMP **(Lee et al., 2003).** These reports indicate a dual action of Lcn2 in the epididymis **(Plant and Zeleznik, 2014)**.

The results also showed that hormonal replacement of rats with physiologic doses of testosterone caused slight elevation of serum Lcn2 versus control, but this increase did not reach statistical significance. Physiologic doses of testosterone following orchidectomy were associated with normal lipid profile versus control and may have caused normal fat distribution and improvement of metabolic disturbance caused by orchidectomy. These effects may result in this partial correction of serum Lcn2.

In the current study, we also noticed a further reduction of Lcn2 in group IV after administration of supra-physiologic dose of testosterone to orchidectomized rats. This reduction was significant compared to both group II and III. Moreover, serum levels of Lcn2 were negatively correlated with serum testosterone levels in this group. Our results were in line with those of **Diamanti-**

Kandarakis et al. (2008) who found low level of Lcn2 in PCOS patients with negative association with testosterone level.

This marked reduction in Lcn2 in this group might be attributed to the effect of supra-physiologic doses of testosterone on cytokine production, as our results showed that injecting orchidectomized rats with supra-physiologic doses of testosterone was associated with a significant decrease in serum levels of IL-1β and TNFα, and these levels were positively correlated with Lcn2. Previous studies reported that supra-physiological concentrations of testosterone suppresses pro-inflammatory cytokines and up-regulate anti-inflammatory cytokines as it attenuates the production of inflammatory cytokines such as TNFα, IL-1β **(Corcoran et al., 2010 and Xu et al., 2015)**, and IL-6 **(Vodo et al., 2013)**. IL-1β showed most profound effect on lcn2 expression and secretion in 3T3-L1 adipocytes **(Zhang et al., 2014)**. Additionally, liver tissue was the most sensitive to TNFα treatment and resulted in an elevation of Lcn2 mRNA levels by more than 2000-folds **(Zhao et al., 2014)**. So, the high level of testosterone in group IV might have decreased Lcn2 level by suppressing its production from the liver and adipose tissue through the inhibitory effect of testosterone on pro-inflammatory cyto-kines.

However, in our study, there was a non-significant change in IL-1β and TNF-α level between groups (I), (II) and (III) which was in line with those of **Chin and Ima-Nirwana, (2017)** who reported that the changes in these cytokine levels might not be significant enough to be detected in vivo.

In addition, **McInnes et al. (2012)** reported that androgen receptor activation in murine 3T3 adipocytes down-regulates retinol-binding protein 4 (RBP4) mRNA. Lcn2 belongs to the same lipocalin super-family members of fatty acid binding proteins and retinol binding proteins with structural similarity **(Lalonde et al., 1994)**. So, a similar effect of testosterone on lcn2 expression from adipocytes might be expected. Besides, high levels of testosterone can decrease adipose tissue mass which is one of the main sources of Lcn2. The absence of significant change in BMI in this group might be due to the anabolic effect of testosterone which increases lean body mass (muscle mass) with concomitant decrease in adipose tissue mass **(Amsterdam et al., 2010).**

Considering lipid profile, in orchidectomized group, serum levels of total cholesterol and LDL showed significant increase versus control. Physiologic doses of testosterone decreased these levels back to control group levels. These findings agreed with the results of previous cross-sectional studies which demonstrated an association between low serum testosterone and high total cholesterol and LDL-C levels **(Barud et al., 2002 and Yao et al., 2011).** Moreover, total cholesterol and LDL-C significantly increase in prostatic cancer patients receiving androgen deprivation therapy (ADT) **(Braga-Basaria et al., 2006 and Yannucci et al., 2006).** This effect may be due to decrease in activity of hepatic lipase (HL) and lipoprotein lipase (LPL) which depends on gonadal

hormones in its function **(Tikkanen and Nikkila, 1987).**

In addition, the increased total cholesterol in orchidectomized group may also be due to increase in acetyl CoA arising from an increase in β-oxidation of fatty acids, and acetyl CoA is a key substrate in the synthesis of cholesterol **(Rang et al., 1995).** Furthermore, LDL-C is the primary transporter of plasma cholesterol. So, the increase in LDL might be secondary to the increase in total cholesterol or reduction in LDL uptake by LDL receptor (PPAR-α and PPAR-γ) due to its down regulation by Low testosterone **(Konstantinos and Christos, 2014)**.

However, in our study, no significant change was found in serum triglyceride or HDL-C in orchidectomized group, which was in line with the results of **Kiel et al. (1989) and Denti et al. (2000)** who found no association between level of serum lipids and endogenous testosterone. Others reported an increase in serum triglycerides and a decrease in HDL-C levels **(Haffner et al., 1993)**.

Moreover, our results revealed significant elevation in total cholesterol, triglycerides, LDL and a significant reduction in HDL in group IV with injection of supra-physiologic doses of testosterone. This atherogenic lipid profile was previously described by **Awad et al. (2012)** when treated adult male albino rats with androgen anabolic steroids. Also, **George (2003) and Gold et al. (2006)** found an increase in triglycerides and total cholesterol level in androgen anabolic steroid (AAS) abusers. This effect of testosterone is induced mainly by induction of hepatic triglyceride lipase (HTGL) which is present in the luminal surface of hepatic endothelium and catabolizes HDL via its phospholipase activity **(Awad et al., 2012).** The activity of, HTGL has been reported to show a significant increase with androgen anabolic steroid therapy **(Applebaum-Bowden et al., 1987).** Besides, it was suggested that serum LDL levels may increase due to the induction of the enzyme HTGL and catabolism of very low density lipoprotein **(Baldo-enzi et al., 1990).**

Additionally, the results of this work showed a significant negative correlation between serum Lcn2 and TC, TG and LDL accompanied by a significant positive correlation with HDL in group (IV). These findings can be explained by the results of **Paton et al. (2013)** who reported that Lcn2 promotes total energy expenditure, lipid clearance and fatty acid oxidation via increased expression of genes involved in β-oxidation including peroxisome proliferator activated receptor-δ. So, the reduction in Lcn2 can affect lipid metabolism and participate in this atherogenic lipid profile.

Our results disagreed with those of **De la Chesnaye et al. (2015)** who demonstrated a decrease in Lcn2 level in patients with type 2 diabetes mellitus with no significant correlation between its levels and lipid profile, and suggested that the reduction in Lcn2 levels depend on the inflammation process through an unknown mechanism. **Choi et al. (2008)** also did not find any relationship between BMI, waist circumference, triglyceride, fasting glucose, and Lcn2 levels in patients with chronic heart disease. The conflicting reports about the correlation between Lcn2 and lipid profile may be

due to differences in species, study design, underlying disease, or feeding behavior.

CONCLUSION

Normal physiologic levels of testosterone are needed for normal lipocalin-2 expression. Disturbance of testosterone level (either by decrease or increase) significantly decreased lipocalin-2 level.

These findings highlight the possible association between serum Lcn2 and serum testosterone levels and the possible adverse effects of testosterone abuse on male fertility.

Further human studies are needed to clarify whether orchidectomy have a similar effect on serum lipocalin-2 in human or not, and to identify different hormonal and humeral factors affecting Lcn2 expression with more concentration on its impact on male reproductive function.

REFERENCES

1. **Aigner F, Maier HT, Schwelberger HG, Wallnofer EA and Amberger A (2007):** Lcn2 regulates the inflammatory response during ischemia and reperfusion of the transplanted heart. Am. J Transplant., 7: 779–788.
2. **Allain C, Poon LS, Chan CS, Richmond W and Fu PC (1974):** Enzymatic determination of total serum cholesterol. Clin. Chem., 20: 470-475.
3. **Amsterdam JV, Opperhuizen A and Hartgens F (2010):** Adverse health effects of anabolic-androgenic steroids. Regulatory Toxicology and Pharmacology, 57: 117-123.
4. **Applebaum-Bowden D, Haffner S M and Hazzard WR (1987):** The dys-lipoproteinemia of anabolic steroid therapy: Increase in hepatic triglyceride lipase precedes the decrease in high density lipoprotein 2 cholesterol. Metabolism, 36: 949-952.
5. **Awad TE, Taha EM, Hassan MS and Amany FY (2012):** Modulatory Effects of Artichock Leave Extract on Nandrolone Decanoate Induced Biochemical Alterations in Rats. Global Journal of Biotechnology & Biochemistry, 7 (2): 68-78.
6. **Baldo-enzi G, Giada F, Zuliani G, Baroni L, Vitale E, Enzi G, Magnanini P and Fellin R (1990):** Lipid and apoprotein modifications in body builders during and after self-administration of anabolic steroids. Metabolism, 39: 203-8.
7. **Barud W, Palusin´ ski R, Beltowski J and Wo´ jcicka G (2002):** Inverse relationship between total testosterone and anti-oxidized low density lipoprotein antibody levels in ageing males. Atherosclerosis, 164: 283–288.
8. **Braga-Basaria M, Muller DC, Carducci MA, Dobs AS and Basaria S (2006):** Lipoprotein profile in men with prostate cancer undergoing androgen deprivation therapy. International Journal of Impotence, 18: 494–498.
9. **Brand JS, Rovers MM, Yeap BB, Schneider HJ, Tuomainen T-P, Haring R, Corona G, Onat A, Maggio M and Bouchard C (2014):** Testosterone, Sex Hormone-Binding Globulin and the Metabolic Syndrome in Men: An Individual Participant Data Meta-Analysis of Observational Studies. PLoS One, 9(7): e100409.
10. **Cakal E, Ozkaya M, Engin-Ustun Y and Ustun Y (2011):** Serum lipocalin-2 as an insulin resistance marker in patients with polycystic ovary syndrome. J Endocrinol Invest., 34(2): 97–100.
11. **Chin K-Y and Ima-Nirwana S (2017):** The Effects of Testosterone Deficiency and Its Replacement on Inflammatory Markers in Rats: A Pilot Study. Int J Endocrinol Metab., 15(1): e43053.
12. **Choi KM, Lee JS, Kim EJ, Baik SH, Seo HS, Choi DS, Oh DJ and Park CG (2008):** Implication of lipocalin-2 and visfatin levels in patients with coronary heart disease. European Journal of Endocrinology, 158: 203–207.
13. **Christoffersen B, Raun K, Svendsen O, Fledelius C and Golozoubova V (2006):** Evaluation of the orchidectomized male Sprague–Dawley rat as a model of the metabolic syndrome and type 2 diabetes International Journal of Obesity, 30: 1288–1297.
14. **Clegg DJ, Benoit SC, Fisher ME, Barrera JG, Seeley RJ and Woods SC (2003):** Sex

hormones determine fat distribution and sensitivity to adiposity signals. Appetite, 52(1):324- 40.

15. **Corcoran MP, Meydani M, Lichtenstein AH, Schaefer EJ, Dillard A and Lamon-Fava S (2010):** Sex hormone modulation of pro-inflammatory cytokine and C-reactive protein expression in macrophages from older men and postmenopausal women. J. Endocrinol., 206: 217–224.

16. **De la Chesnaye E, Manuel-Apolinar L, Zarate A, Damasio L, Espino N, Revilla-Monsalve MC and Islas-Andrade S (2015):** Lipocalin-2 plasmatic levels are reduced in patients with long-term type 2 diabetes mellitus. Int J Clin Exp Med., 8(2): 2853-2859.

17. **Denti L, Pasolini G, Sanfelici L, Benedetti R, Cecchetti A, Ceda GP, Ablondi F and Valenti G (2000):** Aging-related decline of gonadal function in healthy men: correlation with body composition and lipoproteins. Journal of the American Geriatrics Society, 48: 51–58.

18. **Diamanti-Kandarakis E, Livadas S, Kandarakis SA, Margeli** http://www.eje-online.org/content/158/4/525.full - aff-1A and Papassotiriou http://www.eje-online. org/content/158/4/525.full - aff-1 **I (2008):** Serum concentrations of atherogenic proteins neutrophil gelatinase-associated lipocalin and its complex with matrix metalloproteinase-9 are significantly lower in women with polycystic ovary syndrome: hint of a protective mechanism? Eur J Endocrinol., 158(4): 525–531.

19. **Elangovan N, Lee YC, Tzeng WF and Chu ST (2004):** Delivery of ferric ion to mouse spermatozoa is mediated by lipocalin internalization. Biochem Biophys Res Commun., 319(4):1096-104.

20. **Engelberts I, M?ller A, Schoen GJ, Van der Linden CJ and Buurman WA. (1991):** Evaluation of measurement of human TNF in plasma by ELISA. Lymphokine Cytokine Res., 10(1-2):69-76.

21. **Flo TH, Smith KD , Sato S, Rodriguez DJ, Holmes MA, Strong RK, Akira S and Aderem A (2004):** Lipocalin 2 mediates an innate immune response to bacterial infection by sequestrating iron. Nature, 432: 917–921.

22. **Foley PL (2005):** Common Surgical Procedures in Rodents. In: RJDaS M.A., editor. Laboratory animal medicine and management. Office of Animal Research Education and Compliance, University of Virgini, Charlottesville, VA, USA: International Veterinary Information Service, Ithaca NY.

23. **Fried SK and Greenberg AS (2012):** Lipocalin 2: a “sexy” adipokine that regulates 17β-estradiol and obesity. Endocrinology, 153(4): 1582–1584.

24. **Friedwald WT, Levy RI and Fredrickson D (1972):** Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem., 18:499-502.

25. **Gencer M, Gazi E, Hacıvelioğlu S, Barutçu A, Türk?n H, Temiz A, Altun B, Vural A, Cevizci S, Kumcular T and Coşar E (2014):** The relationship between subclinical cardiovascular disease and lipocalin-2 levels in women with PCOS. Eur J Obstet Gynecol Reprod Biol., 181: 99–103.

26. **George AJ (2003):** The actions and side effects of 54. Anabolic Steroids in Sport and Social Abuse Androgeneset Sports, 13(4): 354-366.

27. **Goetz DH, Holmes MA, Borregaard N, Bluhm ME, Raymond KN and Strong RK (2002):** The neutrophil lipocalin NGAL is a bacteriostatic agent that interferes with siderophore mediated iron acquisition. Molec Cell., 10: 1033-1043.

28. **Gold J, Batterham MJ, Rekers H, Harms MK, Geurts TB, Helmyr PM, Silva de Mendonça J, Falleiros-Carvalho LH and Panos G (2006):** Effect of nandrolone decanoate compared with placebo or testosterone on HIV-associated wasting. HIV Med., 7(3): 146-155.

29. **Guo H, Zhang Y, Brockman DA, Hahn W, Bernlohr DA and Chen X (2012):** Lipocalin 2 deficiency alters estradiol production and estrogen receptor signaling in female mice. Endocrinology, 153(3): 1183–1193.

30. **Haffner S, Mykkanen L, Valdez R and Katz M (1993):** Relationship of sex hormones to lipids and lipoproteins in non-diabetic men. J Clin Endocrinol and Metabol., 77:1610–1615.

31. Irahara M, Tamura T, Matuzaki T, Saito S, Yasui T, Yamano S, Kamada M and Aono T (2001): Orexin-A suppresses the pulsatile secretion of luteinizing hormone via β-endorphin. Biochemical and Biophysical Research Communications, 281; 232-236.

32. Jezek D1, Simunić-Banek L and Pezerović-Panijan R (1993): Effects of high doses of testosterone propionate and testosterone enanthate on rat seminiferous tubules--a stereological and cytological study. Arch Toxicol, 67(2):131-40.

33. Kang SS, Ren Y, Liu C-C, Kurti A, Baker KE, Asmann Y, Bu GY and Fryer JD (2017): Lipocalin-2 protects the brain during inflammatory conditions. Molecular Psychiatry, 00, 1–7.

34. Kiel DP, Baron JA, Plymate SR and Chute CG (1989): Sex hormones and lipoproteins in men. American Journal of Medicine, 87: 35–39.

35. Kjeldsen L, Bainton DF, Sengelov H and Borregaard N (1994): Identification of neutrophil gelatinase-associated Lcn as a novel matrix protein of specific granules in human neutrophils. Blood, 83(3):799–807.

36. Kjeldsen L, Johnsen AH, Sengelov H and Borregaard N (1993): Isolation and primary structure of NGAL, a novel protein associated with human neutrophil gelatinase. J Biol Chem., 268(14):10425–10432.

37. Konstantinos NA and Christos SM (2014): Novel concepts in lipoprotein particle metabolism and regulation. Metabolism, 63(1): 1-4.

38. Laaksonen DE, Niskanen L, Punnonen K, Nyysso¨nen K, Tuomainen TP, Valkonen VP, Salonen R and Salonen JT (2004): Testosterone and sex hormone-binding globulin predict the metabolic syndrome and diabetes in middle aged men. Diabet. Care, 27: 1036–1041.

39. La Londe JM, Bernlohr DA and Banaszak LJ (1994): The up-and-down beta-barrel proteins. FASEB J., 8: 1240–1247.

40. Lee YC, Liao Jr C, Li PT, Tzeng WF and Chu ST (2003): Mouse Lcn as an enhancer of spermatozoa motility. Mol Biol Rep., 30:165–172.

41. Luque-Ram?rez M, Mart?nez-Garc?a M? , Montes-Nieto R, Fern?ndez-Dur?n E, Insenser M, Alpa?és M and Escobar-Morreale HF (2013): Sexual dimorphism in adipose tissue function as evidenced by circulating adipokine concentrations in the fasting state and after an oral glucose challenge. Hum Reprod., 28(7): 1908–1918.

42. Marin P, Holmang S, Jonsson L, Sjostrom L, Kvist H and Holm G (1992): The effects of testosterone treatment on body-composition and metabolism in middle-aged obese men. Int J Obes Relat Metab Disord., 16: 991–997.

43. Mart?nez-Garc?a M? , Montes-Nieto R, Fern?ndez-Dur?n E, Insenser M, Luque-Ram?rez M and Escobar-Morreale HF (2013): Evidence for masculinization of adipokine gene expression in visceral and subcutaneous adipose tissue of obese women with polycystic ovary syndrome (PCOS). J Clin Endocrinol Metab., 98(2): E388–E396.

44. Mc Innes KJ, Smith LB, Hunger NI, Saunders PTK, Andrew R and Walker BR (2012): Deletion of the Androgen Receptor in Adipose Tissue in Elevates Retinol Binding Protein 4 and Reveals Independent Effects on Visceral Fat Mass and on Glucose Homeostasis. Diabetes, 61:1072–1081.

45. Muller M, Grobbee DE, den Tonkelaar I, Lamberts SWJ and van der Schouw YT (2005): Endogenous sex hormones and metabolic syndrome in aging men. J Clin Endocrinol Metab., 90: 2618–2623.

45. Naito HK (1989): Triglycerides in clinical chemistry: theory, analysis and correlation. Second edition by Kaplan LA and Pesce AJ. (U.S.A.), P. 997.

47. Nantermet PV, Xu J, Yu Y, Adamski S, Gentile MA, Kimmel DB, Harada S, Gerhold D, Freedman LP and Ray WJ (2004): Identification of genetic pathways activated by the androgen receptor during the induction of proliferation in the ventral prostate gland. J Biol Chem., 279(2): 1310–1322.

48. Novelli EL, Diniz YS, Galhardi CM, Ebaid GM, Rodrigues HG, Mani F, Fernandes AA, Cicogna AC and Novelli Filho JL (2007): Anthropometrical parameters and markers of obesity in rats. Laboratory Animals, 41, 111–119.

49. Paton CM, Rogowski MP, Kozimor AL, Stevenson JL, Chang H and Cooper JA (2013): Lipocalin-2 increases fat oxidation in vitro and is correlated with energy expenditure in normal weight but not obese women. Obesity, 21(12): E640–E648.

50. Phillips GB, Jing T and Heymsfield SB (2003): Relationships in men of sex hormones, insulin, adiposity, and risk factors for myocardial infarction. Metabolism, 52: 784–790.

51. Plant TM and Zeleznik AJ (2014): Major protein families in the epididymis and their regulation. Knobil and Neill's Physiology of Reproduction. Pbl. Academic Press, edition 4, page 735**.**

52. Rang AP, Dale MM and Ritter JM (1995): Pharmacology. Third Edition. Pbl. New York, Churchill Livingstone, 409-410.

53. Rebar RW, Erickson GF and Yen SSC (**1982**): Idiopathic premature ovarian failure: clinical and endocrine characteristics. Fertil. Steril., 37: 35– 41**.**

54. Staprans N, Rapp JH, Pan X-M, Donald L, and Feingold KR (1990): Testosterone Regulates Metabolism of Plasma Chylo-microns in Rats. Arteriosclerosis, 10:591-596.

55. Suzuki K, Lareyre JJ, S?nchez D, Gutierrez G, Araki Y, Matusik RJ and Orgebin-Crist MC (2004): Molecular evolution of epididymal lipocalin genes localized on mouse chromosome 2.Gene, 15: 339:49-59.

56. Tietz N (1998): Fundamentals of Chlinical Chemistry, Pbl. W. B. Saunders, Philadelphia, PA.

57. Tietz NW (1995): Clinical Guide to Laboratory Tests, 3rd Ed., Pbl. W.B. Saunders Company, Philadelphia, PA 19106.

58. Tikkanen, MJ and Nikkila EA (1987): Regulation of hepatic lipase and serum lipoproteins by sex steroids. American Heart Journal, 113: 562-7.

59. Varlamov O, White AE, Carroll JM, Bethea CL, Reddy A, Slayden O, O'Rourke RW, and Roberts CT (2012): Androgen Effects on Adipose Tissue Architecture and Function in Nonhuman Primates. Endocrinology, 153(7): 3100–3110.

60. Vodo S, Bechi N, Petroni A, Muscoli C and Aloisi AM (2013): Testosterone-Induced Effects on Lipids and Inflammation. Mediators of Inflammation, Article ID 183041, 8.

61. Warnick GR, Benderson V and Albers N (1983): Selected methods. Clin Chem., 10: 91-99.

62. Xu MJ, Feng D, Wu H, Wang H, Chan Y, Kolls J, Borregaard N, Porse B, Berger T, Mak TW, Cowland JB, Kong X and Gao B (2015): Liver is the major source of elevated serum lipocalin-2 levels after bacterial infection or partial hepatectomy: a critical role for IL6/STAT3. Hepatology, 61: 692–702.

63. Yan QW, Yang Q, Mody N, Graham TE, Hsu CH, Xu Z, Houstis NE, Kahn BB and Rosen ED (2007): The adipokine Lcn 2 is regulated by obesity and promotes insulin resistance. Diabetes, 56: 2533–2540.

64. Yannucci J, Manola J, Garnick MB, Bhat G and Bubley GJ (2006): The effect of androgen deprivation therapy on fasting serum lipid and glucose parameters. Journal of Urology, 176: 520–525.

65. Yao YC, Cai ZW, Zhao CJ, Wu KL, Wu CX and Han WP (2011): Influence of castration-induced sex hormone deficiency on serum lipid levels and the genes expression in male pigs. Horm Metab Res., 43 (10): 674-80.

66. Yasuoka T, Sasaki M, Fukunaga T, Tsujikawa T, Fujiyama Y, Kushima R and Goodlad RA (2003): The effects of lectins on indomethacin induced small intestinal ulceration. Int J Exp Pathol., 84(5): 231–237.

67. Yilmaz ? , Temur M, Calan M, Kume T, ? zbay PO, Karakulak M and Yapucu S (2017): The relationship between lipocalin-2 and free testosterone levels in polycystic ovary syndrome. Endokrynologia Polska, 68(1):7-12.

68. Zhang J, Wu Y, Zhang Y, Leroith D, Bernlohr DA, Bernlohr DA and Chen X (2008): The role of Lcn2 in the regulation of inflammation in adipocytes and macrophages. Mol Endocrinol., 22: 1416–1426.

69. Zhang Y, Foncea R, Deis JA, Guo H, Bernlohr DA and Chen X (2014): Lipocalin-2 Expression and Secretion Is Highly Regulated by Metabolic Stress, Cytokines, and Nutrients in Adipocytes. PLoS ONE 9(5): e96997**.**

70. Zhao P, Elks CM and Stephens JM (2014): The induction of lipocalin-2 expression in vivo and in vitro. J Biol Chem., 289:5960–9.

تأثير التستستيرون علي اللايبوكالين في مصل الدم في ذكور الجرذان البالغة المخصاة

مها عبد الحميد و نانيس المالكي

قسم الفسيولوجيا الطبية- كلية الطب- جامعة الزقازيق

خلفية البحث: تم التعرف الأولي لهرمون اللايبوكالين كبروتين يفرز من خلايا الدم البيضاء المحايدة. وقد تدخل اللايبوكالين في كثير من الوظائف مثل الالتهاب ونجاة الخلايا و المناعة و الوظائف الحيوية للجهاز البولي والتناسلي، وقد عرف كعامل للتطور و الحماية. وتأثير هرمون التستستيرون علي مستوي اللايبوكالين في ذكور الجرذان البالغة مازال غير معروف.

الهدف من البحث: دراسة تأثير التستستيرون علي مستوي اللايبوكالين في ذكور الجرذان البالغة بعد إزالة الخصيتين.

مواد و طرق البحث: تم استخدام ٣٢ من ذكور الجرذان البالغة، وتم تقسيمهم إلي أربعة مجموعات متساوية كالتالي:

- **المجموعة الأولي (الضابطة):**تعرضت لفتح الجلد بمنطقة كيس الصفن دون المساس بالخصيتين.
- **المجموعة الثانية:** تم إزالة الخصيتين.
- **المجموعة الثالثة:** تم إزالة الخصيتين، ثم تم حقن الجرذان بهرمون التستستيرون بجرعة تحافظ علي المستوي الطبيعي للهرمون في الدم.
- **المجموعة الرابعة:** تم إزالة الخصيتين، ثم تم حقن الجرذان بهرمون التستستيرون بجرعة عالية تحافظ علي مستوي أعلي من المستوي الطبيعي للهرمون في الدم.

النتائج:حدث نقص ذو دلالة إحصائية في مستوي اللايبوكالين في الدم في المجموعة الثانية والثالثة بالمقارنة بالمجموعة الضابطة. وقد أوضحت النتائج حدوث نقص ذو دلالة إحصائية في مستوي اللايبوكالين بالمجموعة الرابعة عند مقارنتها بباقي المجموعات، مع وجود علاقة عكسية ذات دلالة إحصائية بين مستويات هرمون التستستيرون واللايبوكالين في نفس المجموعة.

الخلاصة: المستوي الطبيعي لهرمون التستستيرون مهم للحفاظ علي مستوي طبيعي للايبوكالين في الدم، وأي اضطراب في مستوي هرمون التستستيرون سواء بالزيادة أو النقص يؤدي إلي نقص في مستوي اللايبوكالين بالدم.