

Effect of Glucagon Like Peptide-1 on Serum Kisspeptin Level in Adult Male Albino Rats Treated by Anabolic Androgenic Steroid

DOAA A. ABD EL-MOETY, M.D.*; HANY A. EL-KATTAWY, M.D.* and MOHAMMED A. ATTIA, M.D.**

The Department of Physiology, Faculty of Medicine, Zagazig University and The Department of Pharmacology, Faculty of Medicine, Mansoura University**, Mansoura, Egypt*

Abstract

Background: Anabolic Androgenic Steroids (AAS) are widely used among youth and athletes for improving their physical appearance and performance. However, its use has adverse effects on sexual health and metabolism. Glucagon Like Peptide-1 (GLP-1) is a gut hormone that participates in the neuroendocrine control of hypothalamic-pituitary axis (HPG), and may play an important role in the reproductive functions as a component of GIT-brain axis. Also, the hypothalamic neuropeptide kisspeptin stimulates the Hypothalamic Pituitary Gonadal (HPG) axis and controls gonadotropin secretion. Few researches were performed to study the relationship between each of GLP-1 and kisspeptin with HPG axis.

Aim: To evaluate the potential protective role of (GLP-1) against some altered sexual, metabolic, and histopathologic changes induced by the use of AAS in adult male albino rats.

Material and Methods: 40 adult male albino rats were divided into 4 groups; Group I (control or vehicle treated), Group II (GLP-1 + vehicle treated), Group III (AAS-treated) and Group IV (GLP-1 + AAS). In all groups, Body Weight (BW) was measured and Body Mass Index (BMI) was calculated. Serum testosterone, Luteinizing Hormone (LH), Follicle-Stimulating Hormone (FSH), kisspeptin, glucose and insulin levels were measured, homeostasis Model Assessment of Insulin Resistance (HOMA-IR) was calculated, and lipid profile was estimated. The changes in the histopathological aspects of testis were examined. The testicular weight, testicular coefficient, epididymal sperm count and motility were investigated.

Results: The study revealed that peripheral injection of GLP-1 for 5 weeks to androgen treated-rats in Group IV (GLP-1 + AAS) significantly increased levels of serum kisspeptin, LH, FSH, testosterone and consequently epididymal sperm count and motility compared with rats in Group III (AAS-treated). In addition, GLP-1 treatment induced significant reductions in the values of fasting serum glucose and HOMA-IR, serum total cholesterol and LDL levels with no significant changes in serum insulin, HDL and TG levels in Group IV compared with Group III.

Conclusion: The administered GLP-1 may attenuate some altered sexual, metabolic and histopathological adverse effects induced by AAS use in adult male rats. Therefore, it may be a novel approach for managing AAS abuse.

Key Words: GLP-1 – Kisspeptin – AAS – Testosterone – Sperm – Rats.

Introduction

AAS are synthetic and natural substances analogues to male androgen. They are used with caution in hypogonadism, anaemia of renal failure, osteoporosis and poor growth [1].

Nowadays, AAS are excessively used all over the world. Male gender, younger age and low body confidence all are factors contributing to the use of AAS. Body builders and athletes excessively use AAS. However, AAS abuse has negative and bad adverse effects on general health, sexuality and metabolism [2].

The hypothalamic neuropeptide kisspeptin stimulates the Hypothalamic Pituitary Gonadal (HPG) axis [3]. It controls gonadotropin secretion, fertility and onset of puberty [4]. As it stimulates the release of Gonadotrophin-Releasing Hormone (GnRH) and LH in mammals [5].

The GIT hormone, GLP-1, is mainly produced by the enteroendocrine L cells in the distal ileum and colon, and released by food intake [6]. GLP-1 receptors are widely expressed and induced effects on peripheral tissues, including the heart, the pancreas and the liver as well as the central nervous system [7].

Moreover, it was found that, peripheral administration of GLP-1 in humans, resulting in inducing satiety and body weight reduction as it crossed the blood brain barrier [8].

Correspondence to: Dr. Doaa A. Abdel Moety,
[E-Mail: daabdelmoeti@zu.edu.eg](mailto:daabdelmoeti@zu.edu.eg)

The link between GLP-1 and HPG axis has not been illustrated yet. However, Mac Lusky NJI et al., [9] observed that intracerebroventricular administration of GLP-1 in rats stimulates the LH release and this may indicate a role of GLP-1 in the neuroendocrine control of HPG axis.

So, this work was performed to study the effect of peripheral administration of GLP-1 on serum kisspeptin level, testicular functions and some metabolic parameters and to find the relevance between GLP-1, serum kisspeptin level, and HPG axis in adult male albino rats treated by supraphysiological doses of AAS.

Material and Methods

This study was performed on a total number of 40 healthy adult male local strain albino rats, weighing 180-200gm, obtained from the Animal House of Faculty of Veterinary Medicine, Zagazig University. Animals were kept in steel wire cages, (40 X 28 X 18cm), 5 rats per cage, under hygienic conditions in Animal House of Faculty of Medicine, Zagazig University. This study was performed from September 2017 to November 2017. All animals received care in accordance with the guide to the care and use of experimental animals of Institute of Laboratory Animal Resources [10]. All rats had free access to water and commercial rat standard chow that consisted of 25.8% protein, 62.8% carbohydrates and 11.4% fat [11]. Rats were kept at comfortable temperature (20-24°C) and were maintained on a normal light/dark cycle [12].

Groups: The rats were divided into four equal groups: Each group contains 10 healthy adult male albino rats:

Group I: (Control group): Vehicle-treated group. In which this group, received a vehicle composed of peanut oil with benzyl alcohol (90: 10, V/V) at a dose of 5mg/kg body weight intra-muscular twice a week over a course of 5 weeks [13].

Group II: (GLP-1 + Vehicle-treated) group. In which this group, received the same vehicle of peanut oil with benzyl alcohol at a dose of 5mg/kg body weight intra-muscular and at the same time received intravenous injection of GLP-1 in the tail vein at a dose of 100nmol/kg body weight twice a week for 5 weeks. GLP-1 (7-36) amide (human, rat) Cat. No. 2082 was purchased from (Tocris Bioscience, Burlington, Ontario, Canada) [14].

Group III: (AAS) group: In which the anabolic androgenic steroid, nandrolonedecanoate (Deca-

durabolin) was purchased from (Organon, Roseland, USA), was administered intramuscularly twice a week, at a dose of 5mg/kg body weight over a course of 5 weeks [13,15]. This dose is comparable to that is frequently used by athletes and those who desire to gain weight [16].

Group IV: (GLP-1 + AAS) group. In this group, GLP-1 was administered i.v. at a dose of 100 nmol/kg, and at the same time AAS was administered intramuscularly, at a dose of 5mg/kg, twice a week over a course of 5 weeks [14,15].

Measurement of anthropometric parameters:

Measuring body weight (gm): By using a digital scale, the animal was weighed day before the experiment, twice a week and at the last day. The results were written in a record for each labeled rat.

Calculation of Body Mass Index (BMI) (gm/cm²): The weights and lengths were measured at the end of their experiment, and immediately before they were sacrificed for calculation of the BMI. It can be calculated by dividing body weight (gm)/length² (cm²).

Weights were measured by using a digital scale in grams according to Nascimento et al., [17] and lengths (nose to anus length) were measured in cm. according to Novelli et al., [18].

Measuring Abdominal Circumference (AC) and Thoracic Circumference (TC): By holding the measuring tape around the abdomen just in front of the hind limbs for AC recording and around the chest just behind the forelimbs for TC recording. Then data were plotted in records of each labelled rat [18].

Collection of blood samples: Blood samples were obtained at the time of scarification and were allowed to clot for 2 hours at room temperature and were centrifuged for 20min. at approximately 500rpm. The separated serums were stored at -20°C [19], until used for:

- Estimation of serum testosterone, LH, and FSH levels: According to Tietz [20]. By using rat kits: BC-1115, BC-1031, and BC-1029 respectively, Bio Check Inc. 323 Vintage Park Dr. Foster City, CA-94404.
- Estimation of serum glucose levels (mg/dl) according to Tietz [20].
- Estimation of serum insulin levels (μ U/ml) by Enzyme-Linked Immuno-Sorbent Assay (ELISA) according to Reaven [21].

- Calculation of HOMA-IR: Insulin Resistance (IR) of individual rats was evaluated using the Homeostasis Model Assessment (HOMA-IR) index according to Bonora et al., [22] as follows:

$$\text{HOMA-IR} = \frac{\text{Fasting serum glucose (mg/dL)} \times \text{Fasting serum insulin (}\mu\text{U/mL)}}{405}$$

- Estimation of lipid profile as follows: Total serum cholesterol levels: According to Tietz [20] serum TG levels: According to Fossati and Prencipe [23], serum HDL levels according to Nauck et al., [24] and serum LDL levels was calculated according to Friedewald et al., [25] as follows:

$$\text{LDL} = \frac{\text{TC} - \text{HDL} - \text{TG}}{5}$$

(Kits used for estimation of serum glucose, insulin, cholesterol, TG and HDL levels were purchased from Biosource Europe S.A. Belgium).

- Estimation of serum kisspeptin level: Serum kisspeptin level (ng/ml) was measured according to Ahn JM et al., [26] by using an Enzyme-Linked Immunosorbent Assay (ELISA). Rat Kisspeptin 1 (KISS 1) ELISA Kit. Code: CSB-E13434r Range of detection (0.156-10 ng/ml). Sensitivity: 0.039ng/ml. The kits were obtained from (CUS-ABIO Life science 8400 Baltimore Avenue, College Park, MD 20740, Maryland, USA).

Post mortem examination:

1- Extraction of gonadal tissue: All animals were examined postmortem to determine the weights of the testes. The abdominal wall was opened then the testes were extracted and dissected carefully [27]. Testicular coefficient calculated according to Yan WJ et al., [28] by using the following formula:

$$\text{Testicular coefficient (gm/Kg)} = \frac{\text{The weight of the testes (the mean of the weight of the two testes in gm)}}{\text{Final body weight (kg)}}$$

2- Spermatic parameters analysis: The epididymis of each rat was dissected, removed and minced in 2ml of Hank's Buffer Salt Solution (HBSS) at 37°C [29]. After 5min incubation at 37°C, the cauda epididymis sperm was analyzed using the standard hemocytometric method. The epididymal fluid was drawn up to the 0.5 mark of WBC pipette (White Blood Cell pipette) and the semen diluting fluid (sodium bicarbonate 5g, formalin 1ml, distilled water 99.0ml) was drawn up to '11' mark, and subsequently mixed well. One drop was

added to the haemocytometer chamber and allowed the sperms to settle by keeping haemocytometer in humid place (wet chamber) for 1h. After incubation the number of spermatozoa in the appropriate squares of the haemocytometer was counted under the light microscope at 400X. The sperm concentration refers to the number of spermatozoa/ml fluid, and calculated using the following formula. Sperm count=No. of spermatozoa counted X dilution factor X volume factor/No. of areas counted [30]. The percentage of sperm motility was calculated using the number of live sperm cells over the total number of sperm cells, both motile and non-motile. The sperm cells that were not moving at all were considered to be non-motile, while the rest, which displayed some movement were considered to be motile [31].

3- Gonadal histopathological examination: On the stipulated day after the collection of blood for hormonal assay, laparotomy was performed, and testis were removed and weighed followed by histopathological examination as follow: The testis were dissected out and cleaned with cold physiological saline to remove blood and the adhering tissues. The samples were then fixed in 10% formaldehyde in fresh alcoholic bouin's fluid for 8 hours, and then processed and embedded in paraffin wax, sectioned at 5 μm thickness and stained in hematoxylin-eosin. The sections were examined or observed under a light microscope and the general histological appearance was assessed. The testis histology was performed according to the method used by [32,33].

Statistical analysis:

The data obtained in the present study were expressed as mean \pm SD for quantitative variables and statistically analyzed according to the methods described by Kirkwood [34]. The statistical analysis is done by using SPSS program (19) (SPSS Inc. Chicago, IL, USA).

ANOVA [Post hoc (LSD)] test was used to compare means among more than two groups.

p-value <0.05 was considered statistically significant.

Corrélation coefficient (r): Pearson's correlation analysis was performed to illustrate the relationships between serum kisseptin and all studied parameters among different groups. Pearson's correlation was considered significant at *p*-values <0.05.

Results

Histopathological findings:

Normal testicular tissue from Group I and Group II showing normal seminiferous tubules with central cavity lined by stratified germinal epithelium separated by interstitial cells of leydig. The normal germinal epithelium composed of spermatogonia, secondary spermatocyte, spermatid, and spermatozoa. (H & E X400) Figs. (1A,B).

On the other hand, testicular tissue from Group III showing seminiferous tubules of an adult male

albino rat treated by AAS. There was disarrangement of germinal epithelium with vacuoles, spermatogonia, primary spermatocyte. (H & E X400) Fig. (1C).

In addition, testicular tissue from Group IV from an adult male albino rat treated by GLP-1 & AAS showing normal seminiferous tubules lined by stratified germinal epithelium. The normal germinal epithelium composed of spermatogonia, primary spermatocyte, spermatids, and spermatozoa with absent secondary spermatocyte. (H & E X400) Fig. (1D).

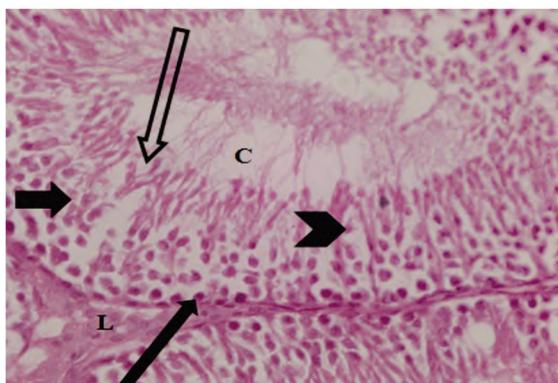


Fig. (1A): A photomicrograph of a section of seminiferous tubules of a control adult male albino rat Group (I) showing circular to oval seminiferous tubules with central cavity (C) lined by stratified germinal epithelium separated by interstitial cells of leydig (L). The normal germinal epithelium (brace) composed of spermatogonia (large arrow), primary spermatocyte (small arrow), secondary spermatocyte (short arrow), spermatid (arrow head), spermatozoa (empty arrow). (H & E X400).

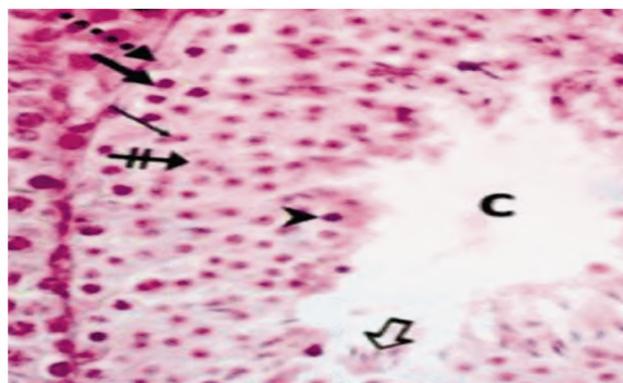


Fig. (1B): A photomicrograph of a section of seminiferous tubules of an adult male albino rat group treated by GLP-1 (II) showing circular to oval seminiferous tubules with central cavity (C) lined by stratified germinal epithelium. The normal germinal epithelium (brace) composed of spermatogonia (Large arrow), primary spermatocyte (small arrow), secondary spermatocyte (crossed arrow), spermatids (arrow head), spermatozoa (empty arrow) and Sertoli cells (striped arrow). (H & E X400).

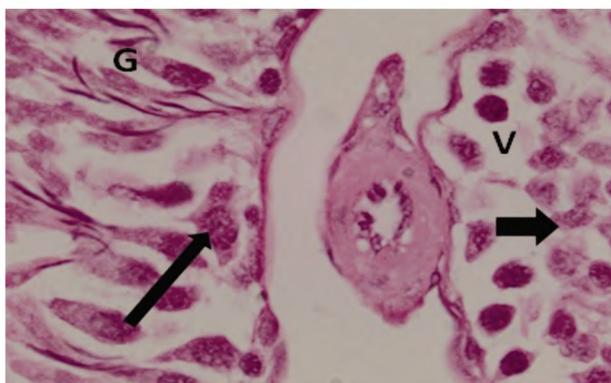


Fig. (1C): A photomicrograph of a section of seminiferous tubules of an adult male albino rat treated by AAS Group (III) showing disarrangement of germinal epithelium (G) with vacuoles (V) spermatogonia (large arrows), primary spermatocyte (small arrows). (H & E X400).

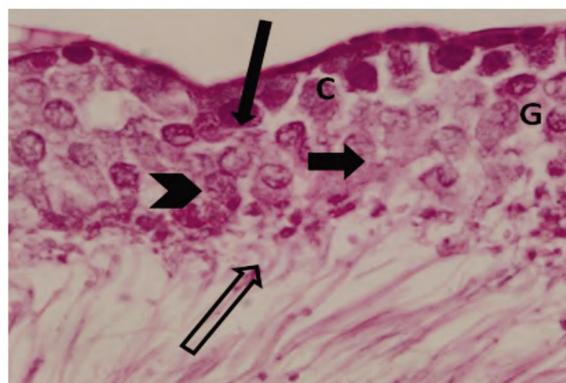


Fig. (1D): A photomicrograph of a section of seminiferous tubules of an adult male albino rat received GLP-1 & AAS Group (IV) showing normal seminiferous tubules lined by stratified germinal epithelium (G). The normal germinal epithelium (brace) composed of spermatogonia (large arrow), primary spermatocyte (small arrow), spermatids (arrow head), and spermatozoa (empty arrow) with absent secondary spermatocyte. (H & E X400).

The present study showed that, serum kisspeptin level (ng/ml) was significantly reduced in Group III (1.94 ± 0.09) when compared to both Group I (2.31 ± 0.11) and Group II (2.29 ± 0.16) ($p < 0.001$, $p < 0.001$ respectively), and significant elevation in its level was detected in Group IV (2.33 ± 0.14) when compared to Group III ($p < 0.001$). However, the level of serum kisspeptin was not significantly changed in Group IV when compared to both Group I and Group II ($p > 0.05$, $p > 0.05$ respectively). No significant change in serum kisspeptin level was recorded in Group II when compared to Group I ($p > 0.05$). Fig. (2A).

Moreover, serum LH level (IU/ml) was significantly reduced in Group III (1.33 ± 0.05) when compared to both Group I (1.41 ± 0.07) and Group II (1.43 ± 0.05) ($p < 0.05$, $p < 0.05$ respectively), and significant elevation in its level was recorded in Group IV (1.45 ± 0.07) when compared to Group III ($p < 0.01$). However, the level of LH hormone was significantly unchanged in Group IV when compared to both Group I and Group II ($p > 0.05$, $p > 0.05$ respectively). No significant change in LH level was observed in Group II when compared to Group I ($p > 0.05$).

Also, serum level of FSH hormone (IU/ml) was significantly decreased in Group III (0.362 ± 0.013) when compared to both Group I (0.378 ± 0.008) and Group II (0.380 ± 0.01) ($p < 0.05$, $p < 0.01$ respectively), and significant increase in its level was recorded in Group IV (0.376 ± 0.005) when compared to Group III ($p < 0.05$). However, no significant change in FSH level in Group IV when compared to both Group I and Group II ($p > 0.05$, $p > 0.05$ respectively). No significant change in FSH level was observed in Group II when compared to Group I ($p > 0.05$). Also, it was noticed that, serum level of testosterone hormone (pg/ml) was significantly decreased in Group III (2.67 ± 0.13) when compared to both Group I (3.1 ± 0.15) and Group II (3.3 ± 0.2) ($p < 0.01$, $p < 0.01$ respectively), and significant elevation in its level was recorded in Group IV (3.32 ± 0.1) when compared to Group III ($p < 0.01$). However, the level of testosterone hormone was significantly unchanged in Group IV when compared to both Group I and Group II ($p > 0.05$, $p > 0.05$ respectively). No significant change in testosterone level was observed in Group II when compared to Group I ($p > 0.05$) Fig. (2B).

The present study recorded a significant reduction in Final Testicular Weight (FTW) (gm) in Group III (1.51 ± 0.11) when compared to both Group I (1.77 ± 0.12) and Group II (1.78 ± 0.08) ($p < 0.01$, $p < 0.01$ respectively), and significant increase

in its value was recorded in Group IV (1.75 ± 0.12) when compared to Group III ($p < 0.01$). However, no significant change in FTW value in Group IV when compared to both Group I and Group II ($p > 0.05$, $p > 0.05$ respectively). No significant change in FTW value was observed in Group II when compared to Group I ($p > 0.05$).

In addition, it was found that testicular coefficient (gm/kg) was significantly reduced in Group III (6.59 ± 0.57) when compared to both Group I (8.63 ± 0.66) and Group II (9.17 ± 0.54) ($p < 0.001$, $p < 0.001$ respectively), and significant elevation in its value was reported in Group IV (7.93 ± 0.6) when compared to Group III ($p < 0.01$). Also, testicular coefficient value in Group IV remained significantly lowered when compared to Group II ($p < 0.01$), and significantly unchanged when compared to Group I ($p > 0.05$). No significant change in testicular coefficient value was observed in Group II when compared to Group I ($p > 0.05$) Fig. (2C).

Moreover, epididymal sperm count (millions/ml) was decreased in Group III (41.9 ± 0.74) when compared to both Group I (45.2 ± 1.9) and Group II (45 ± 1.58) ($p < 0.01$, $p < 0.01$ respectively), and significant increase in its value was recorded in Group IV (44.9 ± 1.75) when compared to Group III ($p < 0.01$). However, no significant change in sperm count in Group IV was observed when compared to both Group I and Group II ($p > 0.05$, $p > 0.05$ respectively). No significant change in sperm count was detected in Group II when compared to Group I ($p > 0.05$).

Also, epididymal sperm motility (%) was reduced in Group III (58.45 ± 8.15) when compared to both Group I (68.45 ± 7.14) and Group II (69.33 ± 8.12) ($p < 0.05$, $p < 0.05$ respectively), and significant increase in its percentage was recorded in Group IV (68.5 ± 6.83) when compared to Group III ($p < 0.05$). However, no significant change in sperm motility in Group IV was observed when compared to both Group I and Group II ($p > 0.05$, $p > 0.05$ respectively). No significant change in sperm motility was detected in Group II when compared to Group I ($p > 0.05$). (Table 1).

The present study showed no significant change in Initial Body Weight (IBW) (gm) in Group III (200.75 ± 15.34) when compared to both Group I (190.12 ± 11.4) and Group II (188.3 ± 12.78) ($p > 0.05$, $p > 0.05$ respectively), and no significant change in its value in Group IV (191.5 ± 14.78) when compared to Group III ($p > 0.05$). However, the value of IBW was significantly unchanged in

Group IV when compared to both Group I and Group II ($p>0.05$, $p>0.05$ respectively). Also, no significant change in IBW was observed in Group II when compared to Group I ($p>0.05$).

However, this study revealed significant increase in Final Body Weight (FBW) (gm) in Group III (228.8 ± 3.89) when compared to both Group I (205.18 ± 3.32) and Group II (194.2 ± 6.53) ($p<0.001$, $p<0.001$ respectively), and significant reduction in its value in Group IV (220.6 ± 14.36) when compared to Group III ($p<0.05$). However, the value of FBW was still significantly higher in Group IV when compared to both Group I and Group II ($p<0.001$, $p<0.001$ respectively). Also, a significant reduction in FBW was observed in Group II when compared to Group I ($p<0.01$).

At the same time, there was a significant increase in Final Body Mass Index (FBMI) (gm/cm^2) in Group III (0.84 ± 0.07) when compared to both Group I (0.59 ± 0.04) and Group II (0.46 ± 0.05) ($p<0.001$, $p<0.001$ respectively), and significant reduction in its value in Group IV (0.7 ± 0.06) when compared to Group III ($p<0.01$). However, the value of FBMI was still significantly higher in Group IV when compared to both Group I and Group II ($p<0.001$, $p<0.001$ respectively). Also, a significant reduction in FBMI was observed in Group II when compared to Group I ($p<0.01$).

In addition, the research showed significant increase in AC/TC ratio in Group III (1.42 ± 0.05) when compared to both Group I (1.18 ± 0.03) and Group II (1.15 ± 0.07) ($p<0.001$, $p<0.001$ respectively), and significant reduction in its value in Group IV (1.33 ± 0.08) when compared to Group III ($p<0.05$). However, the value of AC/TC ratio was still significantly higher in Group IV when compared to both Group I and Group II ($p<0.01$, $p<0.01$ respectively). No significant change in AC/TC ratio was observed in Group II when compared to Group I ($p>0.05$).

Also, the present study showed significant elevation in fasting serum glucose level (mg/dl) in Group III (115 ± 7.9) when compared to both Group I (87.8 ± 1.92) and Group II (86.4 ± 1.14) ($p<0.001$, $p<0.001$ respectively), and significant reduction in its level in Group IV (90.5 ± 5.26) when compared to Group III ($p<0.001$). However, the level of serum glucose was still mild significantly higher in Group IV when compared to both Group I and Group II ($p<0.05$, $p<0.05$ respectively). No significant change in serum glucose level was observed in Group II when compared to Group I ($p>0.05$).

In addition, the research recorded significant increase in fasting serum insulin level ($\mu\text{O}/\text{ml}$) in Group III (17.3 ± 1.86) when compared to both Group I (12 ± 1.58) and Group II (12.4 ± 1.14) ($p<0.001$, $p<0.001$ respectively), and no significant change in its level in Group IV (17 ± 1.3) when compared to Group III ($p>0.05$). However, the level of serum insulin was still significantly elevated in Group IV when compared to both Group I and Group II ($p<0.001$, $p<0.001$ respectively). No significant change in serum insulin level was observed in Group II when compared to Group I ($p>0.05$).

A significant increase in HOMA-IR was recorded in Group III (4.89 ± 0.34) when compared to both Group I (2.6 ± 0.18) and Group II (2.65 ± 0.13) ($p<0.001$, $p<0.001$ respectively), and significant decrease in its level in Group IV (3.79 ± 0.2) when compared to Group III ($p<0.01$). However, the value of HOMA-IR was still significantly higher in Group IV when compared to both Group I and Group II ($p<0.01$, $p<0.01$ respectively). No significant change in HOMA-IR value was observed in Group II when compared to Group I ($p>0.05$). Fig. (2D).

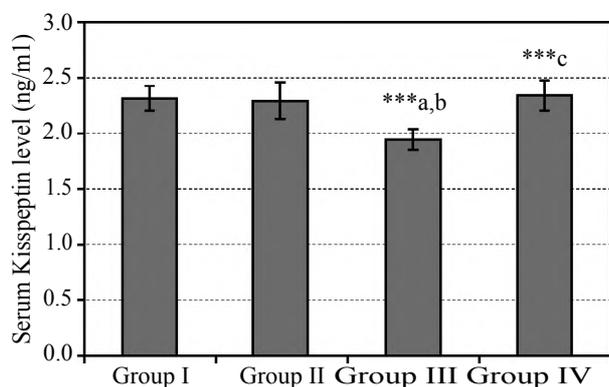
The study showed significant elevation in serum cholesterol level (mg/dl) in Group III (112.8 ± 7.6) when compared to both Group I (75.74 ± 3.18) and Group II (75.9 ± 2.75) ($p<0.001$, $p<0.001$ respectively), and significant reduction in its level in Group IV (104.8 ± 6.98) when compared to Group III ($p<0.05$). However, the level of serum cholesterol was still significantly higher in Group IV when compared to both Group I and Group II ($p<0.001$, $p<0.001$ respectively). No significant change in serum cholesterol level was reported in Group II when compared to Group I ($p>0.05$).

The present study reported a significant reduction in serum High Density Lipoprotein (HDL) level (mg/dl) in Group III (37 ± 2.55) when compared to both Group I (50.2 ± 3.96) and Group II (50.6 ± 3.05) ($p<0.001$, $p<0.001$ respectively). However, no significant change in its level was recorded in Group IV (40 ± 1.58) when compared to Group III ($p>0.05$). And, the level of serum HDL was still significantly reduced in Group IV when compared to both Group I and Group II ($p<0.001$, $p<0.001$ respectively). No significant change in serum HDL level was observed in Group II when compared to Group I ($p>0.05$).

No significant change in serum Triglyceride level (TG) (mg/dl) was detected in Group III (90.1 ± 8.08) when compared to both Group I (86.8 ± 2.39)

Table (3): Correlations between serum Kisspeptin (ng/ml) level and all studied parameters in all groups.

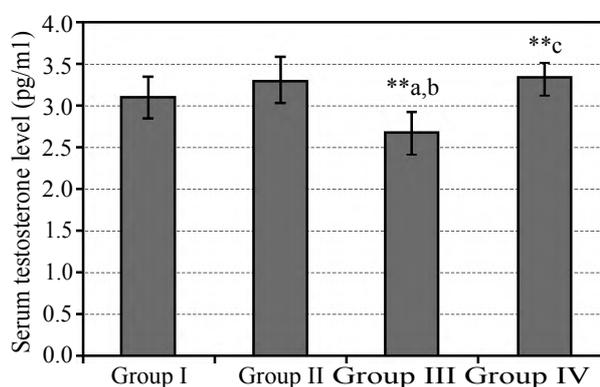
Parameters	Groups	Group I (control or vehicle- treated group)	Group II (GLP-1- treated group)	Group III (AAS- treated group)	Group IV (GLP-1 + AAS treated group)
IBW (gm)		$r=0.311$ $p>0.05$	$r=0.466$ $p>0.05$	$r=0.333$ $p>0.05$	$r=0.284$ $p>0.05$
FBW (gm)		$r=0.219$ $p>0.05$	$r=0.320$ $p>0.05$	$r=0.286$ $p>0.05$	$r=0.165$ $p>0.05$
FBMI (gmlcm ²)		$r=0.512$ $p>0.05$	$r=0.451$ $p>0.05$	$r=0.336$ $p>0.05$	$r=0.327$ $p>0.05$
AC/TC ratio		$r=0.173$ $p>0.05$	$r=0.103$ $p>0.05$	$r=0.155$ $p>0.05$	$r=0.148$ $p>0.05$
Serum testosterone (pg/ml)		$r=0.193$ $p>0.05$	$r=0.175$ $p>0.05$	$r=0.873^{**}$ $p<0.01$	$r=0.845^{**}$ $p<0.01$
Serum LH (IU/ml)		$r=0.211$ $p>0.05$	$r=0.192$ $p>0.05$	$r=0.885^{**}$ $p<0.01$	$r=0.854^{**}$ $p<0.01$
Serum FSH (IU/ml)		$r=0.327$ $p>0.05$	$r=0.338$ $p>0.05$	$r=0.893^{***}$ $p<0.001$	$r=0.896^{***}$ $p<0.001$
Final testicular Wt (gm)		$r=0.135$ $p>0.05$	$r=0.028$ $p>0.05$	$r=0.685^{**}$ $p<0.01$	$r=0.692^{**}$ $p<0.01$
Testicular coefficient (gm/Kg)		$r=0.142$ $p>0.05$	$r=0.171$ $p>0.05$	$r=0.695^{**}$ $p<0.01$	$r=0.587^*$ $p<0.05$
Epididymal sperm count (millions/ml)		$r=0.148$ $p>0.05$	$r=0.111$ $p>0.05$	$r=0.909^{***}$ $p<0.001$	$r=0.891^{**}$ $p<0.01$
Epididymal sperm motility (%)		$r=0.235$ $p>0.05$	$r=0.349$ $p>0.05$	$r=0.889$ $p<0.01^{**}$	$r=0.874^{**}$ $p<0.01$
Serum glucose (mg/dl)		$r=0.533$ $p>0.05$	$r=0.510$ $p>0.05$	$r=0.963^{***}$ $p<0.001$	$r=-0.970^{***}$ $p<0.001$
Serum insulin (μ U/ml)		$r=0.229$ $p>0.05$	$r=0.015$ $p>0.05$	$r=0.103$ $p>0.05$	$r=0.178$ $p>0.05$
HOMA-IR		$r=0.201$ $p>0.05$	$r=0.048$ $p>0.05$	$r=0.981^{***}$ $p<0.001$	$r=-0.975^{***}$ $p<0.001$
Serum cholesterol (mg/dl)		$r=0.127$ $p>0.05$	$r=0.133$ $p>0.05$	$r=0.048$ $p>0.05$	$r=0.078$ $p>0.05$
Serum triglycerides (mg/dl)		$r=0.341$ $p>0.05$	$r=0.424$ $p>0.05$	$r=0.036$ $p>0.05$	$r=0.411$ $p>0.05$
LDL (mg/dl)		$r=0.101$ $p>0.05$	$r=0.008$ $p>0.05$	$r=0.383$ $p>0.05$	$r=0.491$ $p>0.05$
Serum HDL (mg/dl)		$r=0.177$ $p>0.05$	$r=0.281$ $p>0.05$	$r=0.169$ $p>0.05$	$r=0.141$ $p>0.05$

*: $p<0.05$.**: $p<0.01$.***: $p<0.001$.Fig. (2A): Serum Kisspeptin level (ng/ml) expressed as (mean \pm SD) among the four studied groups.***: $p<0.001$.

b: Versus Group II.

a: Versus Group I.

c: Versus Group III.

Fig. (2B): Serum testosterone level (pg/ml) expressed as (mean \pm SD) among the four studied groups.**: $p<0.01$.

b: Versus Group II.

a: Versus Group I.

c: Versus Group III.

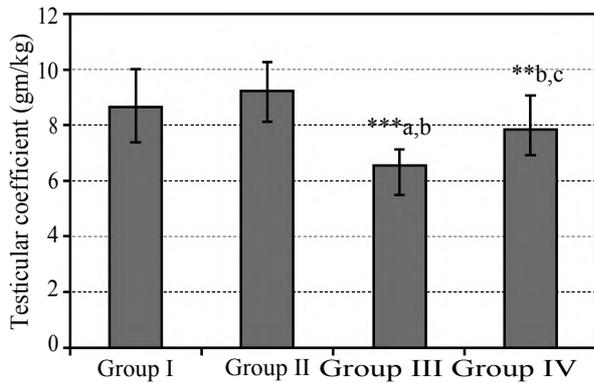


Fig. (2C): Testicular coefficient (gm/kg) expressed as (mean ± SD) among the four studied groups.

p*<0.01. *p*<0.001.
 a: Versus Group I. b: Versus Group II. c: Versus Group III.

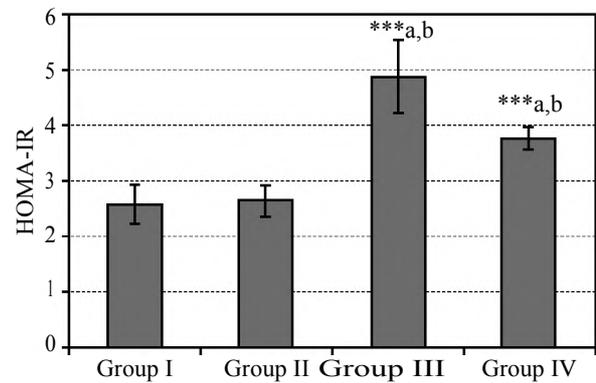


Fig. (2D): HOMA-IR expressed as (mean ± SD) among the four studied groups.

****p*<0.001.
 a: Versus Group I. b: Versus Group II.

Discussion

AAS are steroidal androgens that are similar in their structure and function to testosterone mainly in increasing protein within cells, especially skeletal muscles and also in developing and maintenance of masculine secondary sexual characteristics [35].

Recently, AAS abuse becomes a worldwide problem spreading among young adult males especially athletes to improve their physical appearance and performance [36].

Kisspeptin is a hypothalamic neuropeptide that is considered a powerful stimulator of the release of gonadotrophin releasing hormone (GnRH) [37]. It was found that, kisspeptin neurons synapse on GnRH neurons stimulating the release of hypothalamic GnRH, that trigger the release of pituitary LH and FSH [38,39]. Testosterone is a strong regulator of Kiss1 expression [40]. Androgen receptors are expressed in hypothalamic Kisspeptin neurons not in GnRH neurons [41].

GLP-1 is an anorexigenic hormone, which is released in response to meal intake by the intestinal epithelial endocrine L-cells, and is considered as a physiological regulator of food intake and appetite, and also stimulates insulin release [6].

The relationship between GLP-1 and the HPG axis is still unclear. It was found that GLP-1 participate in the neuroendocrine control of hypothalamic-pituitary axis, food intake, and stress response where intracerebroventricular GLP-1 administration stimulated the secretion of LH, TSH, corticosterone and vasopressin in rats [9].

Therefore, we made a model of an adult male albino rat treated by supraphysiological dose of

AAS for 5 weeks. Then, we investigated some impacts of AAS use on pituitary-gonadal axis, serum kisspeptin level, testicular functions, insulin sensitivity and lipid profile.

For the first time, we assessed if GLP-1 has a potential protective effect against some altered testicular and metabolic functions induced by the use of AAS for 5 weeks in adult male rats looking for an approach for managing AAS abuse.

The present study demonstrated that the serum levels of kisspeptin (ng/ml), LH (IU/ml), FSH (IU/ml) and testosterone (pg/ml) were significantly reduced in Group III (AAS-treated) compared to Group I (vehicle-treated) and Group II (GLP-1 treated). This may be explained by the effect exogenous androgen injection in suppressing the release of GnRH, LH, FSH and subsequently testicular testosterone [35,36]. These findings were in agreement with that of El-Hanbuli et al., [42]. However, in contrast to our findings regarding FSH level; Al-Alwany et al., [43] reported that testosterone injection for six weeks significantly increased the FSH serum level in rats.

This current study showed that peripheral injection of GLP-1 to rats in Group IV (GLP-1 + AAS treated) significantly increased the levels of serum kisspeptin, LH, FSH and consequently testosterone compared to rats in Group III.

Therefore, the administered GLP-1 might correct the changes in kisspeptin, LH, FSH and testosterone levels induced by AAS use. However, kisspeptin, LH, FSH and testosterone levels were not significantly changed in Group IV compared to both Group I and Group II.

Moreover, significant positive correlations were reported in Group III and Group IV between serum kisspeptin level and each of serum levels of LH, FSH, and testosterone.

These findings are supported by Oride et al., [44] who found that expression of rat hypothalamic Kiss-1 mRNA was significantly increased by direct GLP-1 administration in the neurons of brain of rat foetus. Hence, GLP-1 affects GnRH mRNA expression as well.

The relationship between GLP-1 and kisspeptin is very interesting. We found that GLP-1 injection increased the serum level of kisspeptin that was previously decreased by the use of AAS in adult male rats. As kisspeptin is a physiologic regulator to hypothalamic GnRH stimulating its release Roa, et al., [5].

Therefore, it was suggested that the rising effect of GLP-1 on serum kisspeptin level may result in stimulation of HPG axis and increase the release of GnRH as well. This finding is consistent with findings of Beak et al., [45] who showed that GLP-1 stimulated the GnRH secretion in the hypothalamic neuronal cell lines. In addition, central injection of GLP-1 increased plasma LH levels in rats [46].

Moreover, the present study showed that GLP-1 administration in Group II induced no significant differences in the values of serum kisspeptin, LH, FSH and testosterone levels compared to Group I. These findings are in consistent with that of Jeibmann et al., [46] who reported that GLP-1 infusion did not reduce the total production of testosterone but affected its secretory pulsatile pattern, with no significant changes in the pattern of LH release in normal healthy males.

Concerning testicular parameters, the present study demonstrated that administration of supra-physiological doses of AAS for 5 weeks induced significant reductions in final testicular weight (gm), testicular coefficient (gm/kg), epididymal sperm count (millions/ml) and motility (%) in Group III compared to Group I and Group II.

These results are supported with that of Kanayama G et al., and El-Hanbuli HM et al., [42,47] who observed that AAS disturbed the regular production of endogenous testosterone and gonadotropins in human as well as experimental animals.

Furthermore, it has been reported that AAS-treated rats showed significant decline in sperma-

togonia number resulting in reduced sperm count and testicular atrophy [48,49].

The data on the relationship between GLP-1 and reproduction are controversial and scarce. Delayed puberty in female and decreased gonadal weights in male were found to occur in GLP-1 receptor knockout mice. However, the levels of sex hormones were still in normal values in both sexes of GLP-1 receptor knockout mice [9].

In the present study, the values of final testicular weight, testicular coefficient, epididymal sperm count and motility were significantly increased in Group IV compared to Group III indicating a potential role of GLP-1 in improvement of some testicular and reproductive adverse effects induced by AAS use. No significant changes in these values were observed in Group II compared with Group I.

Also, there were significant positive correlations in Group III and Group IV between serum kisspeptin level and each of final testicular weight, testicular coefficient, epididymal sperm count and motility.

Moreover, this study showed that GLP-1 treatment reduced body weight and BMI in Group IV compared to Group III. Therefore, the improvement of final testicular weight, testicular coefficient, epididymal sperm count and motility might follow weight loss. And this can be interpreted according to Hakonsen LB et al., [50] who found that weight reduction could improve sperm count and motility in obese men.

Also, Zhang et al., [51] found that GLP-1 receptor agonist exenatide treatment for 8 weeks resulted in significant increases in sperm motility and activity in high fat diet fed rats when compared with the saline control group.

Moreover, GLP-1-based therapies were found to have anti-inflammatory actions on testis, liver, kidney, lung, and skin by decreasing inflammatory cytokines production and immune cells infiltration [52].

However, in a case report study, GLP-1 receptor agonist Liraglutide was found to cause interrupted sperm production in men probably by affecting gonadotropins, leptin and insulin [53].

Regarding FBW, BMI, AC/TC ratio, this study recorded a significant increase in these studied parameters in Group III compared to both Group I and Group II.

However, when GLP-1 was administered to androgen-treated rats in Group IV, it significantly reduced the values FBW, FBMI and AC/TC ratio compared to rats in Group III. Therefore, GLP-1 could reduce the expected increase in weight, BMI and AC/TC ratio in AAS-treated rats. The administered GLP-1 to vehicle-treated rats in Group II significantly reduced FBW and FBMI without significant change in AC/TC ratio compared to rats in Group I.

These findings are in agreement with that of Shirazia et al., Hunter and Hölscher [54,55] who documented that GLP-1 and its long-lasting analogues could cross the blood brain barrier and suppressed food intake and reduced body weight likely via acting on the central GLP-1 receptors. Moreover, it was demonstrated that GLP-1 receptor agonists significantly decreased body weight, BMI and waist circumference in obese and overweight persons with or without diabetes by Vilsboll et al., and Zhang et al., [56,57]. In humans, it has been found that, peripheral administration of GLP-1 that crosses the blood-brain barrier induced satiety and reduced body weight [58].

Concerning metabolic parameters, this research recorded significant increase in the fasting serum levels of glucose (mg/dl), insulin ($\mu\text{O}/\text{ml}$) and the value of HOMA-IR in Group III compared to both Group I and Group II.

The effects of AAS abuse on glucose metabolism are controversial. In agreement with these findings, George [59] reported that prolonged use of AAS increased insulin resistance and elevated fasting blood glucose levels. However, on the contrary of these findings, normal glucose metabolism was obtained when high AAS doses used in rats [60].

In addition, GLP-1 treatment induced significant reductions in the level of fasting serum glucose and HOMA-IR and had no significant effect on serum insulin level in Group IV compared to Group III. However, fasting serum glucose, insulin levels and HOMA-IR value were still significantly higher in Group IV compared to both Group I and Group II. The values of fasting serum glucose, insulin levels and HOMA-IR did not significantly change in Group II when compared to Group I.

However, Schwetz et al., [61] showed that GLP-1 and Kisspeptin-10 stimulate insulin secretion through activating insulin gene expression and proinsulin biosynthesis.

This research showed that the serum levels of total cholesterol (mg/ml), and Low Density Lipoprotein (LDL) (mg/ml) were increased significantly, and the serum level of High Density Lipoprotein (HDL) (mg/ml) was significantly reduced with no significant change in serum Triglyceride (TG) level (mg/ml) in Group III compared to Group I and Group II.

These results were in agreement with that of Achar et al., [62] who concluded that AAS abuse was associated with increased LDL levels and decreased HDL levels.

On the other hand, Hartgens et al., [63] revealed that total cholesterol was significantly decreased in healthy and diabetic animals treated with testosterone, with no changes in HDL levels.

In addition, GLP-1 administration produced significant decreases in the serum levels of total cholesterol and LDL with no significant change in neither HDL nor TG levels in Group IV compared to Group III. However, the levels of serum cholesterol and LDL were still significantly higher and, the level of serum HDL was still significantly lower in Group IV compared to both Group I and II. In addition, GLP-1 administration did not produce significant changes in any of components of lipid profile in Group II compared to Group I.

And this may be explained according to Lutz et al., [64] who observed that the obesity-induced alterations of lipoprotein metabolism might be modified through pharmacological activation of the GLP-1 system. So, this may be taken in consideration to develop new strategies for treating dyslipidaemia in obesity, diabetes, and other cardio-metabolic diseases.

Discrepancies in the findings of this research and others may be related to the differences in the studied models and species, the variations of the doses of drugs used the methods of administration of these drugs and the duration of treatment.

In conclusion, we have shown that GLP-1 as a GIT hormone has a potential protective role in improving the adverse effects of AAS on metabolic parameters and testicular functions, by increasing serum kisspeptin level through stimulating the pituitary gonadal axis, representing a link between the metabolic status of the body and gonadal action in AAS-treated rats. Therefore, it could present a novel approach for managing AAS abuse. We are still in need to further understand the role of GLP-1 in driving the reproductive functions as a component of GIT- brain axis.

Acknowledgment:

To Professor Doctor Kamal EL-Kashish, Pathology Department, Faculty of Medicine, Zagazig University for performing the histopathological study.

References

- 1- NARAGHI M.A. ABOLHASANI F., KASHANI I., AN-ARKOOL I.J., HEMADI M., AZAMI A., BARBARE-STANI M., AITKEN R.J. and SHOKRI S.: The effects of swimming exercise and supraphysiological doses of nandrolonedecanoate on the testis in adult male rats: A transmission electron microscope study. *Folia Morphol (Warsz)*, 69 (3): 138-46, 2010.
- 2- GWIZDEK K., BRZEK A., BAK-SOSNOWSKA M., DITTFELD A., KNAPIK A. and ZIAJAD: The use of steroids by gym athletes: An attempt to diagnose the problem scale and possible causes. *Journal of Sports Medicine and Physical Fitness* doi: 10.23736/S0022-4707.17.07298-X, 2017.
- 3- CLARKE H., DHILLO W.S. and JAYASENA C.N.: Comprehensive Review on Kisspeptin and Its Role in Reproductive Disorders. *Endocrinol Metab (Seoul)*, 30 (2): 124-41, 2015.
- 4- NAVARRO V.M., CASTELLANO J.M., FERNANDEZ-FERNANDEZ R., TOVAR S., ROA J., MAYEN A., BARREIRO M.L., CASANUEVA F.F., AGUILAR E., DIEGUEZ C., PINILLA L. and TENA-SEMPERE M.: Effects of KiSS-1 Peptide, the Natural Ligand of GPR54, on Follicle-Stimulating Hormone Secretion in the Rat. *Endocrinology*, 146 (4): 1689-97, 2005.
- 5- ROA J., NAVARRO V.M. and TENA-SEMPERE M.: Kisspeptins in reproductive biology: Consensus knowledge and recent developments. *Biology of Reproduction*, 85: 650-60, 2011.
- 6- HERRMANN C., GOKE R., RICHTER G., FEHMANN H.C., ARNOLD R. and GOKE B.: Glucagon-like peptide-1 and glucose-dependent insulin-releasing polypeptide plasma levels in response to nutrients. *Digestion*, 56: 117-26, 1995.
- 7- HAYES M.R., De JONGHE B.C. and KANOSKI S.E.: Role of the glucagon-like-peptide-1 receptor in the control of energy balance. *Physiol. Behav.*, 100 (5): 503-10, 2010.
- 8- ZANDER M., MADSBAD S., MADSEN J.L. and HOLST J.J.: Effect of 6-week course of glucagon-like peptide-1 on glycaemic control, insulin sensitivity, and beta-cell function in type 2 diabetes: A parallel-group study. *Lancet*, 359 (9309): 824-30, 2002.
- 9- MACLUSKY N.J.1., COOK S., SCROCCHI L., SHIN J., KIM J., VACCARINO F., ASASL. and DRUCKER D.J.: Neuroendocrine function and response to stress in mice with complete disruption of glucagon-like peptide-1 receptor signaling. *Endocrinology*, 141 (2): 752-62, 2000.
- 10- Institute of Laboratory Animal Resources: Commission on life sciences and National research council: Guide for the care and use of laboratory animals, 8th Edition. National academy press, Washington DC, 21-55, 1996.
- 11- AHREN B. and SCHEURINK A.J.: Marked hyperleptinaemia after high fat diet associated with severe glucose intolerance in mice. *Eur. J. Endocrinol.*, 139 (4): 461-7, 1998.
- 12- LESOURD B. and MAZARI L.: Nutrition and immunity in the elderly. *Proceedings in the Nutrition*, 58 (3): 685-95, 1999.
- 13- HASSAN A.F. and KAMAL M.M.: Effect of exercise training and anabolic androgenic steroids on hemodynamics, glycogen content, angiogenesis and apoptosis of cardiac muscle in adult male rats, *Int. J. Health Sci.*, (Qassim), 7 (1): 47-60, 2013.
- 14- CHAN H.M., JAIN R., AHREN B., PACINI G. and D'ARGENIO D.Z.: Effects of increasing doses of glucagon-like peptide-1 on insulin-releasing phases during intravenous glucose administration in mice. *Am J Physiol Regul. Integr. Comp. Physiol.*, 300 (5): R 1126-R 1133, 2011.
- 15- NORTON G.R., TRIFUNOVIC B. and WOODIWISS A.J.: Attenuated beta-adrenoceptor-mediated cardiac contractile responses following androgenic steroid administration to sedentary rats. *European Journal of Applied Physiology*, 81 (4): 310-6, 2000.
- 16- POPE H.G. and KATZ D.L.: Affective and psychotic symptoms associated with anabolic steroid use. *American Journal of Psychiatry*, 145 (4): 487-90, 1988.
- 17- NASCIMENTO A., SUGIZAKI M., LEOPOLDO S., LIMA-LEOPOLDO A., NOGUEIR C., NOVELLI E., PADOVANI C. and CICOGNA A.: Misclassification probability as obese or lean in hypercaloric and normocaloric diet. *Biol. Res*, 41: 253-259, 2008.
- 18- NOVELLI E., DINIZ Y., GALHARDI C., EBALD G., RODRIGUES H., MANI F., FERNANDES A., CICOGNA A. and NOVELLIFILHO J.: Anthropometric parameters and markers of obesity in rats. *Laboratory Animals. Ltd. Laboratory Animals*, 41: 111-9, 2007.
- 19- NISHIZAWA H., SHIMOMURA I., KISHIDA K., MAEDA N., KURIYAMA H., NAGARETANI H., MATSUDA M., KONDO H., FURUYAMA N., KIHARA S., NAKAMURA T., TOCHINO Y., FUNAHASHI T. and MATSUZAWA Y.: Androgens decrease plasma adiponectin, an insulin-sensitizing adipocyte-derived protein. *Diabetes*, 51 (9): 2734-41, 2002.
- 20- TIETZ N.W.: *Clinical Guide to Laboratory Tests*, 3rd Ed., pbl: W.B. Saunders Company, Philadelphia, PP 509-80, 1995.
- 21- REAVEN G.M.: Insulin resistance, hyperinsulinemia, hypertriglyceridemia and hypertension. Parallel between human disease. *Diab. Care*, 14 (3): 195-202, 1991.
- 22- BONORA E., TARGHER G., ALBERICHE M., BONADONNA R.C., SAGGIANI F., ZENERE M.B., MONAUNI T. and MUGGEO M.: Homeostasis model assessment closely mirrors the glucose clamp technique in the assessment of insulin sensitivity: Studies in subjects with various degrees of glucose tolerance and insulin sensitivity. *Diabetes Care*, 23 (1): 57-63, 2000.
- 23- FOSSATI P., and PRENCIPE L.: Serum triglycerides determined calorimetrically with an enzyme that produces hydrogen peroxide. *Clin. Chem.*, 28 (10): 2077-80, 1982.

- 24- NAUCK M.A., HOLST J.J. and WILLMS B.: Glucagon-like peptide 1 and its potential in the treatment of non-insulin-dependent diabetes mellitus. *Horm. Metab. Res.*, 29 (9): 411-6, 1997.
- 25- FRIEDEWALD W.T., LEVY R.I. and FREDRICKSON D.S.: Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin. Chem.*, 18 (6): 499-502, 1972.
- 26- AHN J.M., KWON A.R., CHAE H.W., SONG K., KIM D.H. and KIM H.S.: Messenger ribonucleic acid expression of KiSS-1 and serum level of kisspeptin in rat at different developmental stages. *Int. J. Pediatr. Endocrinol.* v. 2015 (Suppl 1): O28, 2015.
- 27- CASTEILLA L., PENICAUD L. and COUSIN B.: Choosing an adipose tissue depot for sampling. Factors in selection and depot specificity. *Methods. Mol. Biol.*, 155: 1-19, 2001.
- 28- YAN W.J., MU Y., YU N., YI T.L., ZHANG Y., PANG X.L., CHENG D. and YANG J.: Protective effects of metformin on reproductive function in obese male rats induced by high-fat diet. *J. Assist. Reprod. Genet.*, 32 (7): 1097-104, 2015.
- 29- IDRIS M.H., BUDIN S.B., OSMAN M. and MOHAMED J.: Protective role of hibiscus sabdariffa calyx extract against streptozotocin induced sperm damage in diabetic rats. *EXCLI Journal*, 11: 659-69, 2012.
- 30- BELSEY M.A., MOSHISSI K.S., ELIASSON R., PAULSEN C.A., CALLEGOS A.J. and PRASAD M.R.: Laboratory manual for the examination of human semen and semen cervical mucus interaction. Press concern, 1-43, 1980.
- 31- KHAKI A., FATHIAZAD F., NOURI M., MALEKI N.A., KHAMNEI H.J. and AHMADI P.: Beneficial effects of quercetin on sperm parameters in streptozotocin-induced diabetic male rats. *Phytother. Res.*, 24 (9): 1285-91, 2010.
- 32- MURTHY N.V., WRAY S.R., MELVILLE G.N., WYNTER H.H., RAM N.V. and HARAN N.V.: Testicular function in rats following immobilization stress. *Int. J. Gynaecol. Obstet.*, 26 (2): 297-9, 1988.
- 33- RAGHAVENDRA V., TANGA F., RUTKOWSKI M.D. and DELEO J.A.: Anti-hyperalgesic and morphine-sparing actions of propofol following peripheral nerve injury in rats: Mechanistic implications of spinal glia and proinflammatory cytokines. *Pain*, 104 (3): 655-64, 2003.
- 34- KIRKWOOD B.R.: Essential medical statistics. *Statistics in Medicine*, 8 (5): 636, 1989.
- 35- DOHLE G.R., SMIT M. and WEBER R.F.: Androgens and male fertility. *World J. Urol.*, 21 (5): 341-5, 2003.
- 36- ODA S.S. and EL-ASHMAWY I.M.: Adverse effects of the anabolic steroid, boldenoneundecylenate, on reproductive functions of male rabbits. *Int. J. Exp. Pathol.*, 93 (3): 172-8, 2012.
- 37- CALLEY J.L. and DHILLO W.S.: Effects of the hormone kisspeptin on reproductive hormone release in humans. *Advances in Biology*. Volume 2014, ArticleID 512650, 2014.
- 38- CLARKSON J. and HERBISON A.E.: Postnatal development of kisspeptin neurons in mouse hypothalamus; sexual dimorphism and projections to gonadotropin-releasing hormone neurons. *Endocrinology*, 147 (12): 5817-25, 2006.
- 39- HAN S.K., GOTTSCH M.L., LEE K.J., POPA S.M., SMITH J.T., JAKAWICH S.K., CLIFTON D.K., STEINER R.A. and HERBISON A.E.: Activation of gonadotropin-releasing hormone neurons by kisspeptin as a neuroendocrine switch for the onset of puberty. *J. Neurosci.*, 25 (49): 11349-56, 2005.
- 40- GEORGE J.T., VELDHUIS J.D., TENA-SEMPERE M., MILLAR R.P. and ANDERSON R.A.: Exploring the pathophysiology of hypogonadism in men with type 2 diabetes: Kisspeptin-10 stimulates serum testosterone and LH secretion in men with type 2 diabetes and mild biochemical hypogonadism. *Clin. Endocrinol. (Oxf)*, 79 (1): 100-4, 2013.
- 41- KAUFFMAN A.S., NAVARRO V.M., KIM J., CLIFTON D. and STEINER R.A.: Sex differences in the regulation of Kiss1/NKB neurons in juvenile mice: Implications for the timing of puberty. *American Journal of Physiology*, 297: E1212-E1221, 2009.
- 42- EL-HANBULI H.M., ABO-SIEF A.F. and MOSTAFA T.: Protective effect of silymarin on the testes of rats treated with anabolic androgenic steroid: A biochemical, histological, histochemical and immunohistochemical study *Journal of Histology & Histopathology*, ISSN 2055-091X, Volume 4, Article 10, 2017.
- 43- AL-ALWANY E.A., AL-SAAD H.K. and HASSAN A.J.: Effect of sustanon on some immunological and hormonal parameters in rats. *Int. J. Chem. Sci.*, 13: 1947-60, 2015.
- 44- ORIDE A., KANASAKI H., MIJDDORJ T., SUKHBATAR U., HARA T., TUMURBAATAR T. and KYO S.: GLP-1 Increases Kiss-1 mRNA Expression in Kisspeptin-Expressing Neuronal cells, *Biology of Reproduction*, 97 (2): 240-8, 2017.
- 45- BEAK S.A., HEATH M.M., SMALL C.J., MORGAN D.G., GHATEI M.A., TAYLOR A.D., BUCKINGHAM J.C., BLOOM S.R. and SMITH D.M.: Glucagon-like peptide-1 stimulates luteinizing hormone-releasing hormone secretion in a rodent hypothalamic neuronal cell line. *J. Clin. Invest.*, 101: 1334-41, 1988.
- 46- JEIBMANN A., ZAHEDI S., SIMONI M., NIESCHLAG E. and BYRNE M.M.: Glucagon-like peptide-1 reduces the pulsatile component of testosterone secretion in healthy males. *European Journal of Clinical Investigation*, 35: 565-72, 2005.
- 47- KANAYAMA G., HUDSON J.I., DELUCA J., ISAACS S., BAGGISH A., WEINER R., BHASIN S. and POPE H.G.: Jr. Prolonged hypogonadism in males following withdrawal from anabolic-androgenic steroids: An under-recognized problem. *Addiction*, 110: 823-31, 2015.
- 48- TAHTAMOUNI L., MUSTAFA N.H., HASSAN E.M., AHMAD I.M., YASIN S.R. and ABDALLA M.Y.: Nandrolonedecanoate administration to male rats induces oxidative stress, seminiferous tubules abnormalities, and sperm DNA fragmentation. *Jordan Journal of Biological Sciences*, 3: 165-74, 2010.
- 49- HARTGENS F. and KUIPERS H.: Effects of androgenic-anabolic steroids in athletes. *Sports Med.*, 34: 513-54, 2004.
- 50- HAKONSEN L.B., THULSTRUP A.M., AGGERHOLM A.S., OLSEN J., BONDE J.P., ANDERSEN C.Y., BUN-

- GUM M., ERNST E.H., HANSEN M.L. and RAMLAU-HANSEN C.H.: Does weight loss improve semen quality and reproductive hormones? Results from a cohort of severely obese men. *Reprod Health*, 8: 24, 2011.
- 51- ZHANG E., XU F., LIANG H., YAN J., XU H., LI Z., WEN X. and WENG J.: GLP-1 Receptor Agonist Exenatide Attenuates the Detrimental Effects of Obesity on Inflammatory Profile in Testis and Sperm Quality in Mice. *American Journal of Reproductive Immunology*, 74: 457-66, 2015.
- 52- LEEI Y.S. and JUN H.S.: Anti-Inflammatory Effects of GLP-1-Based Therapies beyond Glucose Control. *Mediators of Inflammation Journal*, Review Article, Volume 2016 ID 3094642, 2016.
- 53- FONTOURA P., CARDOSO M.C., ERTAL-MARTINS M.C., WERNECK C., SARTORIO C. and RAMOS C.F.: The effects of liraglutide on male fertility: A case report *Reproductive. Bio. Medicine*, 29 (5): 644-6, 2014.
- 54- SHIRAZIA R., PALSDOTTIRA V., COLLANDERA J., ANESTENA F., VOGELA H., LANGLETB F., JASCHKEC A., SCHÜRMAN A., PRÉVOTB V., SHAOA R., JANSSONA J.O. and SKIBICKAA K.P.: Glucagon-like peptide 1 receptor induced suppression of food intake, and body weight is mediated by central IL-1 and IL-6. *Proc. Natl. Acad. Sci. USA*, 110 (40): 16199-204, 2013.
- 55- HUNTER K. and HÖLSCHER C.: Drugs developed to treat diabetes, liraglutide and lixisenatide cross the blood brain barrier and enhance neurogenesis. *B.M.C. Neurosci.*, 13: 33, 2012.
- 56- VILSBOLL T., CHRISTENSEN M., JUNKER A.E., KNOP F.K. and GLUUD L.L.: Effects of glucagon-like peptide-1 receptor agonists on weight loss: Systematic review and meta-analyses of randomised controlled trials. *B.M.J. (Clin. Res. Ed.)*, 344: d7771, 2012.
- 57- ZHANG F., TONG Y., SU N., LI Y., TANG L., HUANG L. and TONG N.: Weight loss effect of glucagon-like peptide-1 mimetics on obese/overweight adults without diabetes: A systematic review and meta-analysis of randomized controlled trials. *J. Diabetes*, 7 (3): 329-39, 2015.
- 58- ZANDER M., MADSBAD S., MADSEN J.L. and HOLST J.J.: Effect of 6-week course of glucagon-like peptide-1 on glycaemic control, insulin sensitivity, and beta-cell function in type 2 diabetes: A parallel-group study. *Lancet*, 359: 824-30, 2002.
- 59- GEORGE A.J.: The actions and side effects of anabolic steroids in sport and social abuse, *Andrologie*, 13: 35, 2003.
- 60- TAKAHASHI M., TATSUGI Y., and KOHNO T.: Endocrinological and pathological effects of anabolic-androgenic steroid in male rats. *Endocrine Journal*, 51: 425-34, 2004.
- 61- SCHWETZ T.A., REISSAUS C.A. and PISTON D.W.: Differential Stimulation of Insulin Secretion by GLP-1 and Kisspeptin-10. *PLoS One*, 9 (11): e113020, 2014.
- 62- ACHAR S., ROSTAMIAN A., and NARAYAN S.M.: Cardiac and Metabolic Effects of Anabolic-Androgenic Steroid Abuse on Lipids, Blood Pressure, Left Ventricular Dimensions, and Rhythm. *Am. J. Cardiol.*, 106 (6): 893-901, 2010.
- 63- HARTGENS F., RIETJENS G., KEIZER H.A., KUIPERS H., and WOLFFENBUTTEL B.H.: Effects of androgenic-anabolic steroids on apolipoproteins and lipoprotein (a). *Br. J. Sports Med.*, 38: 253-9, 2004.
- 64- LUTZ T.A. and OSTO E.: Glucagon-like peptide-1, glucagon-like peptide-2, and lipid metabolism. *Current Opinion on Lipidol*, 27 (3): 257-63, 2016.

تأثير الجلوكاجون شبيه الببتيد-١ على مستوى الكسببتين في مصل دم ذكور الجرذان البيضاء البالغة المعالجة بالستيرويد الإبتنائى منشط الذكورة

خلفية البحث: يستخدم الستيرويد الإبتنائى منشط الذكوره إستخداما واسعا بين الشباب والرياضيين لتحسين مظهرهم وأدائهم البدنى، ومع ذلك آثار سلبية على الصحة الجنسية والتمثيل الغذائى. يعتبر الجلوكاجون شبيه الببتيد-١ أحد هورمونات القناة الهضمية الذى يشارك فى التحكم العصبى للغدد الصماء فى المحور النخامى المهادى والذى ربما يلعب دورا هاما فى الوظائف الإنجابية بإعتباره جزء من محور المخ والقناة الهضمية. أيضا، يقوم الكسببتين وهو ببتيد عصبى مهادى يحفز المحور النخامى المهادى للغدد التناسلية ويتحكم فى إفراز موجبات الغدد التناسلية. تم تنفيذ القليل من الأبحاث العلمية لدراسة العلاقة بين كل جلوكاجون شبيه الببتيد والكسببتين مع المحور النخامى المهادى للغدد التناسلية.

الهدف من البحث: هدفت الدراسة إلى تقييم دور الحماية المحتملة للجلوكاجون شبيه الببتيد-١ ضد التغيرات الجنسية والتشريحية المرضية والأبضية التى تحدث نتيجة إستخدام الستيرويد الإبتنائى منشط الذكوره فى ذكور الجرذان البيضاء البالغة.

مواد وطرق البحث: شملت الدراسة أربعين من ذكور الجرذان البيضاء البالغين التى تم تقسيمهم إلى أربع مجموعات متساوية، المجموعة الأولى (الضابطة) والمجموعة الثانية المعالجة بالجلوكاجون شبيه الببتيد-١ المركب والمجموعة الثالثة المعالجة بالستيرويد الإبتنائى منشط الذكوره والمجموعة الرابعة المعالجة بكل من الجلوكاجون شبيه الببتيد-١ والستيرويد الإبتنائى منشط الذكوره. تم قياس الوزن وحساب كتلة الجسم للجرذان. تم القياس فى مصل الدم لهورمونات التستوستيرون والجسم الأصفر والمحفز للبصيلات وكذلك الكسببتين والجلوكوز والأنسولين، تم حساب تقييم نموذج التوازن من مقاومة الأنسولين وكذلك مستوى الدهون. تم فحص التغيرات التشريحية المرضية للخصى كذلك دراسة وزن ومعامل الخصى بالإضافة إلى عدد وحركة الحيوانات المنوية فى البربخ.

النتائج: كشفت النتائج أن الحقن الطرفى للجلوكاجون شبيه الببتيد-١ لمدة خمس أسابيع للجرذان المعالجة بالأندروجين فى المجموعة الرابعة زيادة كبيرة فى مستويات الكسببتين وهورمونات الجسم الأصفر والمحفز للبصيلات والتستوستيرون فى مصل الدم وبالتالي زيادة فى عدد الحيوانات المنوية فى البربخ والحركية لها مقارنة بالجرذان فى المجموعة الثالثة. وبالإضافة إلى ذلك، أدى العلاج بالجلوكاجون شبيه الببتيد-١ إنخفاضات كبيرة فى قيم الجلوكوز فى مصل الدم ونموذج التوازن من مقاومة الأنسولين، والكوليسترول الكلى والدهون منخفضة الكثافة مع عدم وجود تغيرات كبيرة فى مستويات الأنسولين فى مصل الدم وفى الدهون عالية الكثافة والدهون الثلاثية بالمجموعة الرابعة مقارنة مع المجموعة الثالثة.

الإستنتاج: إستخدام الجلوكاجون شبيه الببتيد-١ قد يخفف بعض تغيير الآثار السلبية فى التأثيرات الجنسية وفى التمثيل الغذائى والتغيرات التشريحية المرضية الناجمة عن إستخدام الستيرويد الإبتنائى منشط الذكوره فى ذكور الجرذان البيضاء البالغين. ولذلك قد يكون نهجا جديدا لإدارة سوء إستخدام الستيرويد الإبتنائى منشط الذكوره.