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Effects of Testosterone Replacement on Insulin Sensitivity, Blood Glucose, Serum Lipids and Vitamin D Concentration in a Rat Model of Andropause

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Abstract: Background: Testosterone facilitates various metabolic processes in the bones, muscles, bone marrow, brain and immune system. Testosterone deficiency may contribute to cardiovascular diseases, central obesity and metabolic dysfunction. Aim: to investigate the effects of testosterone deficiency on blood glucose levels, insulin sensitivity, vitamin D, Homeostatic model assessment of insulin resistance (HOMA-IR), Homeostatic model assessment for beta function (HOMA-IR) and serum lipids in rat models of andropause. Methods, 48 male rats divided into 4 equal groups (n=12/group); a) Control (C) group. b) Orchiectomy (O) group underwent bilateral removal of the testicles. c) Orchiectomy and treated (O+T) group given daily IM testosterone replacement therapy for 7 days, d) Sham and treated (Sh+T) group in which sham operated rats were given the same daily dose of T as O+T group for 7 days. Results: we found that plasma glucose concentration increased significantly ($P<0.001$) in O group. This increase was corrected by testosterone injection in O+T group. In (Sh+T) group, there is a significant reduction in serum glucose ($P<0.001$). We also found that insulin level reduced significantly ($P<0.001$) in O group. This reduction in insulin level was corrected by testosterone. In (Sh+T) group, insulin level increased significantly. 25-OHVD3 was reduced significantly ($P<0.05$) in O group and increased significantly in O+T group. HOMA-IR level decreased significantly ($P<0.001$) and this reduction was corrected by Testosterone. HOMA-β reduced significantly ($P<0.001$) and this reduction was corrected by Testosterone in O+T group. In SH group, there is a marked increase in HOMA-β. We also found insignificant differences between the four groups in serum triglycerides. However, we found very highly significant differences in total cholesterol, LDL and VLDL between C group and O group and very highly significant differences between O+T group and Sh+T group in comparison with O group. We also found significant differences in serum HDL-c in (O) group in comparison with (C) group and between O+T and Sh+T groups in comparison with O group. In conclusion, we found significant reduction in 25-OH(VD), Testosterone, Insulin, HOMA-IR and HOMA-B and significant increase in glucose serum levels in O group and these changes were corrected by Testosterone injection in O+T group. We also found insignificant differences between the four groups in serum triglycerides. However, we found highly significant differences in total cholesterol, LDL, HDL-c and VLDL between C group and O group and very highly significant differences between O+T group and Sh+T group in comparison with O group.

Keywords: Testosterone, Orchiectomized, Insulin, HOMA-IR, HOMA-B, Glucose

1. Introduction

Testosterone is responsible for sexual desire, erection and secondary sex characteristics. Testosterone facilitates various metabolic processes in the bones, muscles, bone marrow, brain and immune system. Therefore, testosterone deficiency leads to metabolic dysfunctions [1].

In men, 80% of total testosterone binds to sex hormone binding globulin and acts as a reserve source. Free testosterone and albumin bound testosterone are biologically active representing 20% of total testosterone [2, 3].

Andropause is a prevalent and serious aging related problem. The gradual decline of testosterone levels with aging may be due to reduction of Leydig cell mass, testicular circulation, hypothalamic GnRH and pituitary gonadotropins [4, 5]. Decreased androgen levels associated with aging is referred as Late-onset hypogonadism. It is manifested by (i) somatovegetative conditions, including fatigue, insomnia, osteopenia, sarcopenia and visceral obesity, (ii) psychological conditions, including disturbed sense of well-being, mood disorders and anxiety and (iii) sexual conditions, including erectile dysfunction and decreased libido [6-8].

Testosterone deficiency may contribute to cardiovascular diseases, central obesity and metabolic dysfunction and its deficiency promoted atherosclerosis by changing lipid metabolism [9]. Visceral obesity leads to gonadotropin suppression and subsequent testosterone deficiency [10]. Clinical studies reported that testosterone levels inversely correlated with total cholesterol and LDL cholesterol levels [11-13]. In addition, animal studies demonstrated highly elevated cholesterol levels in testosterone-deficient male mice [14, 15]. These findings suggested that testosterone serves a valuable role in regulating serum lipids. However, other controversial studies reported the reverse and some of them reported insignificant effects of testosterone [16- 18]. So, for this controversy, we conducted this study to elucidate the effect of cholesterol on lipid profile.

Therefore, this study is carried out to evaluate the effects of testosterone deficiency on blood glucose levels, insulin sensitivity, HOMA-IR, HOMA-B, serum lipids and vitamin D concentration in rat models of andropause.

2. Methods

Animals: A total of 48 male Albino rats (250-300 g) from the experimental animal center in the College of Pharmacy – Zagazig University were used in this study, between June and August 2016. Methods in this study are approved by the National Medical Ethics Committee in faculty of medicine of Zagazig University (Institutional Review Board, IRB). Rats were fed on a normal diet with free access to water, at comfortable temperature, in addition to 12 h light/dark cycle and hygienic issues were considered. Rats were kept one week for acclimatization then randomly divided into 4 equal groups (n=12/group); a) Control (C) group which underwent sham operation, in addition to intramuscular (IM) injection with 0.2 ml of normal saline. b) Orchiectomy (O) group in which all rats were surgically managed by bilateral removal of the testicles in addition to IM injection 0.2 ml of normal saline. c) Orchiectomy and treated (O+T) group in which orchiectomized rats were further given daily IM testosterone replacement therapy for 7 days. d) Sham and treated (Sh+T) group in which sham operated rats were given the same daily dose of T as O+T group for 7 days.

Chemicals: ketamine/xylazine mixture and Testosterone undecanoate purchased from Sigma Chemicals co. Cairo Egypt.

Surgical procedure: Rats were generally anesthetized via intraperitoneal injection of ketamine/xylazine mixture (100/10 mg/kg, respectively) [19] that produces a good anesthesia, and muscle relaxation for 20 min in addition to postoperative analgesia. Anesthetized animals were placed in the supine position, fixed over a sterile small animal operating theatre. Before skin incision, disinfection of the testicular area with iodized alcohol was done. The sham operation was performed through a midline incision in the ventral aspect of the scrotum then dissection of subcutaneous tissues until exploration of testes and suture of the skin. In orchiectomized rats, tunicae were opened, testes were ligated with 3-0 Vicryl placed around the lower end of the spermatic cord then the testes were removed after cutting between two ligations. Subsequently, the testicular skin was sutured and a local antibiotic ointment was applied over the sutures [20]. Animals were left for 2 weeks after surgery for complete healing of wounds and development of andropause [21].

Testosterone hormone replacement therapy: The O+T and Sh+T groups were IM injected with T undecanoate (100 mg/kg) for 1 week after castration, while the other groups with equal volume of 0.9% normal saline instead [22].

Serology: Later on, all rats were euthanized and about 6 mL of blood was collected via cardiac puncture. The blood was put in green-topped mini collection tubes and centrifuged (Sigma Aldrich Eppendorf® Centrifuge 5702) at 12000 rpm for 12 minutes at 4°C, and the plasma was collected and stored at –80°C. Assays for testosterone was done by ELISA kits [23], catalogue number MBS026898 (Mybiosource, USA). 25-OHVD3 was also measured by rat ELISA kits [24] catalogue no MBS288530 (Mybiosource USA). Furthermore, rat insulin ELISA kit (MBS724709, Mybiosource USA) was used to measure the insulin level, while plasma glucose concentration was assessed by the spectrophotometric method [25]. Additionally, Homeostatic model assessment for insulin resistance (HOMA-IR) and Homeostatic model assessment for beta function (HOMA-IR) were calculated [26]. Serum high density lipoprotein (HDL) was estimated by the method of Warnick [27]. Total cholesterol (CH) and triglycerides (TG) were estimated by the methods of Siedel et al. [28] and Foster and Dunn [29], respectively. Low density lipoprotein (LDL) and very low density lipoprotein (VLDL) were calculated by Friedwald's formula [30].

Statistical analysis: The SPSS, version 24 for Windows (SPSS Inc. Chicago, IL, USA), was used. The data were presented as the mean ± SD. An ANOVA with a post hoc test was used to analyze the differences in multiple comparisons.

Pearson correlation coefficient was also used to test the relationships among the study variables. P values < 0.05 were considered to be significant.

3. Results

Table 1. *Biochemical parameters measured for all studied groups.*

Studied parameters	Control	Orchiectomy	Orchiectomy treated	Sham & treated
	n=12	n=12	n=12	n=12
25(OH)-VD (ng/ml)	33.22±7.74	15.00±2.38 P<0.05[a]	73.19±4.45 P<0.001[a] P<0.001[b]	74.40±10.25 P<0.001[a] P>0.05[c]
Testosterone (ng/ml)	7.53±1.38	0.91±0.35 P<0.001[a]	2.94±0.93 P<0.001[a] P<0.001[b]	16.01±2.86 P<0.001[a] P<0.001[c]
Glucose (mg/dl)	95.79±4.55	123.92±5.90 P<0.001[a]	91.89±9.85 P>0.05[a] P<0.001[b]	64.09±8.45 P<0.001[a] P<0.001[c]
Insulin (mIU/ml)	8.85±0.09	4.55±0.95 P<0.001[a]	8.89±0.08 P>0.05[a] P<0.001[b]	10.36±1.54 P<0.05[a] P<0.001[c]
HOMA-IR	2.09±0.09	1.44±0.24 P<0.001[a]	2.02±0.21 P>0.05[a] P<0.001[b]	1.23±0.15 P<0.001[a] P<0.001[c]
HOMA-β	99.06±17.11	27.08±6.60 P>0.05[a]	121.88±41.64 P>0.05[c] P>0.05[c]	810.69±251.50 P<0.001[a] P<0.001[c]

a = *p-value* of significance versus control group, b = *p-value* of significance versus orchiectomy group, c = *p-value* of significance versus orchiectomy treated group.

Table 2. *Serum Lipids (Mean ± SD).*

Studied parameters (mM)	Control	Orchiectomy	Orchiectomy treated	Sham & treated
Triglyceride	1.08 ± 0.19	1.16 ± 0.46	1.09 ± 0.23	0.91 ± 0.53
Total Cholesterol	0.98 ± 0.09	1.89 ± 0.23^{***a}	0.99 ± 0.13^{***b}	0.96 ± 0.39^{***b}
HDL-c	1.12 ± 0.30	0.91 ± 0.16^{*a}	1.11 ± 0.30	1.26 ± 0.50^{*b}
LDL	49.64 ± 1.82	52.74 ± 2.32^{**a}	50.24 ± 1.12^{**b}	48.64 ± 1.32^{***b}
VLDL	15.22 ± 0.48	17.12 ± 0.36^{***a}	15.93 ± 0.37^{***b}	12.22 ± 0.32^{***b}

HDL-c, high-density lipoprotein-cholesterol;
LDL, Low-density lipoprotein
VLDL, Very-Low-density lipoprotein-cholesterol
*a= significant in comparison with control group
*a= significant in comparison with Orchiectomized group

4. Discussion

Testosterone facilitates various metabolic processes in the bones, muscles, bone marrow, brain and immune system. Testosterone deficiency may contribute to cardiovascular diseases, central obesity and metabolic dysfunction [9]. Therefore, this study aims to demonstrate the effects of testosterone deficiency on blood glucose levels, insulin sensitivity, vitamin D and Serum lipids in rat models of andropause.

In our study, we found that plasma glucose concentration increased (from 95.79±4.55 to 123.92±5.90) significantly (P<0.001) in orchiectomized rats in comparison with the control group. This increase was corrected (91.89±9.85) by testosterone injection in Orchiectomy treated group. In sham treated group, there is a significant reduction in serum glucose (64.09±8.45) (P<0.001). These findings were in agreement with RAO PM., et al [10] who found that testosterone stimulates glucose uptake, glycolytic pathway and oxidative phosphorylation. They reported that testosterone participates in lipid homeostatic mechanisms in many tissues, as liver and adipose tissue.

In the present study, we found that insulin level reduced (from 8.85±0.09 to 4.55±0.95) significantly (P<0.001) in orchiectomized rats in comparison with the control group. This reduction in insulin level was corrected by testosterone (8.89±0.08) in Orchiectomy treated group. In sham treated group, insulin level increased (10.36±1.54) significantly in comparison with the last two groups. Our results were in agreement with Zitzmann M. [31] who reported that testosterone deficiency promoted the development of the metabolic syndrome and it has antagonizing effects on the generation of muscle and visceral adipose tissue.

In our study, we also found that 25-(OH)D was reduced (from 33.22±7.74 to 15.00±2.38) significantly (P<0.05) in orchiectomized rats. In addition, we found that testosterone increased 25(OH)D significantly (from 15.00±2.38 to 73.19±4.45) in Orchiectomy treated group. Our findings were in agreement with Zhao et al., [32] who found that Lower

25(OH)D concentrations were associated with lower Sex Hormone Binding Globulin (SHBG) levels and higher free testosterone levels in both men and women. However, our results were in controversy with Haymana et al., [33] who found that 25(OH)D3 was not significantly changed after testosterone replacement therapy (TRT). This controversy can be explained by species differences as well as short term duration used in the latter study.

In the present study, we found that homeostatic model assessment of insulin resistance (HOMA-IR) levels decreased (from 2.09±0.09 to 1.44±0.24) significantly (P<0.001) and this reduction was corrected by Testosterone replacement (2.02±0.21). Our findings are in agreement with Schianca et al., [34] who found that HOMA-IR was related to testosterone and free-testosterone even in patients with normal glucose tolerance. They also reported that at multivariate analysis HOMA-IR was the only variable associated to testosterone (p<0.001) and free-testosterone (p<0.05) plasma concentration. However, our results were in controversy with Haymana et al., [33] who found that HOMA-IR levels were not significantly changed after a short-term testosterone replacement therapy (TRT). This controversy can be explained by species differences as well as short-term duration used in the latter study.

We also found that HOMA-β reduced (from 99.06±17.11 to 27.08±6.60) significantly (P<0.001) and this reduction was corrected by Testosterone replacement (121.88±41.64) in orchiectomy treated group. In sham-operated group, there is a marked increase in HOMA-β (810.69±251.50). Our findings are in agreement with Zitzmann M, [31] who found that testosterone has a protective effect on pancreatic beta cells, this effect exerted by androgen-receptor-mediated mechanisms.

In our study, we also found insignificant differences between the four groups in serum triglycerides. Our finding was in agreement with Christoffersen et al., [35] who found that triglycerides concentration did not differ significantly between the groups. However, we were in controversy with Richard et al., [36] who found a decreased TG concentration in castrated rats.

In the present study, we found very highly significant differences in total cholesterol between Control (C) group (0.98±0.09) and Orchiectomy (O) group (1.89±0.23) (P<0.001). In addition, we found very highly significant differences between Orchiectomy treated (O+T) group (0.99 ±0.13) (P<0.001) and Sham treated (Sh+T) (0.96±0.39) group in comparison with Orchiectomy (O) group. Our finding was supported by Xu et al., [37] who found that orchiectomy lead to increased total cholesterol levels in rats. However, we are in controversy with Christoffersen et al., [35] who found insignificant increase in cholesterol concentration in Orchiectomy (O) group. This controversy can be explained by species differences where their study was performed on Sprage Dawly rats and our study on albino rats. In addition, the diets used in their study were not cholesterol enriched.

We also found significant differences in serum high density lipoprotein-cholesterol (HDL-c) between Orchiectomy (O) group (0.91±0.16) in comparison with Control (C) group (1.12±0.30) and (P<0.05). Also, we found significant differences between Orchiectomy treated (O+T) (1.11±0.23)(P<0.05) and Sham treated (Sh+T) (1.26±0.50) groups in comparison with Orchiectomy (O) group (0.91±0.16). Our results are in agreement with Christoffersen et al., [35] who found a larger increase in HDL-c in the castrated group.

we found highly significant differences in Low Density Lipoprotein (LDL) between Control (C) group (49.64 ± 1.82) and Orchiectomy (O) group (52.74 ± 2.32) (P<0.01). Also, we found highly significant differences between Orchiectomy treated (O+T) group (50.24 ± 1.12) (P<0.001) and Sham treated (Sh+T) (48.64 ± 1.32) group in comparison with Orchiectomy (O) group. Our findings are in agreement with Cai et al., [38] who found that the levels of total cholesterol and LDL cholesterol are increased significantly by castration.

We also found very highly significant differences in Very Low Density Lipoprotein (VLDL) between Control (C) group (15.22 ± 0.48) and Orchiectomy (O) group (17.12 ± 0.36) (P<0.001). Also, we found highly significant differences between Orchiectomy treated (O+T) group (15.93 ± 0.37) (P<0.001) and Sham treated (Sh+T) (12.22 ± 0.32) group in comparison with Orchiectomy (O) group. We are in controversy with Rouver et al., [39] who found that the concentrations of triglycerides and VLDL were decreased after 15 days of castration.

In conclusion, we found significant reduction in 25-OH(VD), Testosterone, Insulin, HOMA-IR and HOMA-B and significant increase in glucose serum levels in orchiectomized rats and these changes were corrected by Testosterone injection. In our study, we also found insignificant differences between the four groups in serum triglycerides. However, we found very highly significant differences in total cholesterol, LDL and VLDL between Control (C) group and Orchiectomy (O) group and very highly significant differences between Orchiectomy treated (O+T) group and Sham treated (Sh+T) group in comparison with Orchiectomy (O) group. We also found significant differences in serum HDL-c (O) group in comparison with (C) group. In addition, we found significant differences between O+T and Sh+T groups in comparison with O group. Further studies need to investigate the clinical applications of testosterone administration in human.

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