The Effect of Testosterone on Liver Tissue and Lipid Profile in Female Rats

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Abstract

Introduction: Androgenic and anabolic action of testosterone mediated through androgen receptor, which have been documented to have significant biological actions in bone, muscle, prostate, adipose tissue and the reproductive, cardiovascular, immune, neural and haemopoietic systems. Aim: Evaluate the action of testosterone on liver histology and lipid profile (cholesterol, triglyceride, LDL, HDL and VLDL). Materials and Methods: Adult female wistar rat (no. 12) and weight (180-200 g) was housed in stain steel cages. Six rats were divided into two groups control group given propylene glycol as vehicle while treated group give testosterone propionate subcutaneous with 100mg/kg dose dissolve in propylene glycol for 30 days. Live histology examination and lipid profile assessment were conducted to compare the differences and the effect of the testosterone. Results: There was no significant differences in all parameters of cholesterol, triglyceride, low-density lipoprotein, high-density lipoprotein and very low-density lipoprotein, while the histology of treated group showed dilatations of the cholesterol sinusoids which led to increase pressure of the hepatic biliary system, with fatty changes and dysplasia of hepatocytes. Conclusion: Testosterone plays an important role in aging by increasing hepatic glycogen stores and alteration of hepatic tissues.

Keywords: Female Rats, Hepatic Tissues, Low-density Lipoproteins.

INTRODUCTION

Testosterone is a steroid hormone and one of the two principal androgens in mature male with androgenic and anabolic effects. Testosterone biosynthesis is an enzymatic sequence process begins from cholestrol and take places in the mature Leydig cells, located in the interstitial compartment of the testis between the seminiferous tubules. These cells are the only cells expressed all enzymes necessary for transformation of cholestrol to testosterone, responsible for production of approximately 95% of the circulating testosterone and consider as the main source of testosterone. The remaining of testosterone production derived from extragonadal sources (adrenal glands, brain). The net contribution of adrenal androgen to circulating testosterone is minor in men but remarkable in women. Cholesterol in the Leydig cell either formed by de novo biosynthesis or derived from endocytosis of low-density lipoproteins (LDLs).

First step in testosterone biosynthesis is the conversion of cholesterol to pregnenolone. This step occurs in mitochondria and involves the shortening of the cholesterol side chain by C22 and C20 hydroxylases followed by cleavage of bound between C20 and C22. The next steps occur in endoplasmic reticulum, either through the ∆4 or through ∆5 pathway. In ∆4 synthesis pathway, pregnenolone converted to testosterone via intermediates progesterone, 17α-hydroxyprogesterone, and androstenedione. While in ∆5 biosynthesis pathway, transformation to testosterone take places through intermediates 17α-hydroxy pregnenolone, dehydroepiandrosterone (DHEA), and androstenedione. The ∆5 pathway is predominant over the ∆4 pathway in human. The pituitary Luteinizing hormone (LH) has receptors on the Leydig cell surface membrane and playing a central regulatory role in testosterone biosynthesis via exerting its effect on steroidogenesis that includes increasing cholesterol availability and activating the rate-limiting enzyme and cholesterol transport proteins. LH is derived from pituitary gland by the episodic secretion of hypothalamic gonadotrophin releasing hormone (GnRH) and secreted into portal bloodstream. The LH is transfers by blood flow from brain into Leydig cell, where it binds to its receptor and star a cascade of biochemical event resulting in production and secretion of testosterone. This cyclic pathway controlled by negative feedback mechanism mediated by testosterone.

Keywords: Female Rats, Hepatic Tissues, Low-density Lipoproteins.
When testosterone level increases, the hypothalamus in brain receives a signal and stops production of GnRH and subsequently the LH production is ceased \[7,8\]. The daily production of testosterone is 5-7 mg with plasma half-life 12 minutes \[5\]. In circulation, only 2% of secreted testosterone is circulate freely, while the majority of it circulated with binding to plasma protein. Sex hormone binding globulin (SHBG) is a high affinity but low- capacity binding protein and responsible for bound 60% - 70% of the circulating testosterone \[9\]. In human, SHBG secreted from Liver, thereby its serum level regulated through the opposing action of hormones on the hepatocytes: estrogens and thyroxin stimulate and androgen and glucocorticoids inhibit SHBG production \[9\].

SHBG serum level also influenced by other factors such as acute or chronic liver disease and androgen deficiency, in which it increases, and the obesity, protein-losing states, non-alcoholic fatty liver disease and, rarely, genetic SHBG deficiency, in which it decreases \[2\]. In rats, SHBG secreted by Sertoli cell into somniferous tubules where it is known as testicular androgen-binding protein \[2,5\]. The majority of secreted testosterone undergoes inactivation once again in the liver by phase I and phase II metabolism. Phase I, testosterone oxidized to inactive metabolites by hepatic oxidase \[2\]. Testosterone considers as CYP3A4 specific substrate and a major hepatic metabolite is 6b-OH TST. 2b-OH TST and androstenedione are two other metabolites formed in the liver by the action of CYP3A4 and CYP2D6, respectively \[10\]. Phase II metabolism involves hepatic conjugations to glucuronides and catalyzed mostly by Uridine diphosphate (UDP) glucuronosyl transferase (UGT) enzymes. the resulted oxidized and conjugated metabolites are inactive and excreted by urinary and/or biliary route \[2\]. Only a small portion of secreted testosterone converted to biologically active metabolites, which are Dihydrotestosterone (DHT) and estradiol \[2\]. Also, these metabolites can be formed from further metabolism of testosterone in the Leydig cell \[5\]. Testosterone is converted to DHT, which is a pure, potent androgen with higher binding affinity and greater potency for androgen receptors, by 5α-reductase enzyme. Type 1 5α-reductase enzyme is expressed in the liver, kidney, skin, and brain, whereas type 2 5α-reductase is expressed mainly in the prostate. Testosterone converted to estradiol by the action of aromatase enzyme \[3\]. Androgens (testosterone and DHT) have an important role in development of male reproductive organs and maturation of secondary sexual characteristics \[5,11\]. Testosterone playing an important role in regulating carbohydrates, proteins, and lipid metabolism and consider as critical physiologic modulator for muscle structure and function \[1\] and some studies shows a relation between testosterone and cognition, emotions and behavior \[3\]. Androgenic and anabolic action of testosterone mediated through androgen receptor, which is a nuclear receptor located on X chromosomes and expressed in a wide range of tissues and as such androgens have been documented to have significant biological actions in bone, muscle, prostate, adipose tissue and the reproductive, cardiovascular, immune, neural and haemopoietic systems \[12\]. Ligand - dependent Androgen receptor actions either dependent or independent on DNA binding. In DNA binding dependent (DBD) action of AR, also referred to as “genomic” or “classical” AR signaling, binding of testosterone to androgen receptors leads to androgen/AR complex. The resulted complex translocates to the nucleus where it binds to androgen receptor elements (AREs) causing modulation in gene transcription \[11\]. Coregulators are specific modulator proteins that regulate the transcriptional activity of androgen via binding to activated receptors and either enhance (coactivator) or repress (corepressor) its ability to transactivate the target gene \[11\]. In non-DNA binding dependent (non-DBD) action of AR, also referred as “non-genomic” or “non-classical” AR signaling, the effect of testosterone mediated within seconds to minutes through activation of second messenger pathway \[11,3\]. The androgen/AR complex activates a second messenger pathway via increasing Intracellular calcium level, causing multiple protein kinases activation \[11,3\] including ERK, Akt and MAPK \[11\] and thereby triggering important signaling cascades \[3\]. The non-DBD pathway also noticed in cells lack a typical androgen receptor but contain testosterone-binding sites on the surface of the plasma membrane \[11,3\], like macrophages \[3\]. These testosterone receptors linked with G-protein and associated with phospholipase C \[3\].

**Material and Method**

**Animal**

Adult female wistar rat (no. 12) and weight (180-200 g) was housed in stain steel cages under control condition (14 h light and 10 h dark cycle) and temperature (24°). Animals had a free access to water and was fed daily. All animal experiment were conducted according to the ethical standard of animal care committee of university of Baghdad- college of pharmacy.

**Testosterone administration**

Rats divided into two groups (no.6 with each group). Control group was injected with propylene glycol which used as a vehicle, while the treated group injected with testosterone propionate (sustanon 250 mg, organon) subcutaneous with 100mg/kg dose dissolve in propylene glycol for 30 days.

**Cholesterol, Triglyceride and Lipoprotein measurement**

After 30 days of testosterone propionate administration to treated group, blood sampling was drawn by heart puncture technique under anesthetic effect of diethyl ether. cholesterol, triglyceride, LDL, HDL and VLDL for both control and treated group was measured by using COBAS
E411 device (ROCHE Company).

Liver Histology
After 30 days of experiment, rats were scarified by cervical dislocation under the effect of diethyl ether anesthesia, liver was collected and store in formalin in a plastic container for histological investigation.

Statistical analysis
All data expressed as mean ± standard error mean (SEM) with a P value <0.05 considered to be significant. Statistical analysis performed using IBM statistical package for social science (SPSS version 25). Figures produced using Microsoft Excel 2016.

RESULTS
1- Lipid profile of control group and treated group shown in table 3.1 lipid profile included (cholesterol, low density lipoprotein, high density lipoprotein, triglycerides, and very low-density lipoprotein) there was no significant differences in the aforementioned measured parameters between control and treated group (P>0.05)

Table 3.1: level of cholesterol, triglyceride, high-density lipoprotein, low-density lipoprotein, and very low-density lipoprotein of the control vs treated group.

<table>
<thead>
<tr>
<th>No.</th>
<th>CHO</th>
<th>TRI</th>
<th>LDL</th>
<th>HDL</th>
<th>VLDL</th>
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<td>25</td>
<td>12</td>
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<td>45</td>
<td>28</td>
<td>9</td>
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<tr>
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<td>58</td>
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<td>20</td>
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<td>29</td>
<td>7</td>
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<tr>
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<td>44</td>
<td>54</td>
<td>21</td>
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</tr>
</tbody>
</table>

Figure 3.1: illustrate the comparison of the lipid profile parameters of control vs treated group. There were no significant differences in all parameters of cholesterol, triglyceride, low-density lipoprotein, high-density lipoprotein and very low-density lipoprotein (94.17±5.58 vs 77.5±8.04, 47.67±4.232 vs 49.17±7.31, 60.67 ±5.65 vs 43± 7.65, 23.5 ±1.17 vs 24.8±1.8, 10± 0.98 vs
9.66±1.4) respectively.

Figure 3.2 showing different sections normal histological structure of the control group

Figure 3.3 showing different sections of liver histology of the treated group. The sections showing dilatations of the hepatic sinusoids which led to increase pressure of the hepatic biliary system, with fatty changes and dysplasia of hepatocytes (arrows)

**DISCUSSION**

Anabolic androgenic steroids (AAS) are synthesized derivatives of the male sex hormone testosterone, firstly developed for therapeutic purposes to provide a high anabolic rate with lesser androgenic side effects. Growing numbers of young athletes are using these agents illicitly to enhance appearance, performance and physical fitness despite the fact their numerous adverse effects and being globally banned. Nowadays, their use still one of the main health problems in sports because these drugs are available and low cost \[13\]. This study showed that treatment of Wistar rats for 30 days did not significantly alter lipid profile of treated group as compared with control group. However, another study showed that treatment of male Wistar rats for three
consecutive months with nandrolone decanoate significantly increase in plasma triglycerides level, furthermore treatment caused a significant decrease in HDL as compared to control group. Interestingly the treatment showed that there was no significant effect of nandrolone decanoate treatment on total cholesterol and LDL, which agrees to what was found in the present study [14].

Nonetheless, these findings of the previous study could explain the results of our study in which the treatment time should be longer to get a significant effect on lipid profile parameters or use different AAS agent to compare between their effects.

Additionally, it was shown that administration of supraphysiologic doses (600 mg/week) of testosterone enanthate combined with strength training exercise for 10 weeks had no significant on lipid profile in normal men [17].

Furthermore, another study shown that Testosterone in low dose has no effect on serum cholesterol concentration, however serum cholesterol concentration decreases in high dose of Testosterone. These studies came in agreement with our findings; some studies have reported a significant reduction in serum cholesterol levels after high doses of testosterone injections [19].

Histopathological studies of the treated group showed dilatations of the hepatic sinusoids, which led to increase pressure of the hepatic biliary system, with fatty changes and dysplasia of hepatocytes. A study in 2017 showed that administration of testosterone for 16 weeks to Wistar rats (at different age groups) cause a significant decrease in liver weight [19].

Testosterone treatment showed significant decrease in hepatocyte area, however increased number of hepatocytes and the number of other liver cells. Chronic hepatic congestion and/or possible cholestasis, characterized by decreased lobular parenchyma components and consequent increase in non-lobular parenchyma, in addition to, an increase of collagen fibers type I (indicating fibrosis) and also showed increased liver glycogen in testosterone treated group [19]. This effect due to positive effect of testosterone on the mitotic capacity of the liver cells secondly testosterone has a negative effect on old animals’ liver as chronic hepatic congestion and/or cholestasis. Finally, testosterone plays an important role in aging by increasing hepatic glycogen stores [19].

CONFLICT OF INTERESTS

There is no conflict of interest.

REFERENCES


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