Role of Testosterone in Glucose Homeostasis in Immobilization Stressed Rats

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Abstract: The purpose of the present study was to examine the role of testosterone in glycemic responses induced by immobilization stress. The study was conducted on male Wistar albino rats divided into 4 groups: control group, testosterone-treated control (unstressed) group, immobilization-stressed group and testosterone-treated immobilization-stressed group. Chronic immobilization stress caused significant decrease in plasma testosterone levels, significant increase in plasma glucose, glucose output by kidneys and significant decrease in glucose uptake by diaphragm. Plasma insulin was significantly decreased and G/I ratio significantly increased. This was associated with impaired β-cell function as indicated by low HOMA-β but absence of insulin resistance as shown by insignificant differences in HOMA-IR. Plasma MDA was significantly increased. Testosterone treatment in immobilization-stressed rats resulted in significant amelioration of β-cell dysfunction as shown by the high HOMA-β together with significant decrease in plasma glucose, glucose output and significant elevation in glucose uptake. Plasma insulin increased significantly and G/I ratio decreased significantly. Plasma MDA decreased significantly. Correlation studies showed that plasma testosterone levels were negatively correlated with plasma glucose levels ($r=-0.536$, $P<0.005$), glucose output by kidneys ($r=-0.451$, $P<0.05$) and plasma MDA levels ($r=-0.383$, $P<0.05$) and positively correlated with plasma insulin levels ($r=0.524$, $P<0.05$), glucose uptake by diaphragm ($r=0.380$, $P<0.05$) and HOMA-β ($r=0.437$, $P<0.05$). Histological examination of pancreas from immobilization-stressed rats revealed degeneration, edema, mononuclear cellular infiltration and cytoplasmic vacuolations. Also, significant increase in caspase-3 immunoreactivity, an apoptotic marker, was observed in pancreatic islets of Langerhans and acinar cells. Testosterone treatment prevented the pancreatic histological damage and attenuated cellular apoptosis. In conclusion, testosterone treatment prevented the development of a diabetes mellitus-like metabolic syndrome associated with immobilization stress. Also, testosterone treatment protected the pancreas against damage and β-cell dysfunction, enhanced insulin secretion and nullified oxidative insult induced by stress. Hence, testosterone could be potentially considered as an adjunct in the treatment of diabetic state in males exposed to stressful situations.
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Key Words: Stress, immobilization, testosterone, glucose, insulin and rats.
Abbreviations: G/I (glucose to insulin), MDA (malondialdehyde), HOMA (Homeostasis model assessment), IR (insulin resistance).

1. Introduction

Stress can be defined as a state of threatened homeostasis and is counteracted by an intricate repertoire of physiologic and behavioral responses that collectively aim to re-establish the disturbed equilibrium (adaptive stress response) [(1)]. Stimuli that challenge homeostasis, designated as stressors, can be divided into 3 general categories: physical, psychosocial and metabolic [(2)]. Inappropriate responses to stressors, as inadequate, excessive and/or prolonged reactions, may turn deleterious and contribute to disease. Chronically imposed or severe stressors can impair various physiologic functions [(3)]. Immobilization stress is a mixture of physical and psychological stressors, restricting movement and isolating the individual from its group [(4)]. Psychological and physiological stressors can disturb neuroendocrine, reproductive, and metabolic functions [(4, 5)]. The alterations in glucose and lipid metabolism induced by stress may contribute to the etiology and development of diabetes mellitus and cardiovascular diseases [(6)].

In males, decreased serum testosterone is one of the first signs of stress [(7)]. Low concentrations of endogenous androgens have been linked with insulin resistance, which is an important upstream driver for metabolic abnormalities such as hyperglycemia, hypertension, or dyslipidemia, and increased cardiovascular risk [(8, 9)]. Men with impaired glucose tolerance were found to have significantly lower levels of total testosterone compared with those with normal glucose tolerance [(10)]. **Keating *et al.*** [(11)] observed that androgen deprivation therapy is associated with an increased incidence of diabetes and cardiovascular disease. On the other hand, administration of testosterone to hypogonadal middle aged men was found to improve insulin sensitivity and glucose homeostasis [(12)].

Thus, the purpose of the current study was to clarify the contribution of testosterone to glucose

homeostasis in male rats exposed to immobilization stress.

2. Material and Methods

Experimental Animals and Protocol:

The study was conducted on thirty two adult male Wistar albino rats, weighing 200-280 g which were obtained from the experimental animal farm in Giza, housed in the Physiology Department Animal House under standard conditions of boarding and were allowed free access to food and water.

For the purpose of the experiment, rats were divided into four groups and total number of rats was 8 per experimental group.

I- Control (unstressed) group: consisted of unstressed rats that were left undisturbed in their home cages.

II- Testosterone-treated control (unstressed) group: consisted of rats that were daily administered testosterone enanthate (Chemical Industries Development, A.R.E.) at a dose 5 mg/kg b.w. intraperitoneally (i.p.) [(13)], 6 days/week for 3 weeks and were left undisturbed in their home cages.

III- Immobilization-stressed group: consisted of rats that were subjected to immobilization stress by restraining separately in tight animal restraining cages (Curtin Matheson Scientific, regular size) in the prone position at room temperature, 4 h/day, 6 days /week for 3 weeks [(14)].

IV- Testosterone-treated immobilization-stressed group: consisted of rats that were daily administered testosterone enanthate at a dose 5 mg/kg b.w. intraperitoneally (i.p.), 6 days/week for 3 weeks, before exposure to immobilization stress by 30 min.

Experimental Procedures:

On the day of experiments, over night fasted rats were weighed and anesthetized i.p. by thiopental sodium 40 mg /kg (EIPICO). The length of the anaesthetized rat was measured from tip of the nose to the anus to calculate body mass index (BMI) according to the following equation: BMI = Body weight (kg) / length (m^2).

A midline abdominal incision was made, and the abdominal aorta was exposed and cannulated. Blood samples were collected in heparinized tubes. Immediately after blood collection, both kidneys were exposed and excised from the renal pedicle and placed in ice cold Krebs Ringer solution for 10 minutes after which cortical kidney slices from both the right and left kidneys were prepared for *in vitro* estimation of glucose output by both kidneys. Then, the diaphragm was exposed, quickly and carefully excised then immediately placed in ice cold Krebs' solution for *in vitro* estimation of glucose uptake by diaphragm. Finally, the pancreas was dissected out and subjected for histological assessment.

Glucose Uptake and Output Assay

In vitro estimation of glucose uptake by the diaphragm was performed according to the method described by **Mohamed *et al.*** [(15)].

In vitro estimation of glucose output by both kidneys was carried out according to the method of **Randall** [(16)] with modifications of **El-Nasr *et al.*** [(17)].

Biochemical Estimations

Blood samples were centrifuged at 3000 rpm for 15 min. to separate plasma. Fresh plasma was used for determination of glucose levels whereas remaining plasma was stored in aliquots and frozen at -20ºC for subsequent determination of testosterone, insulin and malondialdehyde levels.

Plasma testosterone was measured quantitatively by electrochemiluminescence immunoassay (ECLIA) using kits supplied by Roche Diagnostics, USA. The measurement was performed in Oncology Diagnostic Unit, Biochemistry Department, Faculty of Medicine, Ain Shams University.

Plasma glucose was determined enzymatically by a quantitative colorimetric method described by **Trinder** [(18)], using kits supplied by Stanbio-laboratory, U.S.A.

Plasma insulin was measured quantitatively by immunoenzymatic assay using INS-EASIA kit supplied by BioSource Europe S.A., Belgium. The measurement was performed in the Hormone Assay Laboratory, Endocrinology Department, Ain Shams University Hospital.

Plasma malondialdehyde (MDA), an oxidative stress marker, was assayed according to the method of **Esterbauer and Cheeseman** [(19)], as thiobarbituric acid reactive substance.

Homeostasis Model Assessment (HOMA) of Insulin Resistance and Insulin Secretion

Insulin resistance as a measure of insulin action was calculated by using homeostasis model assessment of insulin resistance (HOMA-IR) score that employs the formula: fasting insulin concentration (mIU/l) × glucose (mmol/l)/22.5 [(20)].

HOMA-β (HOMA-beta) as an index of pancreatic beta-cell function, was calculated as [fasting plasma insulin (μIU/ml) × 20] / [fasting plasma glucose (mmol/l) – 3.5] [(20)].

Glucose to insulin ratio (G/I ratio) was calculated as an index for determination of insulin resistance (IR was defined as G/I < 6) [(21)].

Histological Study of Pancreas

Light microscopic study (LM): Specimens from the pancreas were fixed in 10% formalin and dehydrated in ascending grades of alcohol and processed to form paraffin blocks. Serial sections of 5µm thickness were prepared and subjected to Haematoxylin and Eosin stain (H&E) and immunohistochemical staining for caspase-3 for detection of apoptotic pancreatic cells [(22)] using avidin-biotin peroxidase technique using rabbit polyclonal antibody. The reaction appeared as brownish cytoplasmic granules with some nuclear staining.

Morphometric study

1. Histological scoring of pancreas injury was done using H&E-stained sections. The grading was done on a scale of 0 to 4 for edema, hemorrhage, leukocyte infiltration and degeneration. This was done by counting the number of affected foci as follows: 0= absent, 1= mild, 2= moderate, 3= severe and 4= overwhelming [(14)].
2. Number of caspase-3-positive immunoreactive cells in islets of Langerhans and pancreatic acinar cells .

3. Results

Anthropometric measurements

As observed in table (1), the final body weights were significantly increased in control, testosterone-treated unstressed, immobilization-stressed and testosterone-treated immobilization-stressed groups as compared to their initial values ($P<0.005$, $P<0.01$, $P<0.05$ & $P<0.05$ respectively). Final body weights, percentage change in body weight as well as BMI showed insignificant differences among all studied groups.

Plasma testosterone levels

Immobilization-stressed group demonstrated significant decrease in plasma testosterone concentrations *vs.* control group ($P<0.05$). Testosterone levels showed significant increase in testosterone-treated immobilization-stressed group *vs.* control ($P<0.001$), testosterone-treated unstressed ($P<0.05$) and immobilization-stressed ($P<0.001$) groups as well as in testosterone-treated unstressed group *vs.* control group ($P<0.01$) (Table 1 and Fig. 1).

Glycemic studies

As shown in table (2) and fig. (2), immobilization stress significantly increased plasma glucose levels ($P<0.001$) and glucose output by kidneys ($P<0.001$) and decreased plasma insulin levels ($P<0.005$) and glucose uptake by diaphragm ($P<0.001$) *vs.* control group. Testosterone-treated immobilization-stressed group showed significant decrease in plasma glucose levels ($P<0.001$) and glucose output by kidneys ($P<0.001$) and increase in plasma insulin levels ($P<0.01$) and glucose uptake ($P<0.001$) *vs.* immobilization-stressed group but significant increase in glucose output ($P<0.01$) *vs.* testosterone-treated unstressed group.

No significant difference was found in HOMA-IR among the different groups. HOMA-β was significantly decreased in immobilization-stressed group *vs.* control group ($P<0.05$), whereas significantly increased in testosterone-treated immobilization-stressed group *vs.* immobilization-stressed group ($P<0.001$). G/I ratio was significantly increased in stressed group ($P<0.001$) and was significantly decreased by testosterone treatment ($P<0.001$). On the other hand, testosterone-treated unstressed group demonstrated insignificant changes in the different glycemic parameters *vs.* control group.

Plasma MDA levels, oxidative stress marker

As shown in table (1) and fig. (1), immobilization stress increased the plasma MDA levels significantly *vs.* control group ($P<0.001$). In testosterone-treated immobilization-stressed group, the plasma MDA levels significantly decreased *vs.* immobilization-stressed group ($P<0.001$). In testosterone-treated unstressed group, there was no significant difference *vs.* control group.

Correlation Studies

Plasma testosterone levels were negatively correlated with plasma glucose levels, glucose output by kidneys and plasma MDA levels and positively correlated with plasma insulin levels, glucose uptake by diaphragm and HOMA-β (Fig. 3). Plasma MDA levels were negatively correlated with plasma insulin levels ($r=-0.385$, $P<0.05$, $n=32$) and HOMA-β ($r=-0.424$, $P<0.05$, $n=32$).

Histological Study

I. H&E-stained sections

The results revealed by H&E-stained sections of the studied groups are shown in figure (4).

Histological scoring of pancreas injury is displayed in table (3).

II. Caspase-3 immunohistochemical study

As shown in table (4) and figure (5), immunohistochemical staining of the pancreas of control sections demonstrated absence of caspase-3 immunoreactivity in cells of the islets of Langerhans or in the pancreatic acinar cells. Testosterone-treated unstressed rats showed that caspase-3 immunoreactivity of cells of islets of Langerhans and pancreatic acinar cells were comparable to the control

sections. Immobilization-stressed group showed significantly large number of caspase-3 immunoreactive cells of the islets of Langerhans and pancreatic acinar cells *vs.* control group (P<0.001 for both). Testosterone treatment of immobilization-stressed group resulted in significant decrease in the number of caspase-3 immunoreactive cells, in both islets of Langerhans and pancreatic acinar cells (P<0.001 for both).

Correlation Studies: Correlations between plasma levels of testosterone and malondialdehyde and pancreatic histological findings are demonstrated in table (5).

Table (1): Anthropometric measurements and plasma levels of testosterone and malondialdehyde (MDA) in the different studied groups.

	Control	Testosterone-treated unstressed	Immobilization-stressed	Testosterone-treated immobilization-stressed
Initial BW (g)	200.8 ± 6.37	196.3 ± 4.41	199.3 ± 7.26	201.9 ± 7.67
Final BW (g)	221.9 ± 0.4*	215.0 ± 8.56*	214.6 ± 10.7*	211.0 ± 8.09*
% change BW (%)	0.1 ± 0.02	0.09 ± 0.03	0.08 ± 0.03	0.04 ± 0.01
Body mass index (Kg/m²)	4.60 ± 0.13	4.58 ± 0.12	4.55 ± 0.11	4.73 ± 0.1
Testosterone (ng/ml)	2.37 ± 0.41	5.07 ± 0.76 [a]	0.41 ± 0.04 [a]	7.04 ± 1.04 [a b c]
MDA (µmol/L)	1.636 ± 0.05	1.661 ± 0.22	2.922 ± 0.24 [a]	1.764 ± 0.21 [b]

Values are expressed as means ± SEM for eight rats in each group. BW: body weight.
% change is calculated for final body weight from initial body weight.
*: Significance from respective initial BW, calculated by "Student's *t*-test" for paired data at *P*<0.05.
a: Significance calculated by LSD at *P*<0.05 from control group.
b: Significance calculated by LSD at *P*<0.05 from immobilization-stressed group.
c: Significance calculated by LSD at *P*<0.05 from testosterone-treated unstressed group.

Table (2): Glycemic parameters in the different studied groups.

	Control	Testosterone-treated unstressed	Immobilization-stressed	Testosterone-treated immobilization-stressed
Plasma glucose (mg/dl)	95.1 ± 2.82	84.5 ±3.77	158.3 ± 8.47 [a]	95.7 ± 1.98 [b]
Glucose uptake by diaphragm (mg/g/90min)	6.36 ± 0.88	5.01 ± 0.36	3.06 ± 0.37 [a]	5.94 ± 0.39 [b]
Glucose output by kidneys (mg/g/h)	0.729 ± 0.04	0.563 ± 0.03	1.302 ± 0.09 [a]	0.810 ± 0.06 [b c]
Plasma insulin (µIU/ml)	5.813±1.04	3.750±0.45	2.375±0.26 [a]	7.312±1.97 [b]
HOMA-IR	1.380±0.22	0.956±0.10	1.003±0.15	1.730±0.46
HOMA-β	72.1±16.2	102.2±24.4	10.0±1.52 [a]	82.5±24.3 [b]
G/I ratio	17.99±1.75	19.99±2.55	68.59±7.83 [a]	21.66±5.30 [b]

Values are expressed as means ± SEM for eight rats in each group.
a: Significance calculated by LSD at *P*<0.05 from control group.
b: Significance calculated by LSD at *P*<0.05 from immobilization-stressed group.
c: Significance calculated by LSD at *P*<0.05 from testosterone-treated unstressed group.

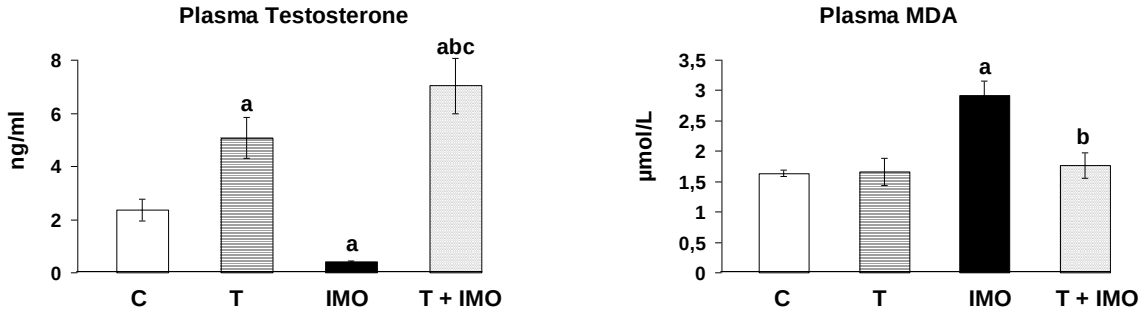

Figure (1): Plasma testosterone and malondialdehyde (MDA) levels of control (C), testosterone-treated unstressed (T), immobilization-stressed (IMO) and testosterone-treated immobilization-stressed (T+ IMO) groups.
Data are expressed as a means ± SEM. a: Significance calculated by LSD at $P<0.05$ from control group. b: Significance calculated by LSD at $P<0.05$ from immobilization-stressed group. c: Significance calculated by LSD at $P<0.05$ from testosterone-treated unstressed group.

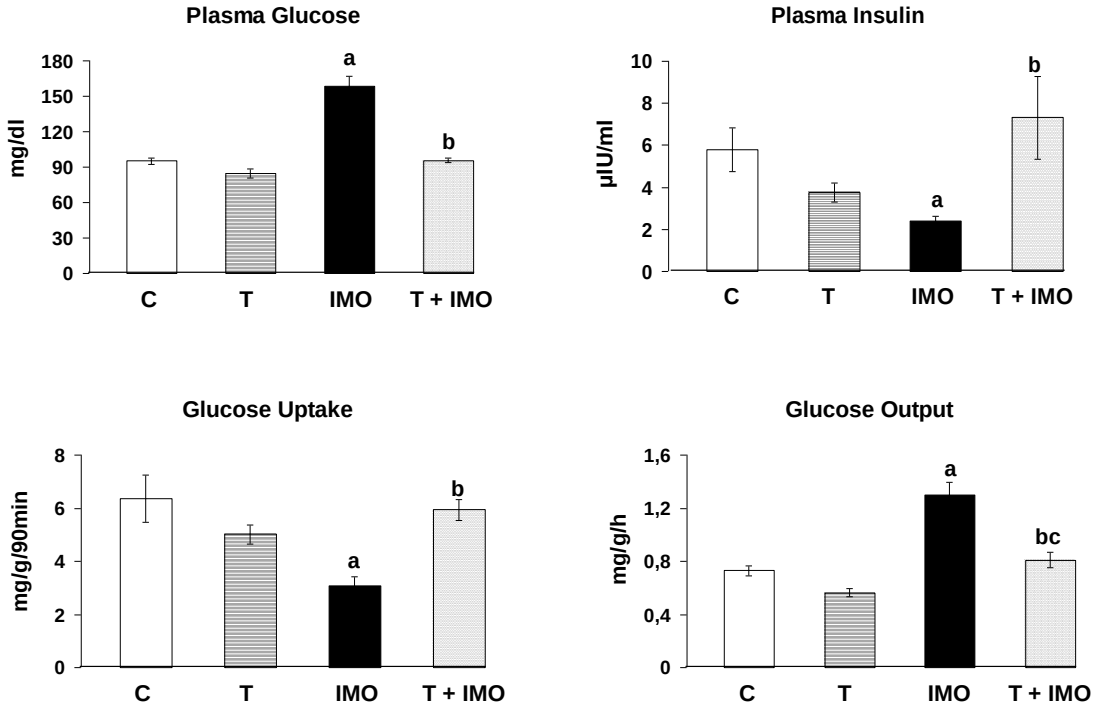

Figure (2): Plasma glucose and insulin levels, glucose uptake by diaphragm and glucose output by kidneys of control (C), testosterone-treated unstressed (T), immobilization-stressed (IMO) and testosterone-treated immobilization-stressed (T+ IMO) groups.
Data are expressed as a means ± SEM. a: Significance calculated by LSD at $P<0.05$ from control group. b: Significance calculated by LSD at $P<0.05$ from immobilization-stressed group. c: Significance calculated by LSD at $P<0.05$ from testosterone-treated unstressed group.

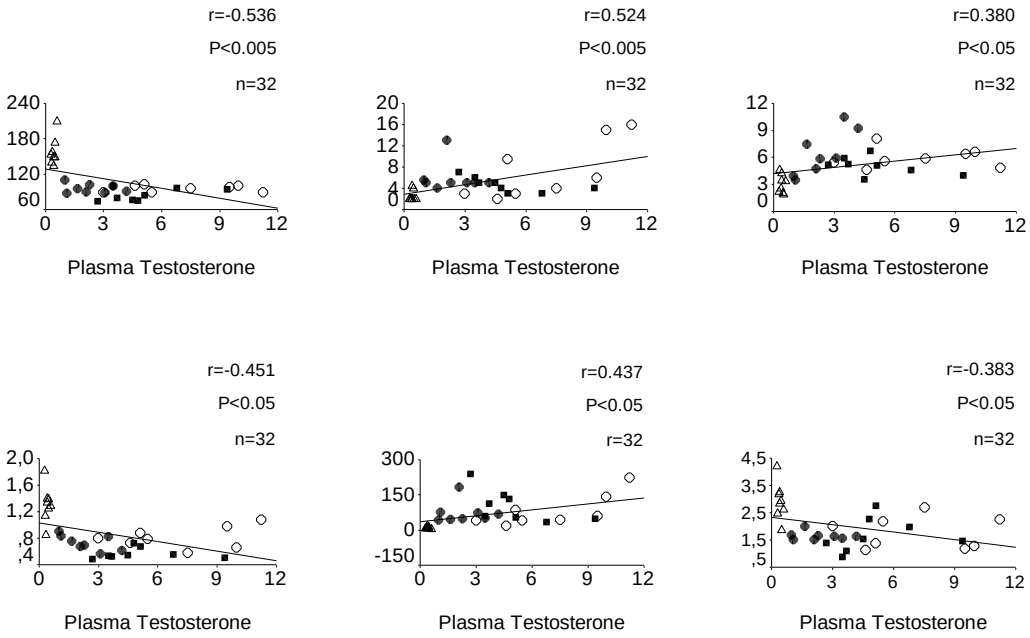

Figure (3): Correlations between plasma levels of testosterone (ng/ml) and plasma levels of glucose (mg/dl) and insulin (μIU/ml), glucose uptake by diaphragm (mg/g/90min), glucose output by kidneys (mg/g/h), HOMA-β and plasma malondialdehyde levels (MDA, μmol/L) in control (●), testosterone-treated unstressed (○), immobilization-stressed (Δ) and testosterone-treated immobilization-stressed (■) groups.

Table (3): Histological scoring of pancreas injury.

	Control	Testosterone-treated unstressed	Immobilization-stressed	Testosterone-treated immobilization-stressed
Edema	0.00 ±0.00	0.00 ±0.00	1.38 ± 0.18 [a]	0.13 ± 0.13 [b]
Hemorrhage	0.00 ±0.00	0.00 ±0.00	0.13 ± 0.13	0.00 ±0.00
Mononuclear cell infiltration	0.00 ±0.00	0.00 ±0.00	1.25 ± 0.16 [a]	0.38 ± 0.18 [a b c]
Degeneration	0.00 ±0.00	0.00 ±0.00	1.50 ± 0.19 [a]	0.38 ± 0.18 [b]

Values are expressed as means ± SEM for eight rats in each group.
a: Significance calculated by LSD at $P<0.05$ from control group.
b: Significance calculated by LSD at $P<0.05$ from immobilization-stressed group.
c: Significance calculated by LSD at $P<0.05$ from testosterone-treated unstressed group.

Table (4): Caspase-3 immunoreactivity in cells of the islets of Langerhans and pancreatic acinar cells.

	Control	Testosterone-treated unstressed	Immobilization-stressed	Testosterone-treated immobilization-stressed
Caspase-3 in islets of Langerhans	0.00 ±0.00	0.00 ±0.00	54.4 ± 1.51 [a]	1.88 ± 0.48 [b]
Caspase-3 in pancreatic acini	0.00 ±0.00	0.00 ±0.00	68.0 ± 0.82 [a]	2.50 ±0.33 [b c]

Values are expressed as means ± SEM for eight rats in each group.
a: Significance calculated by LSD at $P<0.05$ from control group.
b: Significance calculated by LSD at $P<0.05$ from immobilization-stressed group.
c: Significance calculated by LSD at $P<0.05$ from testosterone-treated unstressed group.

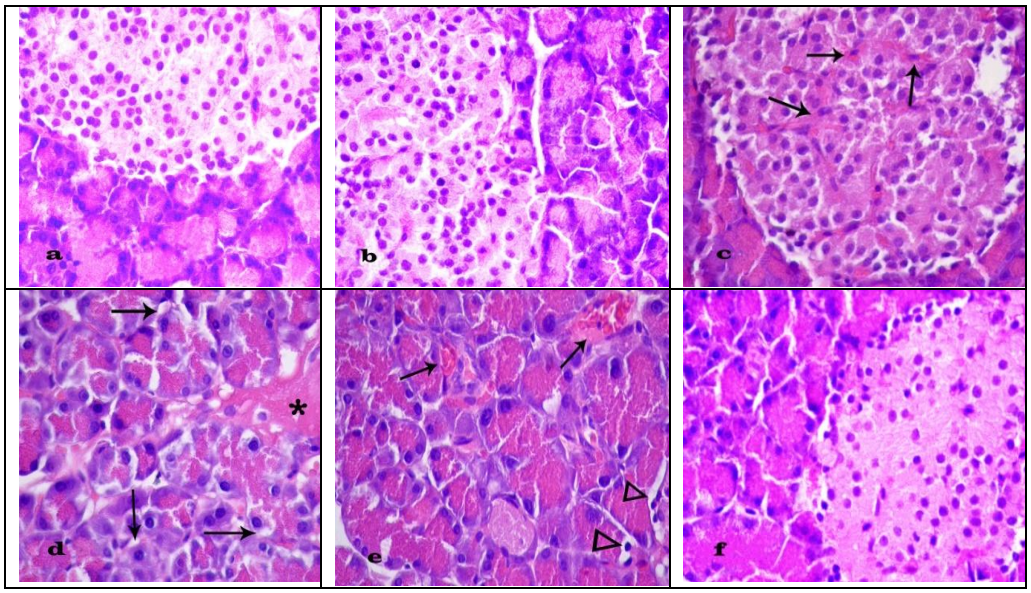

Figure (4): H&E-stained sections of pancreas: a) Control group showing normal structure of the islets of Langerhans and pancreatic acini. b) Testosterone-treated unstressed group showing the structure comparable to the control. c-e) Immobilization-stressed group showing: c) degenerated central islet cells (↑), d) vacuolar degeneration in pancreatic acini (↑) with edema (*) and e) congested blood vessels in between the acini (↑) and inflammatory cell infiltration (Δ). f) Testosterone-treated immobilization-stressed group showing the structure of the pancreatic islets and acini comparable to the control group. (× 640)

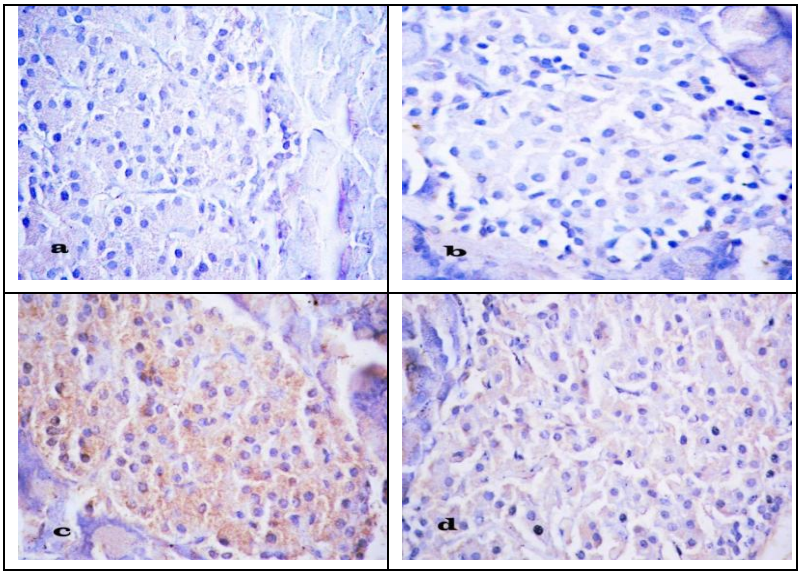

Figure (5): Caspase-3 immunoreactivity in islets of Langerhans and pancreatic acinar cells in control group (a), testosterone-treated unstressed group (b), immobilization-stressed group (c) and testosterone-treated immobilization-stressed group (d). (× 640)

Table (5): Correlation coefficients (r) between plasma levels of testosterone and malondialdehyde (MDA) and pancreatic histological findings

Parameters	Testosterone		MDA	
	r	P	r	P
Pancreatic edema	-0.45	<0.01	0.80	<0.001
Pancreatic hemorrhage	-0.34	NS	0.42	<0.05
Pancreatic mononuclear cell infiltration	-0.20	NS	0.534	<0.005
Pancreatic degeneration & necrosis	-0.51	<0.005	0.59	<0.001
Caspase-3 in islets of Langerhans	-0.60	<0.001	0.72	<0.001
Caspase-3 in pancreatic acinar cells	-0.59	<0.001	0.72	<0.001

The number of rats studied for each parameter is 32. NS: not significant

4. Discussion

The present study examined the contributory role of testosterone hormone in glucose responses in chronic immobilization-stressed male rats. Chronic immobilization-stress elicited perturbation in glycemic homeostasis as shown by hyperglycemia, hypoinsulinemia, decreased glucose uptake by diaphragm and increased glucose output by kidneys. Also, there was impaired pancreatic beta cell function, marked by the low HOMA-β, together with decreased plasma testosterone levels and potentiation of oxidative stress, evidenced by elevation in plasma MDA levels. Meanwhile, the absence of significant changes in HOMA-IR as well as the increased G/I ratio in stressed rats denies the development of insulin resistance in this studied model of chronic immobilization. A large body of human and animal studies supports the notion that stress is implicated in the development of hyperglycemia [23-25]. Testosterone administration to immobilization-stressed rats concurrent with a lowering of plasma glucose levels, glucose output, G/I ratio and plasma MDA levels and an elevation in plasma insulin levels and glucose uptake by diaphragm together with attenuation of pancreatic β-cell dysfunction as shown by the high HOMA-β, suggest that testosterone effectively improves blood glucose control.

Rats in the current work demonstrated decline in plasma testosterone upon exposure to chronic immobilization stress which is consistent with previous studies [26-28]. In males, decreased serum testosterone is one of the first signs of stress [7]. It has been proposed that the high corticosteroid levels, as part of the stress response, may suppress gonadotropins [29] and directly suppress testicular functions [27].

The present data revealed that immobilization stress impaired glucose control. Meanwhile, plasma testosterone showed significant negative correlations with plasma glucose and glucose output but positive correlations with plasma insulin and glucose uptake. These findings are in agreement with previous study by **Thomas *et al.*** [30] who showed a significant inverse correlation between testosterone and fasting blood glucose. Also, **Muthusamy *et al.*** [31] found that castration elevated the blood glucose level and had an inhibitory effect on serum insulin and glucose uptake while these changes were corrected after administration of physiologic doses of testosterone. Thus, it could be suggested that testosterone deficiency is involved in the deranged glucose metabolism observed with immobilization stress and that the high plasma glucose demonstrated appears to be the result of impaired insulin secretion, depressed glucose uptake by skeletal muscle and enhanced gluconeogenesis associated with testosterone deficiency.

The disturbed glucose homeostasis accompanying immobilization stress could also, be ascribed to pancreatic cell dysfunction, especially the β-cell, owing to testosterone lack- as well as oxidative stress- promoted pancreatic cells injury, particularly the β-cells. This view is favoured by histological examination of pancreatic tissue which revealed degeneration of cells of the islets of Langerhans, especially insulin-secreting β-cells, interstitial edema, vascular congestion and mononuclear cellular infiltration as well as by immunohistochemical examination showing pancreatic cells apoptosis as indicated by increased number of caspase-3 positive immunoreactive cells. Plasma testosterone showed significant inverse correlation with pancreatic cells degenerative changes as well as with the apoptotic marker, caspase-3 in both islets of Langerhans and acinar cells, which points to the possible involvement of testosterone absence in pancreatic cells damage.

Furthermore, plasma MDA demonstrated significant positive relationships with the pancreatic degenerative and inflammatory changes as well as with caspase-3 suggesting that these changes could be mediated by oxidative stress. Increased levels of oxygen free radicals may be capable of impairing normal pancreatic structure and function [32]. The pancreatic β-cells are vulnerable to oxidative stress and damage possibly due to its low level of antioxidant enzymes expression [33]. In response to

oxidative injury, pancreatic cells were found to produce and release TNF-α, a proinflammatory cytokine that has been shown to stimulate inflammatory and degenerative changes and apoptosis in pancreatic cells [34-36]. Also, reactive oxygen species (ROS) directly induce apoptosis by damaging DNA [37]. Additionally, the oxidative stress and generation of ROS lead to excessive, unresolved endoplasmic reticulum stress (ER stress), triggering cell death by apoptosis [38, 39]. Cells of the islets of Langerhans, especially β-cells, and pancreatic acinar cells were found to be particularly susceptible to ER stress owing to their highly secretory functions [40, 41]. The presence of cytoplasmic vacuolations seen in islets of Langerhans and pancreatic acinar cells might reflect the existence of ER stress.

Administration of testosterone to immobilization-stressed rats improved the glycemic parameters, prevented pancreatic tissue damage and apoptotic cell death and restored pancreatic β-cells function, as shown by high HOMA-β, suggesting that this hormone exerts a natural protective effect in rat pancreas that was further supported by the significant inverse correlations between plasma testosterone and the apoptotic marker, caspase-3, as well as by the positive correlation between plasma testosterone and HOMA-β. Thus, the present glucose lowering effect of testosterone could be explained by testosterone role in preservation of β-cell integrity and function. Previous reports have indicated that the pancreatic islets can respond to androgens [42]. It is well recognized that steroids act through nuclear receptors that are ligand-regulated transcription factors and participate in many cellular process such as proliferation, differentiation and cell death [43]. The current findings are in accordance with **Rossini *et al***. [44] who reported that the administration of exogenous androgens is likely to prevent the effects of castration on glucose homeostasis, since in multiple-dose streptozotocin diabetes model testosterone administered to control females or orchidectomized males resulted in a glucose response similar to that observed in control males.

The enhancement of glucose uptake by diaphragm coupled with abating of gluconeogenesis observed in stressed rats upon receiving testosterone together with testosterone-induced positive correlation with glucose uptake by diaphragm and negative correlation with glucose output denote that testosterone possesses an insulin-like action that could be an underlying mechanism that accounts for the improved glucose metabolism.

It has been reported that the activities of phosphofructokinase and hexokinase, main glycolytic enzymes, were enhanced by testosterone [45] which could explain the decreased plasma glucose by testosterone in stressed rats.

The decreased glucose uptake by skeletal muscle concomitant with testosterone deficiency in immobilization stressed rats and its restoration to normal level by testosterone treatment could be ascribed to testosterone depletion-promoted loss of cell surface insulin receptors [46] as well as postreceptor defect in glucose transport system, including protein kinase B (Akt) and its phosphoylation, and glucose transporter-4 (GLUT4) expression and its translocation [31]. Previously, castration-mediated impairment in glucose oxidation was found to be coupled with decreased insulin receptor expression in adipose tissue, liver and skeletal muscle [46]. Testosterone deprivation was, also, reported to mediate decrease in the amount of GLUT4 protein in plasma membrane of skeletal muscle and the restoration to normal in rats supplemented with testosterone imply the importance of physiologic level of testosterone to maintain optimum level of GLUT4 population in plasma membrane [31]. In addition, testosterone was, previously, found to increase GLUT-4 protein expression and accelerate GLUT4 translocation to plasma membrane in skeletal muscle cells via enhancement of both Akt and protein kinase C ζ/γ phosphorylations, thus increasing glucose uptake and utilization in skeletal muscle [45], which could provide explanation for the observed decreased plasma glucose level associating testosterone administration in stressed rats.

The encountered finding that testosterone is a plasma insulin enhancer is an additional factor explaining glucose reduction by testosterone. Such enhancing effect is comparable to those reported in the literature showing an enhancing effect of testosterone on insulin secretion [47, 48]. However, in contrast some evidences indicated that testosterone has not significantly affected insulin secretion [49, 50]. Increased plasma insulin could be explained by the direct effect of testosterone on the pancreas [51] assumed from the current positive correlation between plasma levels of testosterone and insulin and, also, by testosterone-induced protection of pancreatic beta cell function observed herein through its direct and antioxidant capabilities. Other proposed mechanisms involve testosterone ability to decrease metabolic clearance rate of insulin [52], impair hepatic capacity to extract insulin [53] and enhance insulin gene expression and release [47].

The hypoglycemic effect of testosterone could, also, be attributed to its antioxidant ability. The antioxidant properties of sex steroid hormones have been shown in different cells and tissues [54, 55]. In tissues as the prostate, testosterone participates in

defensive mechanisms of oxidative stress in rats, increasing the levels of antioxidant enzymes [56, 57]. The reduction of plasma MDA levels in testosterone-treated immobilized rats together with the inverse correlations between plasma MDA levels and both HOMA-β and plasma insulin and the positive relationships between MDA and histological pancreatic damage and caspase-3 support the hypothesis that testosterone protects against oxidative stress-induced pancreatic injury and dysfunction. These data reflect that the abrogation of oxidative stress in stressed rats by testosterone supplementation, attenuated β-cell apoptosis and degenerative changes and resulted in amelioration of β-cell function, thus leading to the recovery of insulin biosynthesis and increased insulin secretion capacity and levels that normalized plasma glucose level through increasing glucose uptake by diaphragm and suppressing glucose output by kidneys.

In conclusion, testosterone treatment to rats exposed to immobilization stress points to its possible role in mediating protection against pancreatic damage and β-cell dysfunction. Such treatment potentiated insulin secretion and nullified oxidative insult, thus, accounting for the beneficial glycemic state imposed by testosterone administration in immobilization stress. Therefore, testosterone could be considered as a supplement to prevent the development of a diabetes mellitus-like metabolic syndrome and could potentially function as a novel hypoglycemic agent for males during stressful situations.

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