Thieme

Testosterone Plasma Concentration is Associated with Insulin Resistance in Male Hypertensive Patients

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ABSTRACT

Background: Low testosterone levels are a common finding among men with Type 2 Diabetes Mellitus (T2DM) and are inversely related to insulin resistance. Whether this relationship holds true in patients with hypertension, but normal glucose tolerance or prediabetes, is unclear.
Methods: We recruited 87 male outpatients with essential arterial hypertension, aged 35–70 years. Anthropometric data were collected, an Oral Glucose Tolerance Test (OGTT) performed, and the homeostasis model assessment of insulin resistance (HOMA-IR) score calculated. Follicle-Stimulating Hormone, Luteinizing Hormone, testosterone, Sex Hormone-Binding-Globulin and free-testosterone were measured. The concentrations of sex hormones were compared between normoglucotolerant, prediabetic and diabetic patients. Non-parametric tests were applied as appropriate to verify differences among groups, while multiple linear regression was used to predict the variability of testosterone and free-testosterone.
Results: Total serum testosterone concentration was significantly lower in T2DM in comparison to normoglucotolerant subjects ($p < 0.01$) and was inversely related to body mass index ($r = -0.25$, $p < 0.01$), waist circumference ($r = -0.27$, $p < 0.01$), pre and post-OGTT plasma glucose ($r = -0.4$, $p < 0.0001$ and $r = -0.29$, $p < 0.01$, respectively), pre and post-OGTT plasma insulin ($r = -0.42$, $p < 0.0001$ and $r = -0.42$, $p < 0.0001$) and HOMA-IR ($r = -0.46$, $p < 0.0001$). Similar associations were observed for free testosterone; HOMA-IR was related to testosterone and free-testosterone even in patients with normal glucose tolerance ($r = -0.47$, $p < 0.01$ and $r = -0.34$, $p < 0.05$, respectively). At multivariate analysis HOMA-IR was the only variable associated to testosterone ($p < 0.001$) and free-testosterone ($p < 0.05$) plasma concentration.
Conclusions: In males with hypertension, the link between insulin sensitivity and hypothalamic-pituitary-gonadal axis is maintained along the entire spectrum of glucose tolerance.

Introduction

A low testosterone level is a common finding among men with Type 2 Diabetes Mellitus (T2DM). Several cross-sectional studies have shown that its prevalence ranges from 25 to 40 % [1–3], reaching 50 % if obesity is associated [4]. The very high prevalence of this association lead the Endocrine Society to recommend the measurement of morning serum total testosterone in patients affected by T2DM who have symptoms of sexual dysfunction, unexplained weight loss, weakness or mobility limitation [5]. It is not known whether low testosterone levels play a causative role or whether they are a consequence of T2DM and/or of its associated clinical features [6]. However, some evidences suggest that testosterone is an important modulator of insulin resistance [7]. In fact, as already reported earlier [8, 9], the Third National Health and Nutrition Examination Survey (NHANES III) disclosed that, in the general population, plasma testosterone concentration is inversely related to HOMA-IR and plasma insulin concentration, 2 proxy measures of insulin resistance [10]. This association has also been confirmed in men with established T2DM [11], and with prediabetic conditions [12, 13], such as impaired fasting glucose (IFG) and impaired glucose tolerance (IGT), all characterized by increased insulin resistance [14, 15].

Furthermore, the role of testosterone in glucose metabolism is supported by evidences from prospective studies, which have convincingly shown that hypogonadism is a risk factor for future development of insulin resistance and T2DM. [16–18]; according to a meta-analysis, men with higher testosterone levels had a 42 % lower risk of T2DM (RR, 0.58; 95 % CI, 0.39–0.87) [19]. Finally, men affected by T2DM and hypogonadotropic hypogonadism, who underwent testosterone replacement treatment, also show an increase in insulin sensitivity [20]. Taken together, these findings suggest that hypogonadism directly affects insulin sensitivity, rather than being merely an associated condition [4, 7].

In the past few years, low testosterone has also been related to other elements of the Metabolic Syndrome, such as arterial hypertension [21]. In this study, we aimed to investigate whether an independent association between the sex hormone profile and insulin resistance exists in men affected by arterial hypertension, across all the different classes of glucose metabolism.

Methods

We collected data from male patients aged 35–70 years referred to a hypertension clinic for essential arterial hypertension. The following exclusion criteria were applied: a) a defined diagnosis of T2DM; b) concomitant endocrine, liver and/or renal diseases; c) treatment with ß-blockers and/or α_1-antagonists and/or thiazides within 6 months before recruitment; d) previous urologic surgical interventions. On this basis, data from 87 patients were collected. All patients gave informed consent to their participation to the study, which was conducted in strict accordance to the Principles of the Declaration of Helsinki. Being a study of clinical practice, no ethical committee approval was requested.

Anthropometric data were collected with patients wearing only light underwear, and included the body mass index (BMI), i. e., body weight divided by the square of the height (kg/m^2) and the waist circumference (WC), measured midway between the lowest rib and the iliac crest when standing. Data on cigarette-smoking were also obtained.

In agreement with the American Diabetes Association 2003 criteria [22], each patient underwent a standard Oral Glucose Tolerance Test (OGTT). This entails the ingestion of 75 g of glucose and measurement of 2 glucose concentrations: Fasting Plasma Glucose (FPG) and 2-h plasma glucose (2hPG) after the glucose challenge, measured along with the corresponding fasting and 2-h plasma insulin concentrations (FPI and 2hPI). Glucose plasma concentration was measured by hexokinase method (ADVIA, Siemens; detection limit 4 mg/dl), while insulin was measured by chemiluminescence (Centaur, Siemens; detection limit 0.5 µUI/ml)

According to the ADA 2003 criteria and the results of the OGTT, a patient was classified as having normal glucose tolerance (NGT) when FPG < 100 mg/dl and 2hPG < 140 mg/dl, T2DM when FPG > 125 and/or 2hPG ≥ 200 mg/dl, IFG when FPG ≥ 100 < 126 mg/dl and 2hPG < 140 mg/dl, IGT when FPG < 100 mg/dl and 2hPG ≥ 140 < 200 mg/dl, and a combination of IFG and IGT (IFG/IGT) when FPG ≥ 100 < 125 mg/dl and 2hPG ≥ 140 < 200 mg/dl. For the purposes of the study, hypertensive subjects with IFG, IGT, IFG/IGT were lumped into a single group called prediabetes (preDM).

For each patient we also calculated the HOMA-IR as follows: (FPI µU/ml × FPG mmol/L)/22.5 [23]. Contextually to OGTT, patients underwent a further morning plasma sampling; follicle-stimulating hormone (FSH), luteinizing hormone (LH), testosterone, sex hormone-binding-globulin (SHBG) were measured by chemiluminiscent immunoassays, and free-testosterone by a radioimmunoassay (Advia Centaur XP, Siemens Healthcare Diagnostics, Ireland/USA).

Based on the testosterone threshold for hypogonadism [24], patients with a plasma concentration below 350 ng/dl were compared to those whose concentration was above this limit. Comparisons between percentages were performed by χ^2 test. Continuous variables were shown as medians with upper and lower quartile (InterQuartile Range, IQR) and compared as appropriate by the Mann-Whitney and Kruskal-Wallis tests, followed by post-hoc analysis. The correlation between continuous variables was analyzed calculating the Spearman Rho correlation coefficient, while multiple linear regression analysis was used to predict the variability of testosterone and free-testosterone. Statistical significance was set at a p-value < 0.05 for all statistical tests. The statistical software package StatSoft 5.1 STATISTICA Inc. (2300 East 14th Street, TULSA, OK 74104, USA) was used for statistical analysis.

Results

We recruited 87 male subjects with a median age of 59 (50–65) years; median BMI was 28.6 (26.0–31.9) kg/m^2 and BMI was ≥ 30 kg/m^2 in 35.6 % of patients (n = 31). Median WC was 99 (93–105) cm, while 37.95 % (n = 33) of subjects had a WC ≥ 102 cm. 57.5 % (n = 50) were active smokers. According to OGTT results, 40 subjects (45.9 %) were classified as NGT, while preDM was diagnosed in 35 (40.3 %; 25 IFG, 3 IGT and 7 IFG/IGT) and T2DM in 12 (13.8 %) patients.

▶ **Table 1** shows the medians (interquartile range, IQR) of FSH, LH, testosterone, free-testosterone and SHBG in NGT, preDM and T2DM groups. Testosterone and SHBG decreased significantly from NGT to T2DM group (p < 0.01 and p < 0.01, respectively). In post-hoc analysis, testosterone was significantly lower in the group of patients with T2DM when compared to NGT group (p < 0.01), while SHBG was significantly lower in preDM than in the NGT group

▶ **Table 1** Hormonal profile according to glucose tolerance stage.

	NGT (n = 40)	PreDM (n = 35)	T2DM (n = 12)	Kruskal-Wallis
FSH (µU/ml)	5.1 (4.4–8.7)	5.1 (3.9–7.9)	7.0 (5.9–10.3)	n.s.
LH (µU/ml)	4.5 (3.5–6.8)	4.3 (2.9–6.5)	4.6 (3.3–9.9	n.s.
TST (ng/dl)	431.5 (330.4–514.2)†	387.0 (344.0–452.0	311.9 (207.8–410.0)	p < 0.01
FTST (pg/ml)	10.7 (8.7–13.7)	11.6 (8.7–12.9)#	8.5 (6.2–11.1)	n.s.
SHBG (nmol/L)	46.5 (40.0–51.6) °	38.9 (33.1–45.7)	30.4 (25.0–61.9)	P < 0.01

▶ **Table 2** Antropometric data and glucose metabolism parameters according to Testosterone levels.

	TST < 350 ng/dl (N = 30) Median (IQR)	TST ≥ 350 ng/dl (N = 57) Median (IQR)	Mann-Whitney test
Age (years)	57.5 (51–65)	59 (50–65)	n.s.
BMI (Kg/m²)	29.4 (27.8–33.4)	28 (25.2–30.2)	$p<0.05$
WC (cm)	100 (94–108)	99 (91–103)	n.s.
FPG (mg/dl)	106 (94–124)	98 (93–107)	$p<0.05$
2hPG (mg/dl)	116 (98–161)	104 (94–139)	n.s.
FPI (μU/ml)	16.4 (12.3–23.2)	10.8 (7–15)	$p<0.0001$
2hPI (μU/ml)	89.1 (48–165.4)	48 (31.1–69.3)	$p<0.01$
HOMA-IR	4.38 (3.4–6.4)	2.65 (1.72–3.8)	$p<0.0001$

($p<0.04$). Furthermore, in T2DM patients free-testosterone was significantly lower in comparison to preDM ($p<0.05$).

In ▶ **Table 2** we compared anthropometric measures and glucose metabolism data between 30 subjects with hypogonadism (testosterone below 350 ng/dl) and 57 subjects with normal plasma testosterone concentration. The former group had median values of BMI ($p<0.05$), FPG ($p<0.05$), FPI ($p<0.0001$), 2hPI ($p<0.01$) and HOMA-IR ($p<0.0001$) significantly higher than the latter group. The percentage of subjects with altered OGTT, although higher in the group of subjects with hypogonadism, was not significantly different between the two groups (63.3% vs. 49.1, $\chi^2=1.6$, n.s.).

As detailed in ▶ **Table 3**, in the study population, total testosterone plasma concentration, free-testosterone and SHBG are inversely and extensively related to glucose metabolism.

The inverse correlations of both testosterone and free-testosterone with HOMA-IR ($r=-0.47$, $p<0.01$ and $r=-0.34$, $p<0.05$, respectively) was confirmed also in the subgroup (N. 40) of NGT subjects.

▶ **Table 4** shows the 2 multivariate analysis models we constructed considering, as regressors, age, BMI, WC and HOMA-IR and, as dependent variable, either testosterone or free-testosterone. In both the analyses, HOMA-IR was the only variable independently and inversely related to testosterone ($p<0.001$) and to free-testosterone ($p<0.05$).

▶ **Table 3** Spearman's correlations between sex hormones and glucose metabolism.

	Total testosterone	Free testosterone	SHPG
BMI (Kg/m²)	$r=-0.25$; $p<0.01$	$r=-0.23$; $p<0.05$	$r=-0.08$; n.s.
WC (cm)	$r=-0.27$; $p<0.01$	$r=-0.28$; $p<0.01$	$r=-0.15$; n.s.
FPG (mg/dl)	$r=-0.40$; $p<0.0001$	$r=-0.26$; $p<0.05$	$r=-0.35$; $p<0.001$
2hPG (mg/dl)	$r=-0.29$; $p<0.01$	$r=-0.21$; n.s.	$r=-0.29$; $p<0.01$
FPI (μU/ml)	$r=-0.42$; $p<0.0001$	$r=-0.28$; $p<0.01$	$r=-0.20$; n.s.
2hPI (μU/ml)	$r=-0.42$; $p<0.0001$	$r=-0.27$; $p<0.01$	$r=-0.15$; n.s.
HOMA-IR	$r=-0.46$; $p<0.0001$	$r=-0.30$; $p<0.01$	$r=-0.27$, $p<0.05$

▶ **Table 4** Multiple regression models: factors associated to testosterone and free-testosterone plasma concentration (after log-transformation).

	Testosterone	Free-testosterone
Age (years)	0.39, p = n.s.	−0.94, p = n.s.
BMI (Kg/m²)	−1.34, p = n.s.	−0.93, p = n.s.
HOMA-IR	−3.97, $p<0.0001$	−2.32, $p<0.05$

Discussion

Our data indicate that, among male patients with hypertension, the sex hormone profile is related to glucose metabolism; in fact the gonadal axis is significantly impaired in patients affected by T2DM. Furthermore, testosterone and free testosterone plasma concentrations are inversely related to insulin resistance, independently from other determinants of glucose metabolism, being this association confirmed in all OGTT classes. Our findings strengthen the hypothesis that testosterone does not act exclusively on sexually-related chemical and attitudinal events, but that it also plays a key role in carbohydrate metabolism [6].

Many observational studies have already documented a high prevalence of hypogonadism in men with T2DM [1–4] or prediabetes [13]. In our study, we decided to investigate selectively a subgroup at high risk for both hypogonadism [25] and insulin resistance [26], hypertensive men. We confirm a strong association between sex hormones and glucose metabolism, also in this subset of subjects: in fact, we observed a progressive reduction of testosterone, free-testosterone and SHBG plasma concentration moving from NGT to T2DM. Furthermore all these 3 hormones were inversely related to HOMA-IR. To date, the mechanism underlying the association between low testosterone levels and insulin resistance has not been completely elucidated [6]; it has been suggested that some confounding factors can mediate this relationship, such as elements of the metabolic syndrome, particularly, obesity [27, 28]. Anyway, our data are in line with previous reports, which agreed in detecting an independent association between insulin resistance and testosterone/free-testosterone plasma concentration [13, 18, 29], which is confirmed after correcting for BMI, age and WC.

As expected according to previous findings, low testosterone levels were not associated to increased gonadotropins plasma concentrations, entailing the condition of hypogonadotropic hypogonadism [5, 17]. It has been reported that insulin promotes gonadotropin release from pituitary cell cultures [30], and that insulin signaling in the brain plays an important role in regulating the reproductive function [31]. Moreover, insulin receptors are expressed

by Leydig cells and insulin enhances testosterone secretion both in vivo and in vitro [32, 33]. Therefore, a low testosterone level in insulin resistant states could indicate a functional defect at one or more levels of the hypothalamic-pituitary-gonadal axis.

In addition to the impact of insulin resistance on testosterone secretion, there is evidence to support an effect of testosterone itself on insulin sensitivity [6, 33]. For example, in male rats, acute castration induces significant insulin resistance by reducing lipolysis, which is reversed by testosterone replacement [34]. In men, low testosterone levels predispose to visceral obesity [35], leading to dysregulation of fatty acid metabolism, which, in turn, promotes insulin resistance [6, 7]. Furthermore, consistent data from prospective studies support the hypothesis that hypogonadism is an independent risk factor for the development of diabetes [16–19]. Finally, although still controversial [5, 6, 36], early interventional studies suggest that testosterone treatment in hypogonadal men with T2DM exerts beneficial effects on insulin resistance and glucose control [37–39]. Taken together, these findings suggest that glucose metabolism and the hypothalamus-pituitary-gonadal axis influence one each other in a complex relationship.

Importantly, we have documented that the inverse association between testosterone and insulin resistance is evident even in case of normal glucose tolerance; in other words, it extends across the full continuum of glucose tolerance. This finding suggests to consider testing the gonadal axis, not only in T2DM, but also in prediabetes and in insulin resistant NGT subjects. Further ad hoc studies are required to investigate whether hypogonadism correction in NGT and prediabetic subjects could prevent progression towards T2DM.

Our study has some limitations: first of all, we decided to focus our attention on patients referred to our clinic because of a diagnosis of arterial hypertension. Therefore, our findings can not be extended to the general population. Furthermore the sample size is limited and larger cohorts should be assessed to better identify and characterize the association between sexual hormonal profile and glucose tolerance, as well as to implement useful management algorithms.

In conclusion, a bidirectional association appears to exist between low testosterone and insulin resistance. In males with hypertension, the link between insulin sensitivity and hypothalamic-pituitary-gonadal axis is maintained along the entire spectrum of glucose tolerance.

Conflict of interest

The authors have no conflict of interest to declare.

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