

ORIGINAL ARTICLE

Glucose tolerance and plasma testosterone concentrations in men. Results of the Asturias Study

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Received 9 January 2010; accepted 11 October 2010

KEYWORDS

Type 2 diabetes;
Glucose intolerance;
Testosterone;
Hypogonadism;
Metabolic syndrome

Abstract

Background and objective: Studies in men have demonstrated a correlation between serum concentrations of androgens and sex hormone binding globulin (SHBG) with the presence of impaired glucose tolerance, diabetes and metabolic syndrome. The aim of this study was to evaluate circulating levels of total testosterone, SHBG, and bioavailable testosterone in the cohort of the Asturias Study and their association with the degree of glucose tolerance and metabolic syndrome.

Patients and methods: The study population consisted of 282 men aged 36 to 85 years old with normal concentrations of total testosterone. The degree of glucose tolerance and the presence of metabolic syndrome were evaluated.

Results: Serum concentrations of testosterone and bioavailable testosterone were negatively correlated with age, body mass index, waist circumference, blood glucose, glycosylated hemoglobin levels and insulin. Serum concentrations of total testosterone, bioavailable testosterone and SHBG were lower in men with glucose intolerance or diabetes than in those with normal glucose tolerance. After multivariate analysis, age and total testosterone levels were independent predictors of the presence of diabetes or glucose intolerance. The risk of glucose intolerance or diabetes mellitus was over 2.5 times higher in men with total testosterone levels in the lowest quartile than in those with total testosterone in the top quartile.

Conclusions: In this general population sample from Asturias, men with lower plasma concentrations of total testosterone – even when within the normal range – have an increased risk of glucose intolerance or diabetes, regardless of age and body mass index.

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PALABRAS CLAVE

Diabetes tipo 2;
Intolerancia a la
glucosa;
Testosterona;
Hipogonadismo;
Síndrome metabólico

Tolerancia a la glucosa y concentraciones plasmáticas de testosterona en varones. Resultados del Estudio Asturias

Resumen

Antecedentes y objetivo: Diversos estudios realizados en varones han demostrado la existencia de una correlación entre las concentraciones séricas de andrógenos así como de proteína transportadora de hormonas sexuales (sex hormone binding globulin o SHBG), y la presencia de alteraciones de la tolerancia a la glucosa, diabetes y síndrome metabólico. El objetivo de este estudio fue evaluar las concentraciones séricas de testosterona total, SHBG y testosterona biodisponible en la cohorte del Estudio Asturias, y su asociación con el grado de tolerancia a la glucosa y el síndrome metabólico.

Pacientes y métodos: Se estudiaron 282 varones adultos de 36 a 85 años de edad con concentraciones séricas normales de testosterona total, evaluándose tanto su grado de tolerancia a la glucosa como la presencia o no de síndrome metabólico.

Resultados: Las concentraciones en suero de testosterona y testosterona biodisponible se correlacionaron negativamente con la edad, el índice de masa corporal, el perímetro de cintura, la glucemia, las concentraciones de hemoglobina glucosilada y la insulinemia. Los hombres con intolerancia a la glucosa o diabetes mellitus tenían concentraciones en suero de testosterona total, SHBG y testosterona biodisponible inferiores a las de los que tenían tolerancia normal a la glucosa. Tras el análisis multivariable, la edad y las concentraciones de testosterona total fueron predictores independientes de la presencia de diabetes mellitus o intolerancia a la glucosa. Los hombres con concentraciones de testosterona total en el cuartil inferior tenían un riesgo de padecer intolerancia o diabetes más de 2,5 veces mayor que el de los que tenían la testosterona plasmática en el cuartil superior.

Conclusiones: En esta muestra de población general de Asturias los hombres con concentraciones en plasma más bajas de testosterona total, aun dentro del rango de normalidad, tienen mayor riesgo de presentar intolerancia a la glucosa o diabetes, independientemente de la edad y del índice de masa corporal.

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Introduction

In both men and women, changes in plasma testosterone levels have been seen to be associated with type 2 diabetes and glucose intolerance, and also with insulin resistance and metabolic syndrome. This association appears to differ depending on sex¹. While in men^{2,3} low plasma testosterone levels are associated to diabetes and an increased insulin resistance, in women it is hyperandrogenism, as occurs in polycystic ovary syndrome⁴, which is associated to diabetes mellitus, impaired glucose tolerance, and decreased insulin sensitivity.

On the other hand, several studies have shown male hypogonadism to be a risk factor for development of insulin resistance, metabolic syndrome, and diabetes⁵, and low testosterone levels, which are associated to a higher insulin resistance, are also commonly seen in men with diabetes⁶.

Various studies and a meta-analysis have shown an inverse correlation of total testosterone blood levels⁷ and the risk of developing type 2 diabetes. However, in the only study conducted on a general population sample, this correlation was only significant for free testosterone or calculated bioavailable testosterone⁸.

We measured serum levels of total testosterone, sex hormone binding globulin (SHBG), and bioavailable testosterone in a sample of a general population aged over 36 years in the Asturias Study⁹ and assessed their relationship

with age, various anthropometric parameters, and the presence or absence of diabetes mellitus, glucose intolerance, and metabolic syndrome.

Materials and methods

The study population was the male population of the cohort of the Asturias Study⁹ at the assessment performed in November 2004. The Asturias Study is a population-based prospective study of diabetes and cardiovascular risk factors started in 1999 in the principality of Asturias. An initial sampling was done in two phases. Fifteen of the 76 basic health areas in Asturias were randomly selected, with a probability proportional to the number of health cards of users aged 30 to 75 years. A total of 125 people were randomly selected from each basic health area. Eighty-seven subjects were excluded for various reasons (type 1 diabetes, pregnancy, severe disease, hospitalization, or use of diabetogenic drugs), and another 162 subjects for unavailability of the necessary contact data. The final sample consisted of 1,626 subjects, of whom 1,034 (63.6%) agreed to participate in the study.

A second assessment was performed in 2004. Of the original cohort, 42 subjects had died and 19 had left Asturias. Another 30 subjects were excluded (pregnancy, severe disease, hospitalization, or use of diabetogenic

drugs). Of the remaining 843 subjects, 700 (74.2%), 321 of them men, participated in this second assessment. Of this male group, 282 men aged 36 to 85 years with total testosterone levels higher than 2.5 ng/mL participated in this study. In all of them, a fasting blood sample was taken and an oral glucose tolerance test was performed administering 75 g of glucose. Samples were centrifuged in situ using a portable centrifuge, carried to the laboratory in a portable ice box, and stored at -40°C . Waist circumference, systolic and diastolic blood pressure, height, and body weight were measured in all subjects.

Glucose was measured using the enzymatic hexokinase method (DPP Hitachi), and subjects were classified as normal, intolerant to glucose or diabetic based on the criteria of the World Health Organization¹⁰ after the oral glucose tolerance test (OGTT). Intolerance was defined as blood glucose levels at 2 h of OGTT ranging from 140 and 200 mg/dL, and diabetes mellitus as levels higher than 200 mg/dL. Plasma levels of total cholesterol, HDL (high density lipoprotein) cholesterol, triglycerides (colorimetric method, DPP Hitachi), glycosylated hemoglobin (HPLC Menarini), and insulin (Sorin radioimmunoassay⁹) were also measured.

Metabolic syndrome was defined based on criteria of the National Cholesterol Education Group - Adult Treatment Panel III (NCEP-ATPIII)¹¹. The Homeostatic Model Assessment (HOMA) index was calculated using the formula: $\text{HOMA} = \text{Insulin (mIU/L)} \times \text{Glucose (mg/dL)} / 405$ ¹². Plasma levels of total testosterone (normal range: 2.5-7.9 ng/mL) and SHBG (normal range: 18-92 nmol/L) were measured using electrochemiluminescence immunoassay (Elecsys 2010, Roche Diagnostics). Inter-assay coefficients of variation were 9.2% and 4% respectively. Bioavailable testosterone was calculated using the Vermeulen algorithm¹³. Glycosylated hemoglobin was measured by HPLC (high performance liquid chromatography), and reference values were 4%-5.9%.

The study was approved by the local ethics committee, and all participants gave their informed consent.

SPSS 15.0 statistical software was used for the statistical study. Descriptive data are given as arithmetic means and standard deviations. Spearman correlations were calculated for continuous variables. Group means were compared using a Student's t test. The sample was divided into quartiles of total testosterone, SHBG, and bioavailable testosterone. A multiple logistic regression analysis was performed, with "impaired glucose tolerance or diabetes" as a dependent variable adjusted for body mass index (BMI) or waist circumference. A value of $p < 0.05$ in the two-tailed test was considered statistically significant.

Results

In our male adult population sample in Asturias, serum levels of total and bioavailable testosterone negatively correlated with age. By decades, serum total testosterone levels gradually decreased with patient age until they stabilized in those aged 75-85 years. However, SHBG levels increased more than 10% with each decade, so that bioavailable testosterone levels decreased more than 10% with each decade up to 75 years, and 5% from 75 to 85 years of age (Fig. 1).

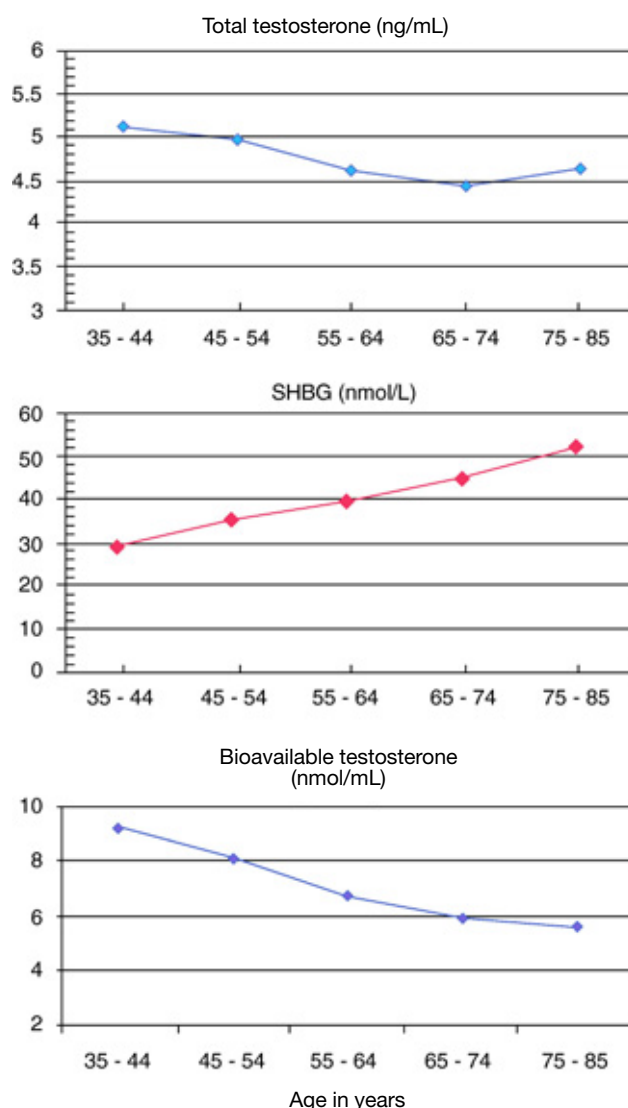


Figure 1 Total testosterone, sex hormone binding globulin, and bioavailable testosterone levels by decade of age. SHBG: sex hormone binding globulin.

A negative correlation of BMI and waist circumference with total testosterone, SHBG, and bioavailable testosterone levels was also seen. These three values were significantly lower in obese men with BMI > 30 as compared to men with a normal weight and BMI < 25 , as shown in Table 1.

Plasma levels of total and bioavailable testosterone were also found to have a significant negative correlation to basal glucose, two-hour glucose, glycosylated hemoglobin (HbA_{1c}), insulinemia, HOMA index, and other variables associated to metabolic syndrome such as systolic blood pressure, and plasma triglyceride levels. There was however no association of SHBG to blood glucose and HbA_{1c} values or the HOMA index.

Seventy of the 282 subjects (24.8%) had metabolic syndrome based on the ATP III criteria and, as shown in Figure 2, serum levels of total and bioavailable testosterone

Table 1 Plasma levels of total testosterone, SHBG, and bioavailable testosterone by body mass index

BMI (kg/m ²)	<25	25-30	>30
Total testosterone (ng/mL)	5.65 ± 1.89	4.63 ± 1.35	4.04 ± 1.43*
SHBG (nmol/L)	50.76 ± 22.05	37.59 ± 14.68	37.57 ± 19.35*
Bioavailable testosterone (nmol/L)	7.48 ± 2.33	7.36 ± 2.39	6.38 ± 2.08*

BMI: body mass index; SHBG: sex hormone binding globulin.

*p < 0.01 versus BMI < 25.

and SHBG were significantly lower in this group as compared to subjects without metabolic syndrome (total testosterone: 4.96 ± 1.59 vs 4.06 ± 1.45 ng/mL respectively; SHBG: 42.53 ± 18.59 vs 34.79 ± 16.88 nmol/L respectively; bioavailable testosterone: 7.36 ± 2.33 vs 6.56 ± 2.27 nmol/L respectively).

Among the 282 men of the cohort, prevalence rates of diabetes and glucose intolerance were 20.2% (57) and 12.4% (35) respectively. Type 2 diabetes mellitus or glucose intolerance were found in 92 patients, 32.6% of the sample. As shown in Table 2, men with glucose intolerance of diabetes mellitus had an older mean age and higher waist circumference and BMI values as compared to those with normal glucose tolerance. Their serum levels of total and bioavailable testosterone and SHBG were also lower.

In our multivariate logistic regression model, both age ($p = 0.017$) and testosterone levels ($p = 0.005$) were independent predictors for the presence of diabetes or glucose intolerance which persisted with BMI and waist circumference as covariates. When total testosterone was introduced as categorical variable as quartiles and the upper quartile was used as a reference, patients with total testosterone levels in the lower quartile had a 2.9-fold greater risk of having diabetes or glucose intolerance as compared to those with levels in the upper quartile (confidence interval: 1,30-6,56).

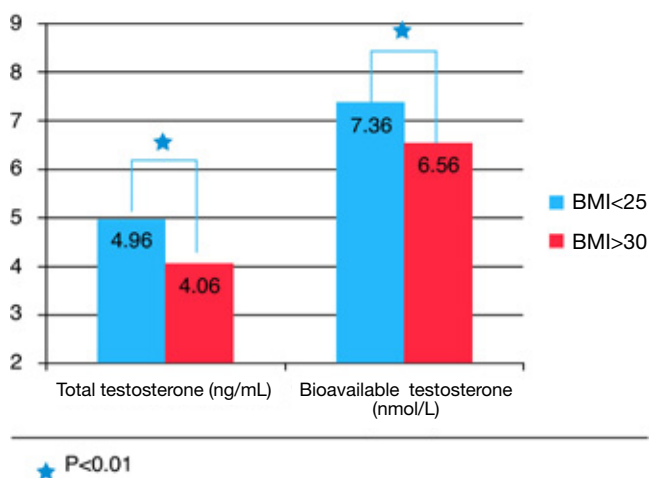


Figure 2 Total and bioavailable testosterone and presence or absence of metabolic syndrome (NCEP-ATP III criteria). BMI: body mass index.

Discussion

This cross-sectional study in men from the Asturias general population supports the results of prior studies^{14,15} showing a gradual decrease in serum levels of total testosterone up to 75 years of age. Such decrease is small, less than 0.5% per year. However, bioavailable testosterone levels show a much greater decrease, more than 1% per year, because of the increase in SHBG levels with age. These results are similar to those found in samples of European¹⁵ and American¹⁶ populations.

In this study, BMI was associated to a significant and progressive decrease in total testosterone levels, in agreement with previous studies¹⁷. This effect of BMI is very important and, in fact, the difference in total testosterone levels between people with normal BMI and obese subjects is greater than seen with age. This effect of BMI appears to be independent from age, because the decrease in total testosterone levels with age occurs in all BMI strata. By contrast, SHBG levels decrease with BMI and this positive correlation does not change with age, which suggests opposite and different effects of age and obesity on them.

Association of obesity to lower levels of both total and bioavailable testosterone appears to be due to a decreased response of pituitary luteinizing hormone due to impairment of the hypothalamic-pituitary-gonadal axis¹⁵ that may be secondary to either inflammatory adipokine production¹⁸ or to insulin resistance and hyperinsulinemia¹⁹. The latter may directly inhibit testosterone secretion by Leydig cells by acting on its specific receptors on these cells²⁰.

Males with hypogonadism, such as patients with Klinefelter syndrome²¹, have a high risk of developing insulin resistance, as well as glucose intolerance and diabetes mellitus, and hypogonadotropic hypogonadism is known to be more common in men with type 2 diabetes mellitus²². However, the relationship between total testosterone serum levels in the normal range and risk of experiencing diabetes or glucose intolerance has not been elucidated yet. This study of a general population sample aged over 36 years with normal total testosterone levels showed total testosterone serum levels to be independently associated to glucose intolerance or diabetes, thus suggesting that low levels of this hormone, even within the normal range, may be a risk factor for this metabolic condition. Although testosterone levels decrease with age and also as BMI increases, and the same occurs with the incidence of diabetes and glucose intolerance, the correlation of total testosterone serum levels to the presence of impaired glucose tolerance in our

Table 2 Age, body mass, waist circumference, and plasma levels of total and bioavailable testosterone and SHBG in patients with normal glucose tolerance and with an impaired glucose tolerance (diabetes and glucose intolerance)

	Total	Diabetes and GI	Normal	p*
N	282	92	190	
Age (years)	58.96 ± 12.25	63.81 ± 11.75	56.61 ± 11.82	<0.001
BMI	28.44 ± 4.07	29.18 ± 4.67	28.08 ± 3.70	0.032
Waist circumference (cm)	98.66 ± 10.53	101.38 ± 11.00	97.35 ± 10.06	0.002
Total testosterone (ng/mL)	4.72 ± 1.61	4.24 ± 1.44	4.95 ± 1.63	<0.001
SHBG (nmol/L)	40.61 ± 18.46	40.07 ± 18.21	41.71 ± 19.01	0.432
Bioavailable testosterone (nmol/L)	7.16 ± 2.34	6.27 ± 2.00	7.60 ± 2.37	<0.001

BMI: body mass index; GI: glucose intolerance; SHBG: sex hormone binding globulin.

*p: statistical significance comparing "normal" to "diabetes and GI".

study was independent from age, added to it as a predictor in our multivariate model, and its predictive value persisted when BMI was introduced in the model, thus suggesting an association that is also independent from the degree of overweight or obesity.

These results in our population agree with those reported by various cross-sectional studies and a meta-analysis⁶ showing an association between low testosterone levels and the presence or development of both metabolic syndrome²³ and type 2 diabetes mellitus or glucose intolerance²⁴⁻²⁶, and which also occurs in men with plasma testosterone levels within the normal range.

When testosterone quartiles were compared in our sample, the age- and BMI-adjusted risk that men with testosterone levels in the lower quartile experience glucose intolerance or diabetes is more than 2.5-fold greater as compared to that in men with testosterone levels in the upper quartile.

This study had many limitations, the main of which was its cross-sectional design, which prevents from establishing the directionality of the associations found. However, prior longitudinal studies^{16,27} appear to confirm the decrease in bioavailable testosterone levels with age. The time sequence between low plasma testosterone levels and occurrence of glucose intolerance cannot be established either from this cross-sectional study, but prospective studies in other populations have shown that low testosterone levels are prior to development of diabetes or glucose intolerance²⁸⁻³¹, and low SHBG levels themselves are also predictors of the risk of diabetes in both sexes³². Follow-up of our cohort could allow us to confirm this hypothesis in the future.

Diabetes mellitus and glucose intolerance are known cardiovascular disease risk factors. In men, consequences on cardiovascular risk of declining total and bioavailable testosterone levels with age have not been clearly established yet. Although some evidence has associated a predictive effect of low testosterone levels^{33,34} on cardiovascular disease in men, additional studies are required to clarify whether part of the increase in cardiovascular risk associated to age is due to development of glucose intolerance in people with lower testosterone levels, and whether therapeutic intervention administering testosterone may have some benefit.

In conclusion, our cross-sectional study of men from the general population of Asturias showed that those with the lowest plasma total testosterone levels, even within the normal range, have a greater risk of experiencing glucose intolerance or diabetes, a risk which is independent from age and BMI.

Conflict of interest

The authors state that they have no conflict of interest.

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