CLINICAL STUDY

Metabolic syndrome and the effect of testosterone treatment in young men with congenital hypogonadotropic hypogonadism

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Abstract

Objective: The relationship between metabolic syndrome (MS) and hypogonadism has always been investigated in study groups confounded with aging, obesity or chronic metabolic disorders. So far, there has been no data about the presence of MS in young hypogonadal patients. Also, there is controversial data about the metabolic effects of testosterone replacement therapy. We investigated the frequency of MS in treatment-naïve, young men with congenital hypogonadal hypogonadism (CHH). We also searched for the effect of testosterone replacement on the metabolic profiles of this specific patient group.

Design: Retrospective analysis.

Methods: A total of 332 patients (age 21.68 ± 2.09 years) were enrolled. The control group included 395 age- and body mass index (BMI)-matched healthy young men (age 21.39 ± 1.49 years). Standard regimen of testosterone esters (250 mg/3 weeks) was given to 208 patients.

Results: MS was more prevalent in CHH ($P < 0.001$) according to healthy controls. The patients had higher arterial blood pressure, waist circumference (WC), triglyceride ($P < 0.001$ for all), fasting glucose ($P = 0.02$), fasting insulin ($P = 0.004$), homeostatic model assessment of insulin resistance (HOMA-IR) ($P = 0.002$) and lower high density lipoprotein (HDL) cholesterol ($P < 0.001$) levels. After 5.63 ± 2.6 months of testosterone treatment, the BMI, WC ($P < 0.001$ for both), systolic blood pressure ($P = 0.002$) and triglyceride level ($P = 0.04$) were increased and the total and HDL cholesterol levels were decreased ($P = 0.02$ and $P < 0.001$ respectively).

Conclusions: This study shows increased prevalence of MS and unfavorable effects of testosterone replacement in young patients with CHH. Long-term follow-up studies are warranted to investigate the cardiovascular safety of testosterone treatment in this specific population.

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Introduction

Metabolic syndrome (MS) is a combination of various premorbid conditions contributing to the development of diabetes and cardiovascular disease. The components of MS which have been defined by several medical groups are the abnormal glucose metabolism, increased abdominal fat, high blood pressure, high triglycerides and low HDL levels (1). The more appropriate method would be to strictly control each of these MS components in order to prevent cardiovascular morbidity and mortality (2).

Hypogonadism, a prevalent clinical situation of the middle and older age population (3, 4), has been reported as a predictor of MS (5–7). However, the majority of the data showing the association between MS and hypogonadism originate from the obese or elderly population with chronic metabolic disorders (8–15). Aging, obesity, chronic disorders and several medications commonly used in daily practice can decrease serum testosterone levels (16–22) and confound the association between hypogonadism and MS. Thus, the unconfounded data of the patients with congenital hypogonadal hypogonadism (CHH) are of utmost importance, as they can provide information about the effects of low testosterone levels on metabolic profiles. High fasting glucose and insulin levels in young men with CHH (23, 24) were reported in two small studies. However, there is no data about the other components of MS in this specific population.

Testosterone replacement is an emerging treatment option for the metabolic abnormalities in patients with hypogonadism (25, 26). However, not all the data is in favor of the benefits of testosterone replacement. Some reports mention that testosterone improves metabolic parameters (27–32), while others do not confirm these data (33–38). Inhomogenities of the study populations, different formulas and follow-up periods and several other confounders such as concomitant drugs or metabolic disorders may be the reasons for these controversies.

Our aim was to search for the prevalence of MS and its components in an unconfounded population of hypogonadism. For this reason, we retrospectively evaluated our database and selected young and treatment-naïve men with CHH. We also searched for the effect of standard dosage of injectable testosterone ester replacement on the metabolic profiles of these patients.

Materials and methods

This retrospective analysis was performed by evaluating the database of the Department of Endocrinology and Metabolism, Gulhane Military Medical Academy School of Medicine, Ankara, Turkey. Military service is compulsory for every young man in Turkey and Gulhane School of Medicine is the tertiary medical center for all the military recruits. A total of 1586 hypogonadal patients were registered between the years 2000 and 2009. Of these, 653 patients were excluded due to previous history of androgen replacement, 214 patients were excluded due to higher testosterone levels (200–300 ng/dl), 28 patients were excluded due to liver, kidney or pulmonary diseases, 76 patients were excluded because of diagnosis other than hypogonadotropic hypogonadism (i.e. primary hypogonadism, panhypopituitarism, pituitary adenoma) and 283 were excluded because of incomplete data about the demographic and the metabolic parameters. Finally, 332 treatment-naïve patients (age 21.68 ± 2.09 years) with CHH were included in the study. The control group included 395 age- and body mass index (BMI)-matched healthy recruits (age 21.39 ± 1.49 years). None of the control subjects had any chronic disorders. All subjects gave informed consent and the Local Ethical Committee of Gulhane School of Medicine approved the study. This study is registered with ClinicalTrials.gov, number NCT01160341.

The height, weight and waist circumference (WC) of the patients and control subjects were measured with their underwear. BMI was computed as the ratio of weight to the square of height (kg/m^2). WC was measured, after the patients exhaled, from the line on the iliac crest which is parallel to the ground. Blood pressure of the patients was measured with an appropriate arm cuff and a mercury column sphygmomanometer, after a resting period of at least 5 min. The mean of the two sitting measurements was used as the systolic and diastolic blood pressure of the patients and controls. Pubertal developments of the patients were assessed according to the Tanner stages. The diagnosis of CHH was based on a failure to undergo spontaneous puberty before 18 years of age and was confirmed by low serum total testosterone levels and normal or low gonadotropin levels. Additional criteria were the absence of a pituitary or hypothalamic mass lesion on magnetic resonance imaging, presence of a gonadotropin response to repetitive doses of GNRH and a normal karyotype (46, XY).

MS was defined according to the National Cholesterol Education Program's Adult Treatment Panel III report, as having any three of the following criteria (WC >102 cm, triglycerides 150 mg/dl, HDL cholesterol <40 mg/dl, blood pressure 130/85 mg/dl, fasting blood glucose 100 mg/dl) (39).

Laboratory measurements

For biochemical analyses, all blood samples were collected from the antecubital vein, between 0800 and 0900 h. after an overnight fasting. The samples were centrifuged for 15 min at 2000 **g**, aliquoted and immediately frozen at $-80\,°C$ for analyses. Fasting plasma glucose, total cholesterol, triglyceride, and high-density lipoprotein cholesterol levels were measured by the enzymatic colorimetric method with Olympus AU2700 auto analyzer using reagents from Olympus Diagnostics (GmbH, Hamburg, Germany). Low-density lipoprotein cholesterol (LDL-C) was calculated by Friedewald's formula (40). The serum basal insulin level and testosterone were measured by the electro-chemiluminescence method with Modular Analytics E170 autoanalyzer using reagents from Roche Diagnostics. Insulin sensitivity was calculated by using the HOMA index by the formula, HOMA-IR $=$ (insulin $\times$ glucose)/405 (41).

Testosterone replacement therapy

Follow-up data was available for the 208 patients who were treated with oil-based injectable blend of four esterized testosterone compounds (Sustanon 250 mg; 30 mg testosterone propionate, 60 mg testosterone phenylpropionate, 60 mg testosterone isocaproate and 100 mg testosterone decanoate) injected for 3 weeks. Some of the patients were not included in the follow-up study, as they were treated with different regimens (i.e. human chorionic gonadotropin in different dosage and periods with or without testosterone compounds; $n=82$). Also, some patients, who were living in the distant regions of the country, were not enrolled in the follow-up study, as they were not able to return for the control visits ($n=42$). The blood samples for the evaluation of the baseline metabolic parameters were taken before the first testosterone injection. The patients were then reevaluated in the 3rd and/or 6th months of treatment. The follow-up visits were arranged on the days before the next testosterone injection. Therefore, the time points for taking the blood samples were similar for all the patients.

Statistical analysis

All data were recorded on a computer database and analyzed using SPSS 11.0 package program (SPSS, Inc., Chicago, IL, USA). Results are expressed as mean$\pm$S.D.

Table 1 The metabolic profiles of the patients with hypogonadism and healthy volunteers.

	Patients (n=332)	Controls (n=395)	P
Age (years)	21.8±2.4	21.7±1.5	0.43
BMI (kg/m^2)	21.9±3.59	21.9±1.01	0.91
WC (cm)	82.6±10.9	76.6±4.56	<0.001
SBP (mmHg)	113±10.1	107±10.9	<0.001
DBP (mmHg)	73.1±8.46	67.6±9.25	<0.001
Total chol (mg/dl)	155±29.3	141±27.0	<0.001
HDL chol (mg/dl)	46.1±11.0	52.6±8.7	<0.001
LDL chol (mg/dl)	89.3±25.1	72.6±23.5	<0.001
TG (mg/dl)[a]	89.0 (68.0–123.0)	69.4 (54.5–90.7)	<0.001
FBG (mg/dl)	85.9±9.32	84.4±7.28	0.02
Insulin (μU/ml)[a]	9.41 (6.45–13.2)	7.62 (5.23–9.23)	0.004
HOMA-IR[a]	2.04 (1.32–2.77)	1.53 (1.05–1.91)	0.002

[a]Results are given as mean (25–75%).

Kolmogorov–Smirnov test was used to determine the distribution characteristics of variables and Levene's test was used to evaluate the equality of variance. Intra-group changes at two time points were analyzed by paired samples t-test or Wilcoxon signed-rank test as appropriate. Inter-group differences were analyzed by χ^2 test, Student's t-test and Mann–Whitney U test as appropriate. Differences were considered significant at $P<0.05$.

Results

The characteristics of the patients and the control subjects are given in Table 1. The metabolic parameters of both the patients and the age- and BMI-matched control subjects were within the normal reference limits. However, when the two groups were compared, the patients with CHH had significantly higher systolic and diastolic blood pressure, WC, triglycerides, total and LDL cholesterol ($P<0.001$ for all), fasting blood glucose (FBG; $P=0.02$) and insulin levels ($P=0.004$) and HOMA indexes ($P=0.002$) and significantly lower HDL cholesterol levels ($P<0.001$). Both groups were also compared according to the presence of MS criteria. The patients with CHH had significantly higher prevalence of MS and its components when compared to those of the control subjects ($P<0.001$; Fig. 1).

The effect of androgen replacement

The follow-up data was available for the 208 patients treated with testosterone ester injection. No significant side effects were reported during the study period. The alterations of the metabolic parameters after a mean follow-up period of $5.63±2.6$ months are given in Table 2. According to the results, the BMI, WC ($P<0.001$ for both), systolic blood pressure (SBP; $P=0.002$) and triglyceride levels ($P=0.04$) were increased and the total and HDL cholesterol levels were decreased significantly ($P=0.02$ and $P<0.001$

respectively). On the other hand, there were no significant alterations in the fasting blood glucose, insulin, HOMA-IR, LDL cholesterol and diastolic blood pressure (DBP) levels. After the treatment period, the mean Tanner stages of the patients were significantly increased from $2.15±0.76$ to $3.23±0.91$ ($P<0.001$).

The alterations in plasma testosterone levels were significantly correlated with the alterations in BMI ($r=0.215, P=0.002$), WC ($r=0.169, P=0.03$) and HDL cholesterol ($r=-0.188, P=0.02$) levels of the patients.

Discussion

According to the results, the metabolic parameters of the young and treatment-naïve patients with hypogonadism were within the normal reference ranges of the adult population. However, when compared with age- and BMI-matched healthy control subjects, the prevalence of MS and its components were significantly higher in the CHH group. The patients with CHH have significantly higher WC, blood pressure, fasting glucose, insulin and triglycerides and significantly lower HDL cholesterol levels in comparison to the healthy control subjects. About 6 months of treatment with oil-based injectable blend of four esterized testosterone compounds (Sustanon 250 mg i.m.) did not improve the metabolic profiles but increased the WCs, systolic blood pressure, triglycerides and decreased the HDL and total cholesterol levels. The implications of the increased prevalence of MS in hypogonadism and the unexpected deterioration of the metabolic profiles after the testosterone replacement are discussed below.

There seems to be an interrelationship between hypogonadism and MS (25). Several cross-sectional studies report increased prevalence of hypogonadism in MS (8, 9, 11), while many others report increased prevalence of MS and diabetes mellitus in subjects with lower testosterone levels (12–15). The increased MS risk in hypogonadism can be explained by several mechanisms. Low testosterone levels increase fat mass and decrease lean muscle, causing insulin resistance and hyperlipidemia (42). Also, the plasma triglyceride clearance may decrease due to the reduced testosterone/estrogen ratio (43). However, the majority of the reports about the association between MS and
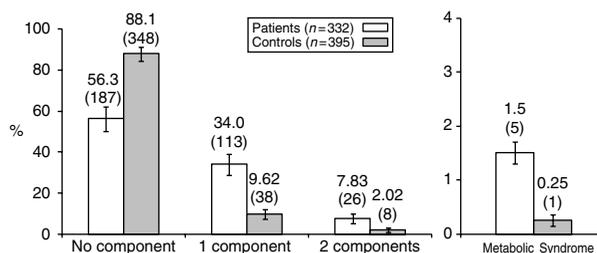
Figure 1 The prevalence of the components of metabolic syndrome in patients and healthy controls given as % (n).

Table 2 The effect of androgen replacement on the components of metabolic syndrome.

	Before treatment (n=208)	After treatment (n=208)	P
BMI (kg/m²)	22.0±3.59	23.1±3.44	<0.001
WC (cm)	82.7±10.9	85.7±9.9	<0.001
Tanner stage	2.15±0.76	3.23±0.91	<0.001
SBP (mmHg)	112±10.6	115±10.2	0.002
DBP (mmHg)	72.1±8.16	72.8±8.42	0.19
Total chol (mg/dl)	156±29.9	151±29.6	0.02
HDL chol (mg/dl)	46.4±11.4	42.6±10.1	<0.001
LDL cho (mg/dl)	89.1±25.5	88.6±25.7	0.79
TG (mg/dl)[a]	89.0 (68.0–124.0)	99.0 (79.5–132.5)	0.04
FBG (mg/dl)	86.4±8.97	85.4±10.3	0.28
Insulin (μU/ml)[a]	9.79 (6.8–13.7)	9.2 (7.3–13.8)	0.25
HOMA-IR[a]	2.04 (1.32–2.78)	2.00 (1.51–2.06)	0.11
Total testosterone (ng/dl)[a]	33.6 (21–43)	215 (59–281)	<0.001

[a]Results are given as mean (25–75%).

hypogonadism are from the elderly or obese populations with chronic disorders (8–15). During the ageing process, testosterone levels constantly decrease, independent of the other clinical variables (16, 17). Moreover, adiposity augments the aromatization of testosterone to estradiol, reducing LH secretion and testosterone levels (18). Obesity also increases glucocorticoid turnover (19), which disturbs the regulation of the hypothalamic–pituitary–adrenal axis (44) and contributes to hypoandrogenism. Also, in most of these reports, the study populations were using chronic medications with the potentials to affect the serum testosterone levels (20–22). In this regard, the data from patients with CHH are of utmost importance, as they may reflect the unconfounded effects of low testosterone levels on metabolic profiles. To our knowledge, there are only two small studies, both of which report higher fasting glucose and insulin levels in young men with CHH, without mentioning the other components of MS (23, 24). Thus, our data are noteworthy as they show poor metabolic parameters in a sizeable group of treatment-naïve patients with CHH, mentioning that hypogonadism can cause MS even in very young ages.

Testosterone replacement is expected to improve the metabolic abnormalities in patients with hypogonadism (25), and for some authors, it may be a novel treatment option in MS (26). However, reports about the metabolic effects of testosterone replacement are controversial. Several studies mention that testosterone replacement improves fasting glucose levels (27, 28), insulin sensitivity (27, 29, 30), arterial blood pressure (31, 32) and total cholesterol levels (27). However, many other studies also show that the effect of androgen replacement on metabolic parameters is either insignificant (33, 34) or unfavorable (35–38). Recently, a prospective study in the elderly hypogonadal men was terminated due to the increased cardiovascular mortality in the testosterone replacement arm (45). However, another prospective randomized study with

a similar design (46) and two recent metanalyses of randomized controlled studies (34, 47) did not show any increase in cardiovascular events. Several reasons can be recalled for the discrepancies, such as different durations, formulations and dosages of androgens and concomitant metabolic disorders and medications (48, 49). So far, there is hardly enough data about the effects of standard testosterone regimens on young, previously untreated CHH patients without confounders. Two recent reports performed in small groups mention that testosterone supplementation improves insulin resistance without significantly affecting the blood lipids, arterial blood pressure or WC (23, 24). However, our results do not support these data and show increased WC, systolic blood pressure and decreased total and HDL cholesterol levels in the study group. We suggest that the size of the study population may be the main factor for the testosterone-induced alterations to become significant. Supportive to our data, a latest metanalysis of randomized controlled studies has also shown that testosterone therapy causes a small but significant decrease in HDL cholesterol levels (47).

The increased arterial blood pressure, triglyceride levels and decreased HDL cholesterol levels due to testosterone replacement may have the potential to cause adverse cardiovascular effects. However, so far, there is no long-term follow-up data that show increased cardiovascular events in this specific population. On the other hand, several reports mention that the decrease in HDL cholesterol levels may not be a harmful effect of testosterone replacement. It has been hypothesized that testosterone leads to trafficking of the cholesterol back from the peripheral tissues to the liver, leading to consumption of HDL. This decrease in HDL cholesterol levels due to testosterone replacement is suggested to exert anti-atherogenic rather than pro-atherogenic effects (50). Moreover, the decrease in total testosterone levels, which has also been shown in previous studies (27), may be regarded as an evidence of the favorable effects of testosterone regiments. It was also reported that the decrease in HDL cholestrol levels after testosterone replacement was due to the decrease in the least atherogenic HDL$_3$ subfraction (51). However, our data cannot verify the above theory, as we have not analyzed the HDL subfractions.

This study may have several limitations. The retrospective design has handicaps compared with a randomized, placebo controlled prospective study. Still, our database provided a large group of young and treatment-naïve CHH patients, which is very helpful to recognize the effect of hypogonadism and its treatment on metabolic parameters. Another limitation may be the formulation of the testosterone regimen. Testosterone ester injections can increase serum testosterone to supraphysiological levels, which may be responsible for the adverse effects on systolic blood pressure and plasma lipids (48). However, two recent randomized, controlled studies, which have also reported decreased HDL

cholesterol levels were performed by using daily transdermal gels (37) or testosterone tablets (38). Therefore, the drug formulation may not be the only reason for the worsening of the metabolic profiles. On the other hand, the alterations in the metabolic parameters, although statistically significant, are minor and may not be clinically important.

In conclusion, the present study has two significant findings. The first one is the increased prevalence of MS and its components in young CHH patients than the healthy control subjects. The second finding is the deterioration of systolic blood pressure, triglycerides and HDL cholesterol levels along with the lowering of total cholesterol levels due to testosterone replacement. These data may be of help to recognize that testosterone replacement may cause unfavorable metabolic effects in young hypogonadal patients. However, whether these metabolic alterations will result in adverse cardiovascular outcomes in the long term is not clear. Prospective studies are needed to find out the long-term effects of testosterone replacement on cardiovascular morbidity and mortality in this specific population.

Declaration of interest

The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

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