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SERUM TESTOSTERONE, FREE TESTOSTERONE INDEX AND SHBG CONCENTRATION FOR REDUCTION OF METABOLIC SYNDROME IN TEHRANIAN MEN

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Abstract

Background. Although androgen deficiency in men is associated with obesity, whether the deficiency is a consequence of the syndrome is still unclear.

Aim. The aim of this study was to determine the association between low levels of sex hormones and development of the metabolic syndrome.

Subjects and Methods. A total of 836 men, aged ≥ 20 years, participants of the Tehran Lipid Glucose Study, were assessed at baseline and after 6.5 years follow-up, based on both ATP III and IDF criteria for occurrence of metabolic syndrome. The association between serum total and free testosterone index and SHBG and metabolic syndrome was investigated using logistic regression models.

Results. After 6.5 years of follow-up, the metabolic syndrome developed in 131 and 207 men based on ATP III and IDF criteria respectively. Multiple logistic regression analysis showed an inverse relationship for total testosterone in the lower tertile concentration, and serum triglycerides, according to both criteria mentioned (OR = 1.6; 95%CI 1.02-2.5). According to ATP III criteria, adjustment of waist circumference eliminated most of the correlations between total testosterone and metabolic syndrome (OR=1.34, 95% CI (0.8-2.3), while SHBG and free testosterone index were not significantly associated with the syndrome. According to IDF criteria, statistical adjustment of waist circumference eliminated most of the correlations between total testosterone and metabolic syndrome [(OR=1.45, 95% CI (0.9-2.3)], and adjustment with triglycerides eliminated all correlations between SHBG and metabolic syndrome (OR=1.5, 95% CI (0.9-2.5).

Conclusions. Androgen deficiency may be related to poorly controlled serum triglycerides and increased waist circumference.

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Key words: metabolic syndrome , total testosterone , SHBG, free testosterone index.

INTRODUCTION

The metabolic syndrome has been established as a precursor state in which patients are at a significantly increased risk of developing type II diabetes mellitus and cardiovascular diseases (CVD) (1, 2) .The pathogenesis of the syndrome is multifactorial, but obesity and sedentary lifestyle and nutritional factors and other yet largely unknown genetic factors interact in the occurrence of the syndrome. Aging is associated with a gradual decline in testosterone levels in men, a decrease which is accompanied by changes in body composition including increase in fat mass, decrease in lean body mass, dyslipidemia, insulin resistance and glucose metabolism dysregulation (2). Epidemiologic evidence shows that in males, sex hormones are related to type 2 diabetes and CVD in some studies (3, 4).

Small intervention trials have demonstrated that testosterone replacement in older men with low serum testosterone compared to young, healthy men, increases lean body mass and decreases fat mass, total cholesterol and LDL without affecting HDL, all of which may be associated with decreased risk of CVD (5,6).

Cross-sectional (7) and longitudinal (8) studies have demonstrated that low serum testosterone and sex hormone binding globulin (SHBG) have been associated with the metabolic syndrome, an association that however has not been tested by either the ATP III or IDF criteria. Hence, using data from the Tehran Lipid and Glucose Study (TLGS), the relationship between sex hormones and the metabolic syndrome was investigated in Tehranian males.

SUBJECTS AND METHODS

The TLGS is a study being conducted to determine the prevalence of non-communicable diseases and the risk factors of atherosclerosis among Tehran's urban population and to develop population based measures and lifestyle modifications to prevent the rising trend of diabetes mellitus and dyslipidemia (9, 10). A multi-stage stratified cluster random sampling technique was used to select 15005 people, aged ≥ 3 years, from district 13 of Tehran, the capital of Iran. The crude response rate was approximately 57.5% and there were differences in terms of age and gender distribution. All protocols and informed consent procedures were approved by the ethical research committee of the Endocrine Research Center. In the present study, of a total of 4397 men, aged ≥ 20 years of age, 1600 men who did not have the metabolic syndrome, according to both ATP III and IDF criteria, were primarily eligible for this study. We excluded 528 men using β blockers, corticosteroids or male sex hormone medication, after appropriate assessment of history, subjects with cirrhosis and renal failure were excluded, 206 others were also excluded for incomplete data, leaving a final sample of 836 men. Assessment of the outcome of interest was undertaken between subjects who did and who did not meet the criteria of metabolic syndrome after 6.5 years follow-up.

Weight and height were measured according to the standard protocol of the TLGS; waist circumference was measured at the narrowest level over light clothing. BMI was computed as the ratio of weight to the square of height (kg/m^2). Blood pressure was measured with a random zero mercury sphygmomanometer. The measurement protocol included two measurements in a sitting position with 5-min intervals and the mean of two measurements was documented as blood pressure.

"High leisure time physical activity" was defined as regular and vigorous activity, at least 3 times per week; "moderate leisure time" as exercising less than 3 times per week and "no activity" was defined as when the activity reported was not enough to meet high and moderate activity definitions (11). Smoking was defined as "never ", "daily" and "occasional" smokers.

Subjects were asked to fast for 12-24 hr before blood sampling which was done between 7:00 and 9:00 a.m. Fasting blood glucose, serum total cholesterol, triglycerides, and high density lipoprotein cholesterol, were measured at the TLGS research laboratory on the day of blood collection. Using the Fried Wald formula, (12) low density lipoprotein cholesterol (LDL-C) was calculated from serum cholesterol, triglycerides and HDL-C, except when triglyceride concentration was greater than 371 mg/dL. Details of clinical and laboratory measurements have been published elsewhere (9,13) .

Total testosterone and SHBG were measured by Eliza (Diagnostic Biochem, Canada Kit). The free testosterone Index (FTI) was calculated as a ratio.Intra and inter -assay coefficients of variations were 8.5, 11.6 and 9.6, 8.6% respectively.

According to the ATP III, the metabolic syndrome was defined as the presence of three or more of the following criteria: Fasting plasma glucose of at least 110 mg/dL, serum triglycerides of at least 150 mg/dL, serum HDL cholesterol <40 mg/dL, blood pressure of at least 130/85 mmHg or antihypertensive medication use, and waist girth > 102cm. According to IDF criteria, the syndrome was defined as a waist girth > 94cm and the presence of two or more of the following criteria: Fasting plasma glucose of at least 100 mg/dL or a clinical diagnosis of diabetes; serum triglycerides of at least 150 mg/dL, serum HDL cholesterol <40mg/dL, blood pressure of at least 130.85 mmHg or antihypertensive medication use.

Statistical analyses

Data are expressed as mean ± SD, except for data that were not normally distributed, in which case median values, ranges and proportions (%) are reported. Differences in clinical and biochemical characteristics of men, at the 6.5 year follow-up developed metabolic syndrome and those who did not were tested for statistical significance, using the Student's t-test and, where indicated, the chi^2 test.

Using logistic regression models, we adjusted for age, smoking, education, physical activity and also for all other risk factors, included in the definition of ATP III and IDF for metabolic syndrome. Serum hormone levels were analyzed as continuous variables and were grouped into the tertiles of their distributions. Logistic regression was used to estimate the odds ratio (OR) and 95% confidence interval (CI) for the presence of each individual

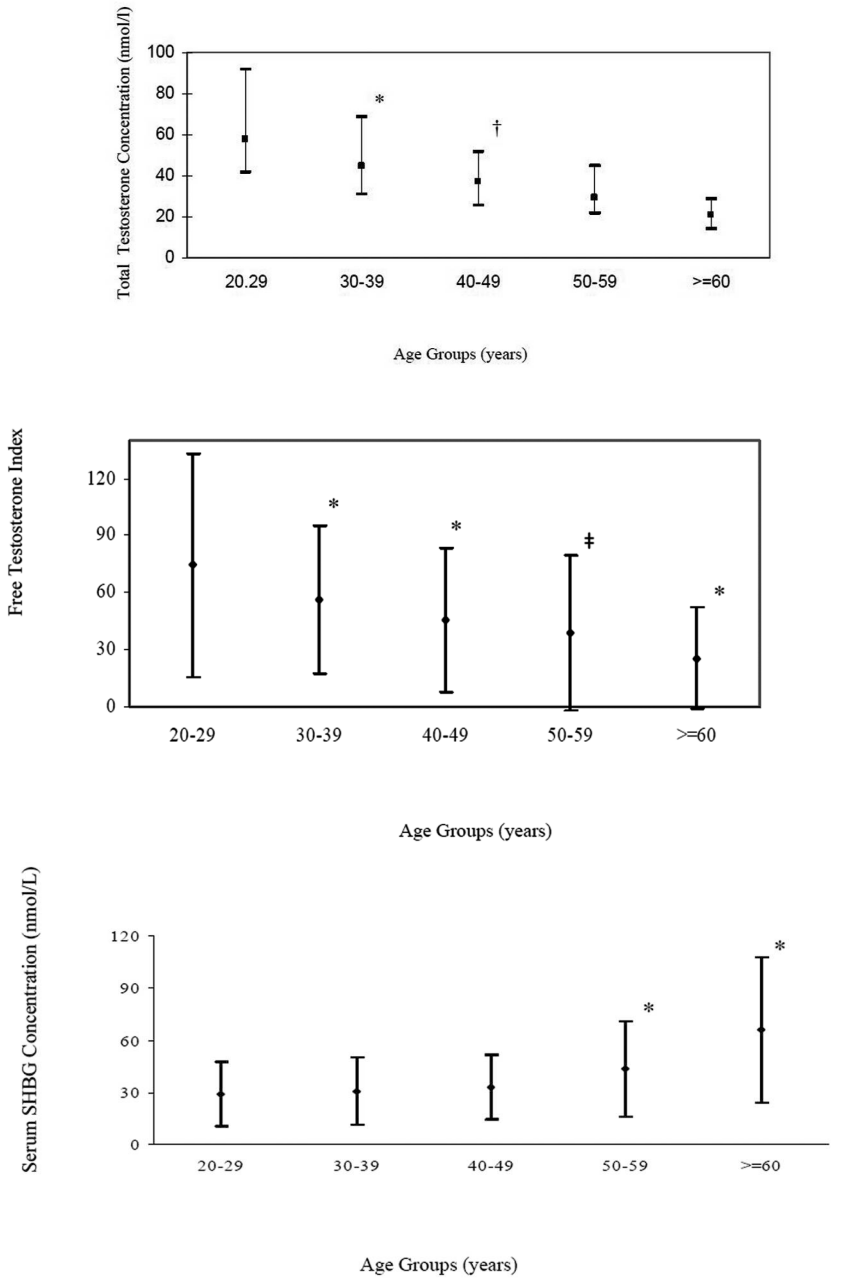

Figure 1. Correlations between plasma androgen levels and age. Serum total testosterone decreases until 50 yrs and remains relatively stable afterwards. The decline in the free testosterone index levels started at an earlier age ($p < 0.001$), SHBG levels however, remained relatively stable until age 40 yr and increased afterwards.*$p<0.001$; † $p<0.0005$; ‡$p<0.004$

Table 1. Baseline characteristics of the studied cohort, without the metabolic syndrome according to ATP III and IDF criteria

	Entire cohort
n	836
Median age (years)	38 (31-50)
Physical activity (%)	
Very active (%)	17.8
Moderately active (%)	17.5
No activity (%)	64.2
Smokers (%)	26.4
Cardiovascular disease (%)	0.7
Median systolic blood pressure (mmHg)	113 (105-121)
Median diastolic blood pressure (mmHg)	74 (69-80)
Mean waist circumference (cm)	84±9.5
Mean BMI (kg/m^2)	24.5±3.4
BMI ≤ 25 kg/m^2 (%)	56.8
BMI > 25 kg/m^2 (%)	43.2
Mean fasting blood glucose (mg/dL)	88±8.5
Median HDL-cholesterol (mg/dL)	39 (35-46)
Median serum triglycerides (mg/dL)	126 (93-173)
Median serum total testosterone (nmol/L)	11 (9-14)
Median free testosterone index	39 (25-62)
Median serum SHBG (nmol/L)	31 (20-43)

risk factor of the metabolic syndrome based on both ATP III and IDF criteria.

For assessment of the associations between sex hormones and the metabolic syndrome based on both criteria, concentration of total testosterone, free testosterone index and SHBG were dichotomized by the lower tertile and entered into logistic regression models adjusting for : 1) without adjustment; 2) adjusted for age; 3) adjusted for age, physical activity, smoking and education; 4) adjusted for the variables in model 3 and waist circumference; 5) adjusted for the variables in model 4 and fasting glucose, triglycerides, systolic and diastolic blood pressure. All statistical analyses were performed using SPSS 15.0 for Windows.

RESULTS

Baseline characteristics of study cohort are presented in Table 1.The median age of the total study group was 38.0yr. 36.4% of participants were smokers, 79% had literacy levels below high school and 56.5% had BMI ≤ 25 kg/m^2. The correlation between plasma androgen levels and age is shown in Figure 1. Serum total testosterone decreased until the age of 50 yr and

Table 2. Baseline characteristics in controls and in men who developed metabolic syndrome according to ATP III and IDF respectively after 6.5 years of follow-up

	Metabolic syndrome according to ATP III at follow-up	Metabolic syndrome according to IDF at follow-up
N (%)	131 (15)	207 (24)
Median age (years)	38 (22-50)	40 (32-49)
Physical activity (%)		
Very active	12.4	14.6
Moderate active	21.7	18.9
No activity	65.9	66.5
Smokers (%)	28.5	29.5
Cardiovascular disease (%)	0.8	0.5
Median systolic blood pressure (mmHg)	117 (110-123)*	115 (107-123)†
Median diastolic blood pressure (mmHg)	76 (71-82)‡	76 (70-82)*
Mean waist circumference (cm)	89±9*	89±7.6*
Mean BMI (kg/m^2)	26±3*	26±3.5*
BMI ≥ 25(kg/m^2) (%)	39.7	57
BMI > 25 (kg/m^2) (%)	60.3	7.7
Mean fasting blood glucose (mg/dL)	89±9.6	89±8.3
Median HDL-Cholesterol (mg/dL)	39 (32-42)*	35 (32-42)*
Median serum triglycerides (mg/dL)	146 (112-214)*	147 (115-220)*
Median serum total testosterone (nmol/L)	10 (9-11)†	10 (23-38)‡
Median free testosterone index	39 (26-57)	41 (24-640)
Median serum SHBG (nmol/L)	31 (19-40.8)	27 (27-64)*

Data are means ± SD, median (inter quarter range), or percent
* P ≤ 0.001, † P ≤ 0.05, ‡ P≤ 0.01

remained relatively stable after that. The decline in free testosterone index levels started at an earlier age (P<0.001); SHBG levels, however, stayed relatively stable until age 40 yr and increased thereafter.

After 6.5 years of follow-up, the metabolic syndrome developed in 131 (15%) and 207 (24%) men according to ATP III and IDF criteria respectively. According to criteria, baseline systolic and diastolic blood pressure,

Table 3.*Adjusted odds ratio and 95% confidence intervals of sex hormones with components of the metabolic syndrome according to ATP III or IDF criteria

	Total testosterone in the lower tertile	Free testosterone index in the lower tertile	SHBG in the lower tertile
Components of metabolic syndrome according to ATP III			
Waist circumference ≥ 102 cm	1.13(0.6-2.1)	1.8 (0.8-2)	1.2 (0.7-1.9)
Triglycerides ≥ 150 mg/dL or medication use	1.39 (0.9-2.1)	0.8 (0.4-1.2)	1.6 (1-2.5) †
HDL < 40 mg/dL or medication use	1.42 (0.9-2.2)	0.8 (0.5-1.28)	1.38 (0.8-2.2)
BP ≥ 130/80 mmHg or medication use	0.7 (0.3-1.3)	1.1 (0.5-2.2)	0.6 (0.3-1.4)
Fasting blood glucose ≥ 110 mg/dL or medication use	0.7 (0.3-1.6)	0.8 (0.4-1.7)	1.1 (0.5-2.4)
Components of metabolic syndrome according to IDF			
Waist circumference ≥ 94 cm	0.9 (0.6-1.6)	0.8 (0.4-1.5)	1.12 (0.6-1.8)
Triglycerides ≥ 150 mg/dL or medication use	1.39 (0.9-2.1)	0.8 (0.5-1.28)	1.6 (1-2.5) ‡
HDL < 40 mg/dL or medication use	1.5 (0.8-2.6)	0.8 (0.5-1.3)	1.38 (0.8-2.2)
Fasting blood glucose ≥ 110 mg/dL or medication use	0.5 (0.6-4.1)	0.8 (0.4-1.7)	1.1 (0.5-2.4)
BP ≥ 130/80 mmHg or medication use	0.7 (0.3-1.3)	1.1 (0.5-2.2)	0.9 (0.5-1.7)

* Odds ratio adjusted for age. Physical activity, education, smoking, waist circumference, fasting blood glucose, triglycerides, HDL-cholesterol, systolic and diastolic blood pressure. † $P<0.05$, ‡ $P<0.01$.

triglycerides, HDL cholesterol, waist circumference and BMI were significantly higher in men with metabolic syndrome compared with those without the condition. No differences were observed for physical activity, age, smoking, and education and fasting glucose.

Table 4. Odd ratio for developing metabolic syndrome, according to ATP III and IDF after a 6.5 year follow-up in 836 men in the lowest tertile of concentration of sex hormones at baseline

	Total testosterone in the lower tertile (nmol/L)	Free testosterone index in the lower tertile	SHBG in the lower tertile (nmol/L)
Metabolic syndrome according to ATP III			
Model 1	1.69(1.04-2.7)†	1.23 (0.7-1.9)	1.32 (0.8-2.7)
Model 2	1.7 (1.03-2.8)†	1.22 (0.7-2.07)	1.44 (0.87-2.4)
Model 3	1.7 (1.1-2.9)†	1.3 (0.7-2.16)	1.46 (0.8-2.4)
Model 4	1.37 (0.9-2.5)	1.54 (0.8-2.7)	0.95 (0.5-1.6)
Model 5	1.34 (0.8-2.3)	1.57 (0.9-2.7)	0.86 (0.5-1.5)
Metabolic syndrome according to IDF			
Model 1	1.77 (1.2-6)‡	0.8 (0.5-1.1)	2.3 (1.15-3.4)§
Model 2	1.92 (1.3-2.9)	0.8 (0.5-1.2)	2.44 (1.4-3.7)§
Model 3	2 (1.34-3)§	0.8 (0.5-1.2)	2.58 (1.64-4.05)§
Model 4	1.5 (0.9-2.3)	0.9 (0.6-1.5)	1.6 (1.02-2.6)‡
Model 5	1.45 (0.9-2.3)	1 (0.6-1.6)	1.5 (0.9-2.5)

* Data or ORs (95% CI).
Model 1: without adjusted.
Model 2: age-adjusted.
Model 3: Adjusted for age, physical activity, smoking education.
Model 4: Adjusted for the variables in model 3 and waist circumference. Model 5: Adjusted for the variables in model 4 and fasting glucose, triglycerides, systolic and diastolic blood pressure. †P<0.05,‡ P<0.01,
§ P<0.001

According to ATP III, participants with metabolic syndrome had lower levels of total testosterone (P=0.02); based on IDF criteria, they had lower levels of both total testosterone (P=0.002) and SHBG levels (P=0.001) (Table 2). In the fully adjusted analysis for age, smoking, physical activity, education, HDL cholesterol, triglycerides, fasting blood glucose, blood pressure and waist circumference, only serum SHBG in lower tertile was found to be negatively associated with triglycerides. According to both criteria, no significant association was found between total testosterone, free testosterone index and the individual components of metabolic syndrome (Table 3).

Associations between the metabolic syndrome and serum total testosterone, free testosterone index and SHBG at baseline are presented in Table 4. According to ATP III criteria, adjustment of waist circumference eliminated most of the correlations

between total testosterone and metabolic syndrome (OR=1.37, 95% CI=0.9-2.5) but it became significant when omitting waist circumference from the model 5, (OR=1.7, 95% CI = 1.04-2.9) (data not shown). SHBG and free testosterone index were not significantly associated with the syndrome. Based on IDF criteria, statistical adjustment of waist circumference eliminated most of the correlations between lower tertile of total testosterone and the metabolic syndrome (OR= 1.5, 95% CI = 0.9-2.3) whereas adjustment with triglycerides eliminated any correlation between SHBG and the metabolic syndrome (OR=1.5, 95% CI=0.9-2.5); the correlation between testosterone and metabolic syndrome however became significant when omitting triglycerides from the model 5 (not shown) [OR= 1.66, 95% CI (1.01-2.7), and the free testosterone index was not significantly associated with the syndromes.

DISCUSSION

This data from a group of Tehranian males show that adjustment of waist circumference eliminated most of the correlations between total testosterone and metabolic syndrome, whereas, linear regression analysis revealed significant association between SHBG levels and high triglycerides. The results suggest that waist circumference and triglycerides may be important confounders for the correlation between plasma sex hormones and metabolic syndrome. In cross-sectional studies, SHBG levels are also inversely correlated with obesity (14,15). In the Massachusetts Male Aging Study, after 9 years of follow-up, obesity was associated with decreased levels of total and free testosterone and SHBG(14).A Canadian study examined the relationship between steroid hormones and lipoprotein level by taking into account the concomitant variation in visceral adipose tissue accumulation measured by computed tomography. Results showed that despite the significant contribution of visceral adipose tissue and metabolic variables, SHBG appeared to be a significant independent correlate of plasma lipoprotein levels in men (16). Using radio labeled oleic acid, Mårin *et al.* found that triglyceride turnover is high in subcutaneous abdominal adipose tissue, testosterone administration increased triglyceride turnover and lipoprotein lipase activity and caused a more rapid turnover of triglycerides in the tissue mentioned, results which demonstrated the marked effects of testosterone on adipose tissue metabolism (17).

In our study, based on both criteria, after adjustment for waist circumference, the association between sex hormones and incident metabolic syndrome did not predict incident metabolic syndrome, a finding in agreement with the Laaksonen *et al.* study, which demonstrated that the association of insulin concentration with subsequent of metabolic syndrome was not significant after adjustment with waist circumference and became significant when omitting waist circumference (8).

Kaplan *et al.* reported that in

patients with and without the metabolic syndrome, testosterone levels decrease significantly with increase in BMI. However, mean testosterone levels, were 10.4 nmol/L and lower in severely obese men than in aging, lean men without the metabolic syndrome. Diabetes or impaired fasting glucose, BMI ≥ 30 kg/m^2, and triglycerides ≥ 150 mg/dL, but not the other components of the metabolic syndrome such as hypertension or HDL-cholesterol<40mg/dL had clinically significant associations with low serum testosterone level. This study provided evidence that increased body weight in men with the metabolic syndrome resulted in lower testosterone levels (18).

Niskanen *et al.* showed that 58 abdominally obese men with the metabolic syndrome, but not necessarily androgen deficiency, placed on a very low-calorie diet for a 9 week period had rapid weight loss (average weight loss of 15 kg) that was sustained after a 12 month period and resulted in significant increase in SHBG and free testosterone levels (19).

Interventional studies of exogenous testosterone supplementation in men with low serum testosterone levels increased lean body mass and decreased fat mass, total cholesterol and low density lipoprotein (20,21). In contrast the Finland study demonstrated that after adjustment for potential confounders, men with total testosterone and SHBG levels in the lower fourth quartile had an increased risk for developing metabolic syndrome (OR=1.7, 95% CI=1.07-2.7 and 1.67, 1.05, 2.62, respectively) (8).

Nevertheless, to justify the minor differences in our results, one reason could be the difference in race and ethnicity. The Boston Area Community Health Survey showed that the odds of metabolic syndrome increased about two-fold for 1 standard deviation decrease in hormone levels and the magnitude of this association was the largest among white men (22).A second reason could be the socioeconomic differences. A recent study in USA showed that after adjusting for age, cardiovascular disease, diabetes, depression, smoking, physical activity, and alcohol , men of a low socioeconomic status had an over two fold increase in risk of erectile dysfunction (23).Third reason could be due to our subjects being overweight (Mean BMI about 26), not obese. Osuna *et al.* reported total testosterone and SHBG concentration were lower in the obese group compared with normal or overweight subjects (14).

Although the waist circumference is simple and convenient for epidemiological studies and provides a useful estimation of the proportion of abdominal or upper-body fat, most of studies reported, low testosterone concentration correlated with greater visceral fat accumulation (15- 24 – 25).

We did not find any relationship between testosterone Index and the risk of metabolic syndrome. It has also been hypothesized that obesity may result in hypogonadism and insulin resistance that may contribute to this scenario. *In vitro* studies in human hepatoma cell lines have demonstrated that decreased SHBG production decreases in the setting of insulin resistance suggesting that in fact SHBG may act as a surrogate marker for insulin resistance (26).

According to experimental studies, metabolic syndrome can lead to development of hypogonadism by various mechanisms including decreased basal

and pulsatile luteininzing hormone secretion, increased sensitivity to negative feedback of androgens (27, 28), suppression of Leydig cell testosterone production (29), increased levels of cytokines, especially IL-6 , TNF α (30) and increase in the enzymes with aromatized activity in fat tissues (31, 32).

Furthermore, in the present study, following adjustment of models, cut points of both lower total testosterone and SHBG levels among 836 men, aged 20-80 years, were 10.2nmol/L and 2.37nmol/L, respectively, similar to findings of another study, which reported the lower total testosterone cut point as being < 11 nmol/L (33).

Of the limitations of our study, one is the single measurement of both sex hormones which reflects long term average less precisely than repeated measurements and the other is that calculated measures of bioavailability and free testosterone were used rather than direct measures. The apparent free testosterone concentration obtained by equilibrium dialysis at 37 C is probably the physiologically most representative method and the most exact for estimating free testosterone. An alternative to the use of labeled testosterone is direct measurement of testosterone in the dialysis. Moreover, there is good evidence that free testosterone is a reliable index of free testosterone (34, 35, 36). Strengths of the present study include the fact that this study is the first to assess the association between circulating sex hormone levels and the presence of metabolic syndrome in a large population of Iranian men, with a wide age range.

In conclusion, we can consider high triglycerides and adiposity are dominant risk factors for developing hypogonadism and indicating the essentiality of life style and dietary interventions to prevent hypogonadism.

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Conflict of interest.

The authors did not report any conflict of interest.

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