The association of serum testosterone and sex hormone-binding globulin with obese men and type 2 diabetic men

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Abstract

Objective: The obesity and type 2 diabetes are the most common diseases in our time; therefore we decided to study the association between these diseases and the levels of serum testosterone and sex hormone-binding globulin as sexual biomarkers for men.

Material and methods: Ninety men aged from 45-60 years old were included in this study and were divided into 3 groups included 30 normal used as control, 30 obese and 30 with type 2 diabetes. Lipid profiles, fasting glucose, glycated hemoglobin and insulin as well as total testosterone and sex hormone-binding globulin were measured.

Results: We found that serum total testosterone and sex hormone-binding globulin concentrations were lower significantly in obese men and in men with type 2 diabetes than in normal ones after adjustment for age and body mass index. Also total testosterone and sex hormone-binding globulin concentrations showed significant and positive correlation with high density lipoprotein-cholesterol concentration and a significant and negative correlation with low density lipoprotein-cholesterol, glucose, glycated hemoglobin, insulin, triacylglycerol and cholesterol concentrations after adjustment for age and body mass index. **Conclusion:** Our data indicate that lower total testosterone and sex hormone-binding globulin concentrations were associated with unfavorable lipid profiles and insulin resistance and was a strong predictor of the risk of type 2 diabetes and obesity in aged men.

Keywords: Testosterone; Sex hormone-binding globulin; Obesity; Type 2 diabetes**.**

Abbreviations: BMI, body mass index; ELISA, enzyme-linked immunosorbent assay; HbA1c, glycated hemoglobin; HDL-C, high density lipoprotein-cholesterol; IR, insulin resistance; LDL-C, low density lipoprotein-cholesterol; MMAS, Massachusetts Male Aging Study; MetS, metabolic syndrome; SHBG, sex hormone-binding globulin; TAG, triacylglycerol; T2D, type 2 diabetes; Tt, total testosterone; WHO, world health organization.

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Introduction

It has been suggested that obesity is associated with testosterone in men[1]. Also it was found that total testosterone (Tt) in men is affected by excessive body weight compared to men with a normal body mass index (BMI)[2]. Indeed, one of intervention studies has confirmed that both diet -and surgically- induced weight losses are associated with improvement in testosterone levels[3].

Serum sex hormone-binding globulin (SHBG) has been reported to be closely associated with lipid profiles. In men, SHBG were postulated to be affected by unfavorable lipid levels[4]. Also in male adults, it has been shown that SHBG were associated with BMI and abdominal circumference[5].

It has been suggested that testosterone levels in men may be linked to the development of a number of the clinical characteristics associated with type 2 diabetes (T2D) and metabolic syndrome (MetS) which is defined as a cluster of insulin resistance (IR), hyperglycemia and visceral obesity[6]. Furthermore, it has been suggested that Tt levels are associated with insulin concentration and IR in men with T2D[7].

It has been reported that there was a relationship between serum SHBG and T2D where it was postulated that serum levels of SHBG may be affected unfavorably by the risk of T2D in men, and this unfavorable SHBG levels may precede the development of impaired glucose metabolism[8]. So the purpose of this study is to evaluate the serum levels of Tt and SHBG in obese men and type 2 diabetic men as sexual biomarkers and to know the effect of these diseases on its concentrations.

Material and Methods

Study population

Ninety men their ages ranged from 45-60 years old were selected from the outpatient's department of Menoufiya University hospital and were classified into 3 groups. First one was 30 healthy men referred to as control; second group was 30 obese men with BMI ranged between 30-34.9 kg/m^2. According to world health organization (WHO) criteria[9], obesity was classified as class I for a BMI between 30 and 34.9 associated with a moderate risk. While third group was 30 type 2 diabetic men. According to WHO criteria[10], diabetes was defined by either fasting plasma glucose levels ≥126 mg/dL, 2-h post-load glucose levels ≥200 mg/dL after a 75 g oral glucose tolerance test, or diabetes diagnosed by physicians. Subjects with Hypogonadism, Hypopituitarism, renal or hepatic failure and those who used exogenous hormone, opium or medication which might affect sex hormone level were excluded from the study. The principles laid down in the Declaration of Helsinki have been complied with. Also this study has been approved by the relevant local ethics committee, and all the subjects have given informed consent to participate in the research.

Anthropometric and Biochemical Measurements

BMI was calculated as the weight divided by the squared height (kg/m²). After overnight fasting, venous blood samples were collected between 8 and 10 AM. Blood samples were collected in tubes containing EDTA for measuring fasting plasma glucose and glycated hemoglobin (HbA1c) levels, the remaining frozen serum samples were sent to laboratory and were stored in deep freezers at −70°C until used for measuring cholesterol, triacylglycerol (TAG), high density lipoprotein-cholesterol (HDL-C), insulin, Tt and SHBG.

Fasting plasma glucose level was measured by the glucose oxidase method using a commercial kit (Spinreact, Santa Coloma, Sant Esteve De Bas, Spain). HbA1c level was measured by quantitative colorimetric method using a commercial kit (Stanbio Glycohemoglobin, Boerne, Texas, USA). Serum cholesterol and TAG levels were measured enzymatically using a commercial kit (Spinreact, Santa Coloma, Sant Esteve De Bas, Spain). Serum HDL-C level was measured after precipitation of apolipoprotein B-containing lipoproteins with phosphotungstic acid and Mg using a commercial kit (Spinreact, Santa Coloma, Sant Esteve De Bas, Spain). Serum LDL-C level was calculated by Friedewald's formula[11]. Serum insulin concentration was measured by enzyme-linked immunosorbent assay (ELISA) using a commercial kit (DRG Insulin ELISA ,Gmbh, Germany), sensitivity 1.76 µIU/ml, intraassay CV 1.8–2.6% and interassay CV 2.9–6.0%. Serum Tt concentration was measured by ELISA using a commercial kit (DRG Testosterone ELISA ,Gmbh, Germany), sensitivity 0.083 ng/ml, intraassay CV 3.28–4.16% and interassay CV 4.73–9.94%. Serum SHBG concentration was measured by ELISA using a commercial kit (DRG SHBG ELISA ,Gmbh, Germany), sensitivity 0.77 nmol/l, intraassay CV 4.0–9.0% and interassay CV 3.1–8.0%.

Statistical analysis

Statistical analyses were performed using the SPSS software package, version 20 on IBM compatible computer. Quantitative data were expressed as mean & standard deviation (X±SD) and analyzed by applying Anova test for comparison of three groups of normally distributed variables and three groups of not normally distributed variables by applying Kruskal-Wallis test; Post Hoc test was used to obtain comparison between every two groups (multiple comparison tests).

Spearman correlation was used to assess relationship between not normally distributed quantitative variables. P-value at 0.05 was used to determine significance regarding: P-value > 0.05 to be statistically insignificant, P-value ≤ 0.05 to be statistically significant and P-value ≤ 0.001 to be high statistically significant.

Results and Discussion

The present study highlighted two major observations: obesity and T2D in men aged from 45-60 years old were associated with an overall reduction in Tt and SHBG levels; this decreasing in Tt and SHBG levels was associated with unfavorable lipid profiles, hyperglycemia and IR which are components of MetS.

Multiple cross-sectional studies have consistently found negative linear correlations between Tt levels and adiposity in men[12]. Multiple observational studies in community-dwelling men suggested that obesity leads to decrease testosterone. In the prospective Massachusetts Male Aging Study (MMAS), moving from a non-obese to an obese state resulted in a decline of testosterone levels comparable to that of advancing 10 years in age[13]. Similar findings have been reported in cohort studies of men from Europe[14] and Australia[15].

Another study showed that Tt and SHBG correlated inversely and significantly with BMI suggesting that the decline of testosterone and SHBG could be partly explained by the increase in central and overall obesity[13, 16].

Our results were in agreement with those previous studies where we showed that there was a significant decrease in testosterone level in obese group compared to control group (Table 1, Fig. 1) and also showed a significant decrease in SHBG level in obese group compared to control group (Table 1, Fig. 2).

Table 1 Comparison of biochemical parameters studied in three groups (obese, type 2 diabetic and control)

Biochemical parameters	Control group (n=30)	Obese group (n=30)	Type 2 diabetic group (n=30)	P value	Post Hoc test
Testosterone (ng/ml)	7.56 ± 1.19	4.64± 1.90	4.94± 2.13	< 0.001	P1 0.52 P2 < 0.001 P3 < 0.001
SHBG (nmol/l)	41.22 ± 7.16	18.97 ±7.96	24.32± 8.55	< 0.001	P1 0.01 P2 < 0.001 P3 < 0.001
Insulin (µIU/ml)	11.79± 2.07	26.54±8.71	27.38± 5.99	< 0.001	P1 0.60 P2 < 0.001 P3 < 0.001
TAG (mg/dl)	138.65 ± 1.08	183.76 ± 1.49	182.44 ± 2.84	< 0.001	P1 0.02 P2 < 0.001 P3 < 0.001
Cholesterol (mg/dl)	180.04 ± 1.16	232.19 ±1.42	230.92 ± 2.06	< 0.001	P1 0.007 P2 < 0.001 P3 < 0.001
HDL-C (mg/dl)	47.02± 0.74	42.07± 1.51	43.04± 0.92	< 0.001	P1 0.003 P2 < 0.001 P3 < 0.001
LDL-C (mg/dl)	94.75± 0.64	123.09 ± 1.66	122.11± 1.56	< 0.001	P1 0.02 P2 < 0.001 P3 < 0.001
Glucose (mg/dl)	95.21± 9.65	100.29 ±8.73	153.14 ±5.72	< 0.001	P1 < 0.001 P2 0.03 P3 < 0.001
HbA1C (%)	5.00 ± 0.39	5.63 ± 1.19	7.60 ± 0.59	< 0.001	P1 < 0.001 P2 0.007 P3 < 0.001

[a] Note P1: between obese group and type 2 diabetic group, P2: between obese group and control group, P3: between type 2 diabetic group and control group. Where P value is non significant (NS) at P-value > 0.05, significant (S) at P-value ≤ 0.05 and highly significant (HS) at P-value ≤ 0.001.

Table 1 showed the comparative study between three groups (obese, type 2 diabetic and control) regarding studied biochemical parameters levels. Post Hoc test was also done to show statistical difference between every two groups of them apart.

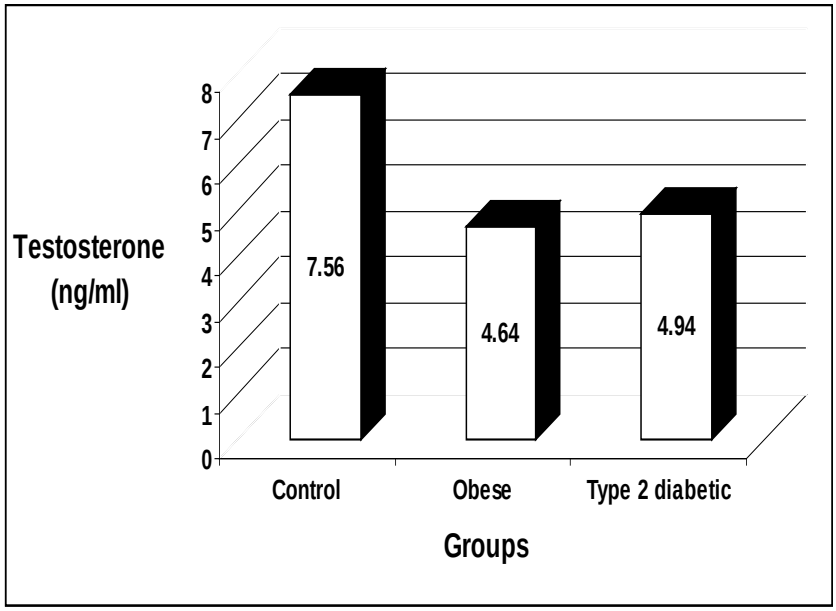

Figure 1 Testosterone level in patients and control groups. Testosterone level was significantly reduced in obese group and type 2 diabetic group compared to control group with (P value < 0.001) while there was non significant difference between obese group and type 2 diabetic group with (P value = 0.52).

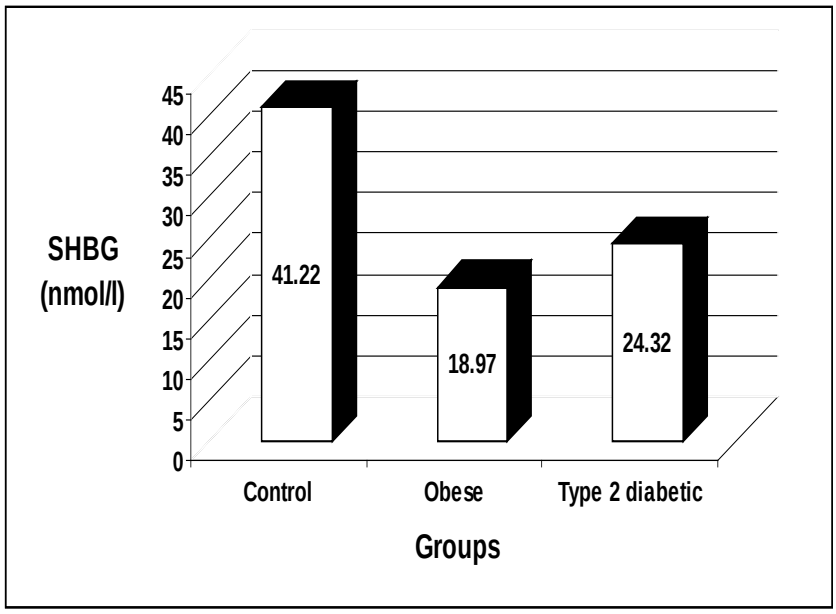

Figure 2 SHBG level in patients and control groups. SHBG level was significantly reduced in obese group and type 2 diabetic group compared to control group with (P value < 0.001) and there was significant reduced in obese group compared to type 2 diabetic group with (P value = 0.01).

A cross-sectional study of 314 Chinese men found that older obese men (defined by BMI >28 kg/m2 for these Asian men) had a lower testosterone level compared to age-matched lean men[17]. Our results supported this study especially that this study took the same range of BMI as in our research.

From analyses to our study, we found that lower levels of Tt and SHBG were associated with unfavorable lipid profile as shown in (Table 1) which showed significant decrease in Tt and SHBG level in obese group compared to control group and this reduction appeared to be associated with significant increase in TAG, cholesterol, LDL-C levels and with significant decrease in HDL-C level. Also we found that Tt and SHBG correlated directly with HDL-C and inversely with TAG, cholesterol and LDL-C as shown in (Tables 2, 3) and all these relationships were statistically significant.

Table 2 Relationship between testosterone level and other measured biochemical parameters

Other biochemical parameters	Testosterone (ng/ml)	
	r	P value
SHBG (nmol/l)	0.40	< 0.001
Insulin µIU/ml	- 0.50	< 0.001
TAG (mg/dl)	- 0.56	< 0.001
Cholesterol (mg/dl)	- 0.47	< 0.001
HDL-C (mg/dl)	0.41	< 0.001
LDL-C (mg/dl)	- 0.55	< 0.001
Glucose (mg/dl)	- 0.46	< 0.001
HbA1c (%)	- 0.31	0.003

[b] Note P value is non significant (NS) at P-value > 0.05, significant (S) at P-value ≤ 0.05 and highly significant (HS) at P-value ≤ 0.001.

Table 2 showed the relationship between testosterone level and other measured studied biochemical parameters.

Table 3 Relationship between SHBG level and other measured biochemical parameters

Other biochemical parameters	SHBG (nmol/l)	
	r	P value
Insulin µIU/ml	- 0.59	< 0.001
TAG (mg/dl)	- 0.74	< 0.001
cholesterol (mg/dl)	- 0.75	< 0.001
HDL-C (mg/dl)	0.70	< 0.001
LDL-C (mg/dl)	- 0.76	< 0.001
Glucose (mg/dl)	- 0.46	< 0.001
HbA1c (%)	- 0.35	0.001

[c] Note P value is non significant (NS) at P-value > 0.05, significant (S) at P-value ≤ 0.05 and highly significant (HS) at P-value ≤ 0.001.

Table 3 showed the relationship between SHBG level and other measured studied biochemical parameters.

These analyses to our study were in line with results of other studies. One of these studies has demonstrated increased serum TAG and insulin[18] and decreased HDL-C concentrations[19] in men with low Tt or SHBG levels. Other showed a positive correlation between serum testosterone and HDL-C in healthy and diabetic men[20].

The mechanisms of how testosterone and SHBG affect the lipid profile are not completely understood. Low levels of testosterone are associated with abdominal obesity, which leads to increased aromatase activity and hence more testosterone is converted to estradiol[21]. This lower level of testosterone may lead to increased activity of lipoprotein lipase, causing an increased fatty acid uptake and TAG storage in adipocytes. This, in turn, results in an increase in fat mass, which correlates with increased IR[22]. In addition, decreasing SHBG level is associated with higher serum insulin level resulting from the risk for insulin resistance[23].

The association between low levels of testosterone and diabetes mellitus has recently received substantial attention[24]. A systematic review and meta-analysis by Ding et al. revealed associations between low testosterone level and both incident and prevalent diabetes[25]. These findings were in

consistent with our results as shown in (Table 1) and (Fig. 1) which showed significant decrease in testosterone level in type 2 diabetic group compared to control group and this reduction appeared to be associated with significant increase in glucose level and also with significant increase in HbA1c level.

Few studies have examined the associations between androgens and HbA1c in men. In 391 elderly men, a negative correlation between Tt and HbA1c was found[26]. Fernandez-Real et al. also reported a negative correlation between SHBG and HbA1c in 39 men with various degrees of obesity and glucose tolerance[27]. Our results supported these studies as shown in (Tables 2, 3) which showed that testosterone and SHBG levels are inversely correlated with HbA1c levels, and these relationships were statistically significant.

Cross-sectional studies have reported an association between low serum SHBG concentration and an increased risk of development of T2D. Of these, 23 cross-sectional studies were included in a 2006 systematic review and meta-analysis, which reported that a higher concentration of SHBG was associated with a lower risk of development of T2D[25]. Our results are in agreement with findings of these studies as shown in (Table 1) and (Fig. 2) which showed significant decrease in SHBG level in type 2 diabetic group compared with control group.

Low testosterone is associated with impaired insulin sensitivity which in turn was strongly related to MetS[28]. A systematic review and meta-analysis supported that endogenous Tt was lower in subjects with MetS compared with those without[29]. Also lower levels of Tt and SHBG were associated with MetS in Korean men, and this negative relationship of sex hormones and MetS is also proved in another study[30]. So the low Tt and SHBG might be better androgen biomarkers for diabetes and MetS and this suggestion consistent with another study[31].

Osuna et al. also revealed that SHBG and Tt were negatively correlated with insulin levels[23]. This result was in agreement with our results as shown in (Table 1) which showed significant decrease in Tt and SHBG levels in type 2 diabetic group compared to control group and this reduction appeared to be associated with significant increase in insulin level. Also (Tables 2, 3) revealed that Tt and SHBG levels were inversely correlated with insulin levels and these relationships were statistically significant.

Regarding the relationship between insulin resistance and SHBG, it has been documented that insulin is an important regulator of SHBG production in the liver. Pasquali et al. showed that inhibition of insulin secretion by means of diazoxide induces an increase in SHBG levels, both in obese men and in men with normal body weights[32]. Thus, it has been proposed that SHBG levels could be a valuable marker of insulin resistance or hyperinsulinemia in humans.

Conclusions

In conclusion, we showed that Tt and SHBG concentrations were lowered in obese men and type 2 diabetic men and these lowered levels could be considered as good biomarkers of the risk of T2D and obesity in aged men.

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