CLINICAL STUDY

Lower sex hormone-binding globulin is more strongly associated with metabolic syndrome than lower total testosterone in older men: the Health in Men Study

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Abstract

Background: Reduced circulating testosterone and sex hormone-binding globulin (SHBG) are implicated as risk factors for metabolic syndrome. As SHBG increases with age while testosterone declines, we examined the relative contributions of SHBG and testosterone to the risk of metabolic syndrome in older men.

Methods: We conducted a cross-sectional study of 2502 community-dwelling men aged ≥ 70 years without known diabetes. Metabolic syndrome was defined using the National Cholesterol Education Program-Third Adult Treatment Panel (NCEP-ATPIII) criteria. Early morning fasting sera were assayed for total testosterone, SHBG and LH. Free testosterone was calculated using mass action equations.

Results: There were 602 men with metabolic syndrome (24.1%). The risk of metabolic syndrome increased for total testosterone < 20 nmol/l, SHBG < 50 nmol/l and free testosterone < 300 pmol/l. In univariate analyses SHBG was associated with all five components of metabolic syndrome, total testosterone was associated with all except hypertension, and free testosterone was associated only with waist circumference and triglycerides. In multivariate analysis, both total testosterone and especially SHBG remained associated with metabolic syndrome, with odds ratios of 1.34 (95% confidence interval (CI): 1.18–1.52) and 1.77 (95% CI: 1.53–2.06) respectively. Men with hypogonadotrophic hypogonadism (total testosterone < 8 nmol/l, LH ≤ 12 IU/l) had the highest prevalence of metabolic syndrome (53%, $P < 0.001$).

Conclusions: Lower SHBG is more strongly associated with metabolic syndrome than lower total testosterone in community-dwelling older men. SHBG may be the primary driver of these relationships, possibly reflecting its relationship with insulin sensitivity. Further studies should examine whether measures that raise SHBG protect against the development of metabolic syndrome in older men.

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Introduction

Cardiovascular disease (CVD) and reduced circulating testosterone are common afflictions of older men. Significant risk factors for atherosclerosis, the most common underlying process in CVD, include obesity, insulin resistance and dyslipidaemia, often described as the metabolic syndrome. Whether the age-related decline in circulating testosterone contributes to these risk factors is an important question because the answer may provide a rationale for testosterone therapy in many men. Nevertheless, the evidence for such intervention is contradictory (1–4).

A number of studies have shown that serum testosterone levels are related to insulin resistance (5–7), other features of the metabolic syndrome (8–11) and CVD (12, 13). However, most (~98%) circulating testosterone is reversibly bound to albumin and sex hormone-binding globulin (SHBG). Thus, the unbound fraction of testosterone is dependent on the concentrations of these proteins as well as the total testosterone. It has therefore been hypothesised that unbound, or 'free' testosterone and non-SHBG-bound ('bioavailable') testosterone may be better reflections of prevailing testosterone biological activity than the total testosterone (14). It is also clear that the concentration

of SHBG in serum is modulated by insulin concentrations or insulin resistance, such that higher insulin concentrations or insulin resistance are associated with lower SHBG concentrations (15, 16). This relationship may well confound the relationship between total testosterone and metabolic syndrome. Of those studies that have examined both total and free or bioavailable testosterone (5, 6, 8, 9, 11–13), only four (6, 8, 9, 12) have found significant associations between free or bioavailable testosterone and aspects of the metabolic syndrome.

It is well recognised that with advancing age SHBG increases, while free testosterone declines more rapidly than total testosterone (17–19). Thus, SHBG should be considered together with changes in total or free testosterone to determine the most relevant measures of androgen status in older men. Clarifying whether free testosterone concentration provides any advantage over measurement of total testosterone, and whether a strong association exists between SHBG and metabolic syndrome independently of testosterone, could help to identify older men at risk of CVD. To illuminate the associations between androgen variables and the metabolic syndrome during male ageing, we studied these features in a large sample of community-dwelling older men.

Subjects and methods

Study participants

The Health in Men Study is a prospective follow-up of older men who participated in a trial of screening for abdominal aortic aneurysms (20). Briefly, between April 1996 and January 1999, community-dwelling men resident in Perth, Western Australia, aged 65–83 years were randomly selected from the electoral roll and invited to take part, enrolment to vote being compulsory for Australian citizens. Of the 17 432 eligible men, 12 203 (70%) participated, completing a risk factor questionnaire covering aspects of medical history and lifestyle relevant to CVD. Between October 2001 and August 2004, surviving men were invited to participate in a follow-up study. Of ~9000 surviving men, 4263 (representing 35% of those initially screened) completed a follow-up visit that included another health questionnaire, and a single early morning blood sample. Of these, sera were available for assay of hormone status in 4165 men. From these subjects we excluded men taking testosterone replacement, non-fasting men, those being treated with androgen deprivation therapy, those with prostate cancer and those reporting diabetes or the use of glucose-lowering drugs ($n=1663$), leaving 2502 eligible subjects with fasting blood samples. Height (in centimetres), weight (in kilograms) and blood pressure were measured using standard procedures. The Human

Research Ethics Committee of the University of Western Australia approved the study protocol.

Laboratory assays

Blood samples were collected between 0800 and 1030 h after the subjects had fasted overnight. Serum was processed immediately and stored at -80°C until assayed. Serum total testosterone, SHBG and luteinizing hormone (LH) were determined by chemiluminescent immunoassays on an Immulite 2000 analyzer (Diagnostic Products Corp-Biomediq, Doncaster, Australia). Between-day imprecision (coefficient of variation) was 11.2 and 8.9% at 7.2 and 18 nmol/l, 6.7 and 6.2% at 5.2 and 81 nmol/l, and 6.4 and 5.8% at 2.3 and 19 IU/l for testosterone, SHBG and LH respectively. The working range of the testosterone assay was 0.7–55 nmol/l; the sensitivities of the SHBG and LH assays were 2 nmol/l and 0.1 IU/l respectively. Free testosterone, specifically the portion not bound to either SHBG or albumin, was calculated from total testosterone and SHBG using mass action equations as described by Vermeulen *et al.* (21). Fasting serum glucose, total and high-density lipoprotein (HDL) cholesterol and triglycerides were estimated using a Roche Hitachi 917 analyzer (Roche Diagnostic GmbH). Between-day imprecision was 2.9 and 2.2% at 4.8 and 15.2 mmol/l, 2.3 and 2.1% at 3.2 and 6.7 mmol/l, 2.4 and 2.5% at 0.8 and 1.7 mmol/l, and 4.8 and 2.4% at 0.9 and 2.0 mmol/l for glucose, cholesterol, HDL and triglycerides respectively.

Definition of metabolic syndrome

Metabolic syndrome was defined according to the NCEP-ATPIII classification as three or more of: waist circumference >102 cm, fasting plasma glucose ≥ 5.6 mmol/l, fasting serum triglycerides ≥ 1.7 mmol/l, HDL cholesterol <1.03 mmol/l and blood pressure $\geq 130/85$ mmHg (22). We used the most recent modification of the definition, which lowered the threshold for an abnormal glucose concentration to ≥ 5.6 mmol/l (23). Men with a previous diagnosis of hypertension or taking antihypertensive therapy were regarded as fulfilling the criterion for hypertension.

Exclusion of men with known diabetes

Some of the men in the original cohort with a diagnosis of diabetes had type 1 diabetes rather than type 2 and this information was not specifically recorded. Furthermore, fasting triglyceride and HDL concentrations were not available for the substantial fraction of men with diabetes who were not fasting at the time of blood sampling. For these reasons we excluded all men with known diabetes from the study.

Classification of hypogonadal men

Men were classified as having primary hypogonadism if their total testosterone was <8 nmol/l and LH >12 IU/l, Leydig cell impairment if total testosterone was 8–15 nmol/l and LH >12 IU/l, and hypogonadotrophic hypogonadism if total testosterone was <8 nmol/l and LH ≤ 12 IU/l (24).

Statistical analysis

Data were analysed with the statistical package Stata, version 10.0 (StataCorp, College Station, Texas, USA 2007). Participants were divided into two groups according to whether they met the criteria for metabolic syndrome. We used *t*-tests to compare differences between the groups with regard to body composition, blood pressure, fasting glucose, SHBG, total and free testosterone. The Mann–Whitney *U* test was used to assess the remaining continuous variables as these were not normally distributed, and categorical variables were compared with the Pearson χ^2 test. Binary logistic regression analyses were undertaken to explore hormonal associations with the metabolic syndrome and its components, and to obtain a graphical representation of the predicted probabilities of metabolic syndrome for a given hormone level. Because the relationship between all hormones and the metabolic syndrome did not appear to be linear, spline functions were applied to the analyses. SHBG appeared to be most strongly associated with the metabolic syndrome and its components, and was entered into a multivariate model with total testosterone to determine whether the association remained after adjustment. Variables were adjusted for each other, age and the square of testosterone and SHBG to account for linear and non-linear relationships. All odds ratios (ORs) indicate the incremental risk associated with a one-unit decrease in Z score. $P < 0.05$ were considered statistically significant.

Results

Characteristics of study participants with and without metabolic syndrome

The characteristics of the 2502 subjects, stratified as to their metabolic syndrome status, are shown in Table 1. The prevalence of metabolic syndrome was 24.1%. There was no significant difference in the ages of the men with and without metabolic syndrome.

Men with metabolic syndrome had significantly lower mean serum testosterone, calculated free testosterone and SHBG than those without metabolic syndrome ($P < 0.001$ for each). The difference in LH between the two groups of men was not statistically significant.

Probability of metabolic syndrome according to hormone status

The relationships between the probability of having metabolic syndrome and total and calculated free testosterone, SHBG and LH are shown in Fig. 1. The risk increased markedly at total testosterone <20 nmol/l and SHBG <50 nmol/l. For calculated free testosterone, there was increased risk below 300 pmol/l, but the increase in risk was much less marked than for total testosterone. There was a small increase in risk as LH fell below 4 IU/l.

Risk of individual components of the metabolic syndrome according to hormone status

Table 2 shows the prevalence of each of the components of the metabolic syndrome in this sample of men, while Table 3 shows the increase in risk of each metabolic syndrome component for one-unit decrease in Z score in the hormone marker. For SHBG, the odds ratios were significantly increased for each component, and for total

Table 1 Characteristics of 2502 older men with and without metabolic syndrome.

	MetS – ve (<i>n</i> =1900)	MetS + ve (<i>n</i> =602)	<i>P</i> value
Age (years)	76.45 (74.16–79.22)	76.22 (74.25–78.84)	0.226
BMI (kg/m ²)	25.36 $\pm$ 3.03	29.03 $\pm$ 3.59	<0.001
Waist (cm)	95.45 $\pm$ 8.33	106.34 $\pm$ 8.35	<0.001
WHR	0.95 $\pm$ 0.06	1.01 $\pm$ 0.07	<0.001
Systolic BP (mmHg)	146.0 $\pm$ 20.0	150.3 $\pm$ 19.2	<0.001
Diastolic BP (mmHg)	74.0 $\pm$ 10.1	76.5 $\pm$ 10.0	<0.001
Glucose (mmol/l)	5.29 $\pm$ 0.52	5.80 $\pm$ 0.88	<0.001
TG (mmol/l)	1.0 (0.7–1.3)	1.7 (1.2–2.2)	<0.001
HDL (mmol/l)	1.4 (1.3–1.7)	1.1 (1.0–1.4)	<0.001
LDL (mmol/l)	3.0 (2.4–3.5)	3.0 (2.4–3.5)	0.787
Total cholesterol (mmol/l)	5.0 (4.3–5.6)	5.0 (4.3–5.7)	0.280
Total testosterone (nmol/l)	16.7 $\pm$ 5.7	14.0 $\pm$ 4.9	<0.001
Free testosterone (pmol/l)	291.2 $\pm$ 90.8	274.6 $\pm$ 88.2	<0.001
SHBG (nmol/l)	45.5 $\pm$ 17.0	36.8 $\pm$ 14.0	<0.001
LH (IU/l)	4.27 (2.97–6.41)	4.07 (2.77–6.20)	0.059

All values are expressed as mean $\pm$ s.d., except for age, lipids and LH, which are median (interquartile range). BMI, body mass index; WHR, weight height ratio; BP, blood pressure; TG, triglycerides; HDL, high density lipoprotein; LDL, low density lipoprotein; SHBG, sex hormone-binding globulin; LH, luteinizing hormone.

Figure 1 Relationships between probability of metabolic syndrome and total and free testosterone, sex hormone-binding globulin SHBG and luteinizing hormone LH concentrations in 2502 older men.

testosterone this was true for all components except hypertension. For free testosterone, however, the ORs were significantly increased only for waist measurement > 102 cm and serum triglycerides ≥ 1.7 mmol/l.

Multivariate analysis

We performed multivariate logistic regression analysis to determine whether total testosterone or SHBG was more strongly associated with metabolic syndrome. Table 4 shows that in both univariate and multivariate analyses, lower SHBG was more strongly associated with metabolic syndrome than lower total testosterone, but that both these markers remained associated in the multivariate analysis.

Risk of metabolic syndrome in hypogonadal men

Table 5 shows that men characterised as having Leydig cell impairment did not have an increased risk of metabolic syndrome. Men with hypergonadotrophic hypogonadism had a higher risk of metabolic syndrome, which was not statistically significant. By contrast, those with hypogonadotrophic hypogonadism had a significantly higher risk of metabolic syndrome. The number of subjects in these groups, especially the

second, is small which would reduce the power available to detect a significant association.

Discussion

We found that both SHBG and total testosterone were significantly and independently associated with metabolic syndrome in community-dwelling non-diabetic older men, but that SHBG had stronger relationships with the metabolic syndrome and each of its components when compared with total testosterone. Other studies in men spanning from middle to old age found that associations of lower total testosterone and lower SHBG with metabolic syndrome were comparable in magnitude (8, 9, 11, 25). The study by Maggio *et al.* (26) showed inverse correlations between metabolic

Table 2 Distribution of components of the metabolic syndrome among 2502 older men.

Component	n	%
Waist circumference > 102 cm	787	31.5
Triglycerides ≥ 1.7 mmol/l	464	18.6
HDL < 1.03 mmol/l	335	13.4
Hypertension ^a	2195	88.5
Glucose ≥ 5.6 mmol/l	811	32.4

^aSystolic BP ≥ 130 mmHg, diastolic BP ≥ 85 mmHg, ever diagnosed with hypertension, or treatment for hypertension.

Table 3 Univariate associations between a one-unit decrease in Z score for total and free testosterone, sex hormone-binding globulin (SHBG) and presence of the components of the metabolic syndrome.

	Waist circumference > 102 cm		Triglycerides ≥ 1.7 mmol/l		High density lipoprotein < 1.03 mmol/l		Hypertension		Glucose ≥ 5.6 mmol/l	
	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
SHBG	1.66	1.50–1.85	1.94	1.69–2.22	1.51	1.31–1.75	1.17	1.05–1.31	1.21	1.10–1.32
Total testosterone	1.69	1.53–1.87	1.69	1.50–1.91	1.28	1.13–1.46	1.10	0.98–1.24	1.21	1.10–1.32
Free testosterone	1.29	1.17–1.41	1.20	1.08–1.34	1.00	0.89–1.12	1.00	0.89–1.14	1.07	0.99–1.17

syndrome and both total testosterone and log SHBG specifically in older men, with the latter reaching a greater level of statistical significance. Our results, showing a stronger association with metabolic syndrome for SHBG than for total testosterone are consistent with these studies. These observations suggest that the apparent association between testosterone and metabolic syndrome is secondary to the association between low SHBG and metabolic syndrome. Thus, interventions aimed at preventing metabolic syndrome in older men might be better directed at measures that increase SHBG rather than preventing or treating reduced total testosterone.

By contrast, calculated free testosterone was not independently associated with metabolic syndrome. Previous studies in which lower bioavailable testosterone (6, 8, 12) and lower calculated free testosterone (6, 9) were associated with metabolic syndrome involved primarily middle-aged men. Other authors have found that free testosterone is not associated with metabolic syndrome in older non-diabetic men (11, 26). This is of interest, as with increasing age total testosterone declines while SHBG rises (17–19, 27–29). The age-related increase in SHBG has been cast in a negative light as, at constant total testosterone, higher SHBG would be expected to reduce circulating free testosterone by providing binding sites for testosterone. However, in addition to its relationship with metabolic syndrome, lower SHBG is also associated with increased concentrations of smaller, denser low density lipoproteins and increased mortality from CVD (30, 31). Additionally, SHBG correlates with adiponectin, and lower levels of adiponectin are associated with metabolic syndrome (32, 33). Furthermore, a higher ratio of testosterone to SHBG predicts increased risk of developing metabolic syndrome (25), and in one study lower free testosterone was associated with reduced

mortality from ischaemic heart disease (34). On the other hand, in older men higher free testosterone possibly preserves cognitive function (35–37). Thus, there may be divergent associations of androgens in ageing men, free testosterone correlating with cognitive function and total testosterone and SHBG with metabolic health. The relationships between free and total testosterone with a range of health outcomes should be considered in order to determine the relevance of the 'free' hormone in this context.

In older men, total testosterone and SHBG are positively and strongly correlated with $r=0.6$ (19, 29). Thus, despite both total testosterone and SHBG remaining associated with metabolic syndrome in our multivariate analysis, the primary driver for the association could be SHBG since it was the more strongly associated. This is consistent with the fact that SHBG and insulin resistance are negatively correlated and metabolic syndrome is a reflection of insulin resistance (6, 8, 11). Alternatively, given the cross-sectional nature of our study, insulin resistance could be the primary determinant of both lower SHBG and metabolic syndrome, with the association between testosterone and metabolic syndrome being a reflection of the relationship between SHBG and testosterone. Also, total testosterone decreases in moderately obese men in keeping with reduced SHBG-binding capacity, while free testosterone only decreases in severely obese men where LH pulse amplitude is reduced (38). These observations provide support for reduced SHBG and to a lesser extent total testosterone, but not free testosterone, being markers of metabolic syndrome. Further studies would be needed to determine whether SHBG possesses any value as a surrogate marker for insulin sensitivity in tracking responses to improvements in lifestyle such as weight reduction, healthier eating and increased exercise.

Table 4 Univariate and multivariate associations between a one-unit decrease in Z score for total testosterone and sex hormone-binding globulin (SHBG) and presence of the metabolic syndrome.

	Univariate			Multivariate ^a		
	OR	95% CI	P value	OR	95% CI	P value
Total testosterone	1.78	1.59–1.99	<0.001	1.34	1.18–1.52	<0.001
SHBG	2.04	1.80–2.31	<0.001	1.77	1.53–2.06	<0.001

^aAge, total testosterone, SHBG and the squares of total testosterone and SHBG were entered into the multivariate model.

Table 5 Prevalence of metabolic syndrome in men with different categories of hypogonadism.

	MetS –ve		MetS +ve		P value
	n	%	n	%	
Leydig cell impairment Total testosterone 8–15 nmol/l LH > 12 IU/l	61	76	19	24	0.947
Hypergonadotrophic hypogonadism Total testosterone < 8 nmol/l LH > 12 IU/l	18	62	11	38	0.079
Hypogonadotrophic hypogonadism Total testosterone < 8 nmol/l LH ≤ 12 IU/l	33	47	37	53	< 0.001

Finally, our findings indicate that metabolic syndrome seems particularly associated with hypogonadotrophic hypogonadism, with a prevalence of 53% in men with total testosterone < 8 nmol/l and LH ≤ 12 IU/l. By contrast, the prevalence in men with hypergonadotrophic hypogonadism (testosterone < 8 nmol/l and LH > 12 IU/l) was only 38%. While the latter difference was of borderline statistical significance possibly due to the relatively small number of men involved, it is nevertheless consistent with the study by Bojesen *et al.* which found that metabolic syndrome was present in 44% of men with Klinefelter's syndrome (39). Thus, men with hypo- and hypergonadotrophic hypogonadism appear to have an increased prevalence of metabolic syndrome. However, further investigation is warranted to determine whether these conditions distinguished by differing LH concentrations might possess distinct implications for cardiovascular health. It is possible that the weaker correlation of hypergonadotrophic hypogonadism with metabolic syndrome reflects the absence of impaired hypothalamo-pituitary gonadotrophin-releasing hormone (GnRH) function, as previously described for obese men.

Strengths of this study are the size of our cohort (2502 participants), the fact that its members were community dwelling rather than selected on the basis of some other health condition, and its involvement of men older than 70 years. Fasting blood samples were collected in the morning from each of our participants to allow analysis of fasting HDL and triglycerides, and to minimise the potential effects of circadian variation on testosterone concentrations.

Limitations of this study include the cross-sectional nature of the analysis and the use of a single blood sample. Blood sampling at a single time point offers a reasonable estimate of usual testosterone levels (40), and the scope of the study did not extend to repeated blood sampling or to assay of other hormones such as oestradiol. Also, we cannot comment on the relationship between hormones and metabolic syndrome in older men with diabetes as these men were excluded from the analysis.

Data derived using one testosterone immunoassay should not be extrapolated widely without considering

the potential for different testosterone assays to give varying results (41). Additionally, calculation of free testosterone using the Vermeulen method may not correlate precisely with directly measured circulating free testosterone (42). However, immunoassay of total testosterone and calculation of free testosterone are widely accepted methods used extensively in large studies where measurement of total testosterone by mass spectrometry and free testosterone by equilibrium dialysis is impractical (8, 11, 19, 29).

In conclusion, lower SHBG is more strongly associated with metabolic syndrome than lower total testosterone in community-dwelling men above 70 years age. This may reflect the relationship between insulin sensitivity and SHBG. These findings support examination of interventions that raise circulating SHBG as a possible strategy for preventing the development of the metabolic syndrome in older men.

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