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Testosterone, SHBG and the metabolic syndrome: a systematic review and meta-analysis of observational studies.

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Abstract

Background: Accumulating evidence suggests a sex-dependent role of circulating testosterone in the metabolic syndrome (MetS).

Methods: We conducted a meta-analysis of observational studies (PubMed and EMBASE – May 1, 2010) relating MetS to determinants of testosterone status [total testosterone (TT), free testosterone (FT) and sex hormone-binding globulin (SHBG)].

Results: Fifty-two studies were identified, comprising 22 043 men and 7839 women and presenting relative risk (RR) estimates or hormone levels for subjects with and without MetS. Endogenous TT and FT levels were lower in men with MetS (TT mean difference = -2.64 nmol/L; 95% CI, -2.95, -2.32, FT standardized mean difference = -0.26 pmol/L; 95% CI, -0.39, -0.13) and higher in women with MetS (TT mean difference = 0.14 nmol/L; 95% CI, 0.07, 0.20, FT standardized mean difference = 0.52 pmol/L; 95% CI, 0.33, 0.71) compared with those without. Similarly, men with higher TT levels had a lower MetS risk (RR estimate = 0.38; 95% CI, 0.28, 0.50) while TT increased the risk of MetS in women (RR estimate = 1.68; 95% CI, 1.15, 2.45). In both sexes, higher SHBG levels were associated with a reduced risk (men: RR estimate = 0.29; 95% CI, 0.21, 0.41, women: RR estimate = 0.30; 95% CI, 0.21, 0.42).

Conclusion: This meta-analysis supports the presence of a sex-dependent association between testosterone and MetS: TT and FT levels are lower in men with MetS, whilst they are higher in women with MetS. There are no indications for a sex-specific association between SHBG and MetS. In both men and women, MetS is associated with lower SHBG levels.

Key words: Testosterone, SHBG, metabolic syndrome, systematic review, meta-analysis, observational studies

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Introduction

The metabolic syndrome (MetS) is a constellation of metabolic risk factors (including hypertension, dyslipidemia, abdominal obesity and impaired glucose metabolism), that is associated with a two-fold increased risk of cardiovascular disease, and an even higher risk of type 2 diabetes [1,2]. Over the past years, various definitions of MetS have been introduced, of which those proposed by the National Cholesterol Education Program - Adult Treatment Panel III (NCEP ATP III) [3], the World Health Organization (WHO) [4] and the International Diabetes Federation (IDF) [5] are the most widely used. The prevalence of MetS increases with age and is higher in men than in women [6]. MetS associated risks seem to vary according to sex, with MetS being a stronger risk factor for cardiovascular disease in women than men [7,8].

Besides sex differences in prevalence and prognosis, factors associated with the occurrence of MetS may also vary by gender. Previous studies have suggested a role for sex hormones in the development of MetS. Androgen-deprivation therapy in prostate cancer patients [9], and low total testosterone levels (TT) in hypogonadal men [10,11] have been associated with the metabolic syndrome. On the other hand, MetS and its individual components are common in hyperandrogenic conditions in women, such as the polycystic ovary syndrome (PCOS) [12,13]. Sex hormone-binding globulin (SHBG), a testosterone transport protein that affects the circulating levels of free testosterone (FT), has also been linked to MetS. Low SHBG levels have been observed in both men and women with MetS [14,15]. However, little is known about possible sex differences in this association. Furthermore, several studies have examined the relationship between FT and MetS, although their findings have been inconsistent in men [16-18] and women [15,19,20].

To systematically asses the associations of MetS with TT, SHBG and FT, and to investigate possible sex differences in these associations, we conducted a meta-analysis of observational studies relating endogenous TT, SHBG and/or FT levels to the metabolic syndrome in men and women separately.

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Methods

Data Sources and Searches

We performed this meta-analysis according to the guidelines of the Meta-analysis of Observational Studies in Epidemiology group [21]. A systematic search of PubMed and EMBASE (1966 – May 1, 2010) was conducted for English-language articles using the key words *metabolic syndrome, insulin resistance syndrome* and *syndrome X* combined with *testosterone, sex hormone-binding globulin, shbg, androgens, sex hormones* and *sex steroids*. In addition, reference lists of retrieved articles were searched.

Study Selection

Studies were selected by two investigators (J.S.B., Y.T.v.d.S.), using the following criteria: 1. observational studies including TT, SHBG and/or FT as *determinant* and MetS as *outcome*. 2. MetS defined as the presence of at least 3 of the following 5 components: obesity (based on waist circumference, waist to hip ratio or BMI), elevated triglyceride levels, low high density lipoprotein cholesterol levels, impaired glucose metabolism (based on fasting glucose or insulin levels, presence of insulin resistance or diagnosis of diabetes) and hypertension (based on systolic and diastolic blood pressure measurements). 3. studies conducted in adults or adolescents. 4. availability of a measure of association (mean plus SD of hormone levels in subjects with and without MetS and/or a relative risk estimate (odds ratio (OR), relative risk (RR), hazard ratio (HR), prevalence ratio (PR)). 5. studies not selecting participants on the basis of existing diabetes mellitus or cardiovascular disease.

If multiple reports used the same population for calculating association measures, we only included the analysis based on the largest number of participants.

Data Extraction and Quality Assessment

The following data were extracted from each included study: 1. study characteristics (first author, year of publication, country of data collection, study design, length of follow-up if longitudinal, MetS definition (and if applicable its modification), method of free testosterone assessment, exclusion

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criterion regarding type 2 diabetes and variables incorporated in multivariable analyses); 2. study sample characteristics (sex, mean age and BMI, PCOS status in women, number of subjects with and without MetS, mean and SD (derived if SE or 95% CI reported) of TT, SHBG and FT in subjects with and without MetS and relative risk (RR) estimates).

The primary measure of association was the mean difference in TT, SHBG and FT levels between subjects with and without MetS. For the calculation of mean differences, medians and geometric means were assumed to equal means. If studies provided ranges or interquartile ranges instead of SDs, approximate SDs were derived using data extraction methods of Higgins [22] and Hozo [23] et al.

For studies relating TT, SHBG and FT to MetS risk, RR estimates were included as a secondary measure of association. ORs, RRs, HRs and PRs adjusted for the largest number of confounders were extracted. Adjustments for other hormones and components part of the MetS definition were omitted, as these might obscure true associations. Since individual studies reported RR estimates based on various cut-off levels (tertiles, quartiles or specific thresholds) or as a 1 SD increase in testosterone and SHBG, RR estimates were transformed to a uniform scale (comparing the highest versus lowest tertile of TT, SHBG and FT) using the method of Danesh et al. [24]. According to this method, the log relative risk estimate comparing the highest versus lowest tertile can be estimated as 2.18/2.54 times the log relative risk estimate comparing the highest versus lowest quartile, or assuming a normal distribution, as 2.18 times the log relative risk estimate for a 1 SD increase in TT, SHBG or FT. From the study of Laaksonen et al [25] log ORs for the highest versus lowest tertile were obtained by multiplying the dichotomized log ORs by 2.18/1.695.

The quality of each study was assessed against the following criteria: 1. population-based sample. 2. exclusion of subjects on hormonal therapy. 3. use of fasting blood samples for assessment of MetS components. 4. adjusted analysis for potential confounders. An extra criterion was added for studies including men: 5. blood sample collection for hormonal assessment in the morning. Studies with a population based sample were defined as those including subjects from the community, who were not institutionalized, clinic based or known to have MetS. Each criterion was graded as ‘yes’, ‘no’ or ‘unclear’.

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Attemps were made to contact authors when further information was needed for meta-analytic calculations. We contacted 13 authors for missing data of whom 9 provided additional data [15,26-33]

Data Synthesis and Analysis

Measures of association were analyzed for men and women separately, unless results showed no clear indications for an interaction by sex. To compare TT and SHBG levels between subjects with and without MetS, pooled analyses were performed using unstandardized mean differences of TT and SHBG. For the comparison of FT levels, standardized mean differences (mean differences divided by the pooled standard deviation) were used, because individual studies used various methods for FT assessment.

Between-study heterogeneity was quantified by the I^2 statistic [22]. Random-effects models of DerSimonian and Laird [34] were applied in obtaining pooled estimates of association measures.

Univariable metaregression analyses including sex as covariate were conducted to assess sex differences in TT, SHBG and FT levels between subjects with and without MetS. Within each sex, univariable metaregression analyses for predetermined variables (age, BMI, MetS criteria, exclusion of type 2 diabetes, PCOS status, study design, adjustment for covariates and method of FT assessment) were performed to investigate their impact on the association measures and between-study heterogeneity. For these analyses, studies were stratified according to mean age (< 55 years vs ≥ 55 years), mean BMI (< 25 kg/m^2 vs ≥ 25 kg/m^2), MetS definition used (NCEP ATP III vs other criteria (WHO, IDF, EGIR)), exclusion of diabetic patients (yes vs no), study design (cross-sectional (CS) vs longitudinal (LO)), adjustment for covariates (yes vs no) and method of FT assessment (direct measurement vs algorithms). Age and BMI were also entered as continuous terms in metaregression analyses. In women, studies were further classified according to the number of PCOS patients included (< 50% vs ≥ 50%). The prevalence of PCOS ranges from 5 to 10% in reproductive women, depending on ethnicity and the criteria being used [35]. In studies not excluding PCOS patients explicitly, the relative number of PCOS patients was assumed not to exceed this percentage range.

Multivariable metaregression analyses including sex and each of the predetermined variables (except for PCOS status) were conducted to investigate whether the interaction effect of sex changed after

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adjusting for age, BMI and control for age. Univariable and multivariable metaregression analyses were not considered when there were fewer than 10 studies available.

To investigate the impact of each quality parameter separately, sensitivity analyses were conducted in which studies not meeting the individual criteria were excluded. Since direct radioimmunoassay (RIA) is a less reliable method for measuring free testosterone levels [36], the impact of this assay was also investigated in sensitivity analyses. To asses the presence of possible publication bias, funnel plots were drawn and correlations between standardized association measures and their corresponding SEs were analyzed using Egger's test [37]. In case of publication bias, the "trim and fill" method of Duval and Tweedie [38] was used to correct for this bias. All analyses were conducted using STATA 11.1 (StataCorp., College Station, Tex., U.S.A.).

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Results

Study selection

The study selection process is described in Figure 1. Our initial search yielded 596 articles. Of these, 428 articles were excluded based on abstract review. After full text review, an extra 116 studies were excluded because of lack of measure of interest (n = 91), lack of standard MetS definition (n = 7), inappropriateness of reported association measure for inclusion (n = 8), multiple publication (n = 7), unavailability of full text (n = 2) and no correct stratification of MetS (n = 1), leaving 52 studies eligible for inclusion, 32 including men, 19 including women and 1 study including both men and women.

Characteristics and quality of studies

Study characteristics are summarized in Tables 1 and 2. In men, 26 studies were cross-sectional, 5 were longitudinal and 1 study used a case-control design. In women, 19 studies were cross-sectional and 1 study used a case-control design. Nine studies included PCOS patients. Of these, 5 studies used the NICHD criteria to define PCOS [73], 3 studies used the Rotterdam criteria [74] and in 1 study PCOS criteria were not specified. From 45 studies mean differences were derived; 17 studies provided relative risk estimates. Ten studies included both measures of association and 4 studies provided mean differences for two populations separately. In analyses, these populations were considered as individual studies.

Most of the studies used the NCEP ATP III criteria to define MetS and some applied modified versions of criteria (Supplementary Table 1). Four studies reported mean differences for more than one MetS definition. From these studies, only the NCEP ATP III definition was considered in the pooled estimate of the mean difference. In univariable metaregression analyses, mean differences corresponding with all definitions were included. An overview of the study quality and methods of FT measurement is presented in Supplementary Tables 2 and 3.

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Total Testosterone

Studies presenting TT levels in subjects with and without MetS included 14 319 men and 3904 women in total. Men with MetS had lower levels of TT (mean difference = -2.64 nmol/L; 95% CI, -2.95, -2.32), whereas women with MetS had higher levels of TT (mean difference = 0.14 nmol/L; 95% CI, 0.07, 0.20) compared with those without (Figure 2A). In multivariable metaregression analyses this sex-dependent association remained significant ($P < 0.001$) after adjusting for study level differences in age, BMI, diabetes status and control for age.

In men, there was evidence of substantial between-study heterogeneity (I^2 = 89.1%), which was not explained by BMI, diabetes status, control for age or study design. However, in stratified and metaregression analyses TT mean differences were smaller in studies applying NCEP ATP III criteria (P = 0.03) (Table 3). Furthermore, metaregression analyses including age as continuous term showed a trend (P = 0.08) towards a stronger association in younger men. In women, no significant heterogeneity was observed (I^2 = 28.5%), though the association between TT and MetS appeared to be stronger in women without PCOS (P = 0.02) (Table 3). In sensitivity analyses, differences in study quality did not influence associations between TT and MetS in both men and women.

Studies incorporating TT relative risk estimates comprised 13 974 men and 4063 women. Pooled analyses of RR estimates showed a reduced MetS risk with increasing TT levels (RR estimate highest versus lowest TT tertile = 0.38; 95% CI, 0.28, 0.50) (Figure 3A). An opposite association was observed in women (RR estimate highest versus lowest TT tertile = 1.68; 95% CI, 1.15, 2.45). Although the number of studies on which the pooled RR estimates are based are small, these data are consistent with a sex difference in the association of MetS with TT. Substantial heterogeneity was observed among RR estimates in both men (I^2 = 88.5%) and women (I^2 = 66.6%). In men, analyses stratified for study design showed that associations were stronger in cross-sectional studies (RR estimate highest versus lowest TT tertile = 0.31; 95% CI, 0.23, 0.41) than longitudinal studies (RR estimate highest versus lowest TT tertile = 0.64; 95% CI, 0.53, 0.79). In women, no sources of heterogeneity could be identified.

Funnel plots did not disclose publication bias among studies reporting mean differences (men: Egger's test = -1.21; 95% CI, -2.49, 0.06 and women: Egger's test = -0.09; 95% CI, -1.88, 1.70) and RR

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estimates (men: Egger's test = -2.03; 95% CI, -5.81, 1.75 and women: Egger's test = 2.05; 95% CI, -0.60, 4.70) (Supplementary Figures 1A and 1B). Although there was no strong evidence for publication bias in RR estimates, visual inspection of the funnel plot showed some asymmetry in women. Because of the small number of studies (N = 4), this plot was difficult to interpret.

Sex Hormone-Binding Globulin

Studies reporting SHBG levels in subjects with and without MetS comprised 10 537 men and 4006 women. In both sexes, SHBG levels were lower in subjects with MetS (men: mean difference = -9.77 nmol/L; 95% CI, -12.26, -7.29; women: mean difference = -19.39 nmol/L; 95% CI, -23.81, -14.98) than in those without (Figure 2B). Overall, the inverse association between SHBG and MetS was stronger in women than men (P = 0.003). In multivariable metaregression analyses this sex difference remained consistent after adjusting for study level differences in age, BMI, diabetes status and control for age.

Substantial between-study heterogeneity was observed in both men (I^2 = 97.6%) and women (I^2 = 85.5%). In men, this heterogeneity was partly explained by differences in age. Univariable metaregression analyses including age as a dichotomous term showed that the association between SHBG and MetS tended to be more pronounced in men aged 55 years and older (P = 0.08). This effect of age, however, disappeared when age was entered as a continuous term. In women, the association appeared to be stronger in those with a BMI < 25 kg/m^2 (Table 3). This effect of BMI was also observed in metaregression analyses including BMI as a continuous term (P = 0.04) Sensitivity analyses showed no effect of study quality on the associations between SHBG and MetS in both men and women.

Studies providing data on SHBG relative risk estimates comprised 10 057 men and 3868 women. Analysis of RR estimates showed similar inverse associations between SHBG and MetS risk in men (RR estimate highest versus lowest SHBG tertile = 0.29; 95% CI, 0.21, 0.41) and women (RR estimate for highest versus lowest SHBG tertile = 0.30; 95% CI, 0.21, 0.42) (Figure 3B), without evidence of a sex difference (P = 0.74). There was heterogeneity among RR estimates in men (I^2 =

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80.7%) which remained unexplained in stratified and metaregression analyses. In women, no substantial heterogeneity was observed (I^2 = 37.1%).

There were indications for publication bias among studies reporting mean differences in men (Egger's test = 3.73; 95% CI, 0.18, 7.27). Funnel plots showed asymmetry and pointed to missing studies in the lower left-handed corner, indicating a lack of studies reporting large SHBG differences with high precision (Supplementary Figure 1A). In women, no publication bias was observed (Egger's test = -2.03; 95% CI, -4.92, 0.86). Egger's test did not detect publication bias among studies reporting RR estimates (men: Egger's test = -1.87; 95% CI, -6.50, 2.88, women: Egger's test = -1.57; 95% CI, -3.35, 0.19), but in women the funnel plot showed some asymmetry (Supplementary Figure 1B).

Free Testosterone

Studies presenting FT levels in subjects with and without MetS included 8750 men and 1744 women in total. A sex difference was found (P = 0.004), such that women with MetS had higher FT levels (mean difference = 0.52; 95% CI, 0.33, 0.71), while men with the metabolic syndrome had lower levels of FT than those without (mean difference = -0.26; 95% CI, -0.39, -0.13) (Figure 2C). This sex-dependent association remained significant in multivariable analyses.

Substantial between-study heterogeneity was observed in both men (I^2 = 79.9%) and women (I^2 = 61.1%). In men, heterogeneity was partly explained by the different MetS criteria used across studies. As for TT, the inverse association with FT tended to be weaker among studies using NCEP ATP III criteria (P = 0.08) (Table 3). Furthermore, the association between MetS and FT differed according to the mean age of the study population (P = 0.01), with a stronger association being observed in younger men (Table 3). In women, no sources of heterogeneity were identified. In sensitivity analyses, exclusion of studies using RIA did not change the observed associations materially. Associations were also not affected by differences in study quality.

Studies reporting FT relative risk estimates comprised 7281 men. Consistent with the findings for TT, high FT levels were associated with a reduced MetS risk, albeit not statistically significant (RR estimate highest versus lowest FT tertile = 0.64; 95% CI, 0.41, 1.01) (Figure 3C). There was evidence of substantial between-study heterogeneity (I^2 = 86.4%), of which no sources could be identified. One

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study in women reported a RR estimate for FT, albeit not significant (RR estimate highest versus lowest FT tertile = 1.24; 95% CI, 0.67, 2.31).

No publication bias was detected among studies providing FT mean differences in men (Egger's test = -1.19; 95% CI, -3.25, 0.88) and RR estimates in men (Egger's test = -2.69; 95% CI, -10.55, 5.16). In women, funnel plots disclosed publication bias among studies reporting mean differences (Egger's test = 2.36; 95% CI, 0.51, 4.21), indicating a lack of small studies reporting small FT differences (Supplementary Figure 1A).

Discussion

Results of this meta-analysis support the presence of a sex-dependent association between endogenous testosterone and MetS. TT levels were lower in men MetS, whilst they were higher in women with MetS. There was also some evidence for a sex-specific association between free testosterone and MetS with FT levels being lower in men with MetS, while being higher in women with MetS. Interestingly no sex specific association was observed for SHBG. In both sexes, MetS was associated with a decrease in SHBG levels. Although the mean difference in SHBG levels between those with and without MetS was larger in women, this sex difference was lost after taking potential confounders into account in pooled analyses of RR estimates.

Some limitations of our meta-analysis need to be considered while interpreting the findings. First of all, we could only partly explain between-study heterogeneity. In metaregression analyses we observed that at least some of the heterogeneity in men was explained by differences in age, MetS criteria and study design. In older men the associations of TT and FT with MetS tended to be less pronounced. This effect of age has been reported previously [43] and may be attributed to the age-related decline in testosterone, resulting in a lower contrast in TT and FT with increasing age. Associations of TT and FT with MetS were also weaker when NCEP ATP II criteria were used. These criteria differ from other criteria in degree of emphasis of the individual MetS components. While the NCEP ATP III criteria put equal emphasis on the five MetS components, other criteria assign greater value to a particular component: impaired glucose metabolism (WHO and EGIR) and presence of abdominal

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obesity (IDF). Therefore, this differential effect of MetS criteria suggests that abdominal obesity and impaired glucose metabolism are important mediators of the observed associations between testosterone and MetS in men. Furthermore, analyses stratified for study design showed stronger associations in cross-sectional studies. This may indicate that the 'rare disease assumption' does not apply to the metabolic syndrome, with odds ratios from cross-sectional studies overestimating the actual association. In women, the association between TT and MetS was weaker in PCOS patients High baseline levels of testosterone in this specific patient population may result in lower interindividual variation and low power to detect an association. Metaregression analyses further showed that the association between SHBG and MetS was more pronounced in leaner women, suggesting that in obesity SHBG is only one of the contributing factors. Another potential source of between-heterogeneity in both men and women is the variety of methods used for measuring free testosterone levels [81,82]. FT values vary between different algorithms and FT measurements by RIA have been criticized due to a lack of accuracy [36]. However, sensitivity analyses showed that the use of RIA did not have a major impact on the association between FT and MetS. In spite of material heterogeneity, we decided to pool the data from all studies. While pooling of heterogeneous studies may affect the validity of the pooled estimates, the results of individual studies were largely compatible with the pooled estimates and pointed in the same direction as the overall estimate.

Another concern is the presence of potential publication bias among studies reporting SHBG mean differences in men and FT mean differences in women. However, evaluation of this publication bias by the "trim and fill" method showed that imputation of missing studies did not significantly alter the observed associations of SHBG and FT with MetS. It is important to recognize that asymmetry is not necessarily the result of publication bias, but can also be caused by between-study heterogeneity.

A final limitation is the major contribution of cross-sectional studies to our meta-analysis, which precludes us from drawing firm conclusions about temporal associations. In men, findings from four longitudinal studies [18,25,43,48] support a causal role for testosterone in the MetS etiology. Experimental studies have demonstrated that testosterone has beneficial effect on glucose and fat metabolism in male rats [83-86]. Moreover, intervention studies in hypogonadal have shown improvements in individual components [87,88] and even reversal of MetS following testosterone therapy [89,90]. However, associations

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in the opposite direction have been reported as well. In obese men, weight loss and maintenance cause an increase in testosterone and SHBG levels [91,92] and experimental data show suppressive effects of adiposity and insulin on testosterone production in men [93-95]. Furthermore, MetS has been associated with an increased risk of hypogonadism in middle aged men [96]. Hence, complex, bidirectional relationships between testosterone and MetS seem to be plausible. In women, evidence for a causal role of testosterone in MetS is limited. This is reflected by the lack of longitudinal studies in this meta-analysis. Nevertheless, some recent findings suggest that testosterone may be a risk factor in women as well. In a prospective study [97], low SHBG and high testosterone levels at baseline were found to be associated with an increased MetS risk. Furthermore, high testosterone levels have been associated with increased risk of diabetes in postmenopausal women [98] and a decrease in insulin sensitivity in female rats [99]. On the other hand, metformin therapy and weight loss reduce androgen excess in women [100,101], while insulin stimulates the ovarian production of testosterone [102].

Since TT and SHBG are correlated, it is also unclear whether the observed associations between SHBG and MetS reflect an independent effect of SHBG. However, increasing evidence from epidemiological studies support the involvement of SHBG in MetS [10,25,97] and diabetes etiology[98,103,104]. Moreover, polymorphisms in the SHBG gene have recently been shown to affect not only SHBG levels but also type 2 diabetes risks in men as well as in women [98,105], suggesting a potential causal role for SHBG in pathophysiological mechanisms.

Pooled estimates of our meta-analysis are comparable (regarding strength and direction) with those previously reported for type 2 diabetes by Ding et al. [106]. This once more suggests a predominant role for glucose metabolism in the associations of testosterone with MetS and further indicates that the sex-dependent role of testosterone is not restricted to type 2 diabetes, but also exist in preceding conditions such as MetS, and may even be found in earlier stages of disease. Although the exact mechanisms underlying the sex specific associations between testosterone and MetS are not completely understood, similar sex-specific effects of testosterone have been observed in animal models. Low testosterone levels following castration in male rats, for instance, have been linked to obesity, insulin resistance and dyslipidemia [84,107,108]. Whereas prenatal and postnatal administration of testosterone has adverse effects on various MetS components in female rats [109-111].

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The lack of a sex specific association between SHBG and MetS is not fully understood. Nevertheless, recent findings from genetic studies [112,113] provide some explanation. In these studies, one particular SHBG SNP, rs1799941, was found to have no effect on total testosterone levels in women while raising testosterone levels in men. Based on these data, it has been hypothesized that women with genetically lower SHBG levels are exposed to proportionally more of the adverse effects of the biologically active unbound testosterone, such as increasing risk of MetS and diabetes in women. On the other hand, in men there is recent evidence that bound testosterone may be biologically active. If this is the case, then men with lower total testosterone due to lower SHBG will be exposed to less of the protective metabolic effects of androgens, despite similar levels of unbound or free testosterone and also experience higher risk of MetS and diabetes [105]. Thus similar 'genetic' levels of SHBG may affect MetS risk in men and women differently, by altering the levels of testosterone in a sex-specific manner. Further research is necessary to elucidate the role of SHBG in the pathophysiology of MetS and diabetes.

In conclusion, findings of this meta-analysis support the presence of a sex-dependent association between TT and MetS, with high endogenous TT lowering MetS risk in men, while increasing MetS risk in women. There are also indications for a sex difference in the association between FT and MetS. Higher SHBG levels are associated with a lower MetS risk in both men and women. Differences in age, BMI, MetS criteria, PCOS status and study design account for some of the variability observed. The comparability of our pooled estimates with those available for type 2 diabetes suggests a major contribution of impaired glucose metabolism to the observed associations. To further clarify the causal nature of the observed associations, more large-scale longitudinal studies are required, in women in particular. However, longitudinal studies are not perfect as early disease processes before the actual diagnosis of MetS may influence the level of testosterone and SHBG as well. Therefore, additional tools, such as Mendelian randomization studies and intervention studies, are needed to establish causation.

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Key messages

1. Associations between endogenous testosterone and the metabolic syndrome (MetS) are sex-specific with total and free testosterone levels being lower in men with MetS, while being higher in women with MetS.
2. There are no indications for a sex-specific association between SHBG and MetS. In both men and women MetS is associated with lower SHBG levels.
3. The large contribution of cross-sectional studies (particularly in women), stresses the need for more longitudinal studies, Mendelian randomization studies and intervention studies to establish the causal nature of the observed association between testosterone, SHBG and MetS.

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Table 1A Characteristics of studies reporting on TT, SHBG and/or FT levels in men with and without MetS

Nr.	Source	Country	Study design	Participants	Mean age	Mean BMI	Adjusted for age	MetS No.		Mean (SD), nmol/L Total Testosterone		Mean (SD), nmol/L SHBG		Mean (SD), pmol/L Free Testosterone	
								Yes	No	MetS$^+$	MetS$^-$	MetS$^+$	MetS$^-$	MetS$^+$	MetS$^-$
1	Katabami et al, 2010 [39]	Japan	CS	Nondiabetic men	46	23.7	No	70	204	-	-	-	-	40.6 (13.9) [c]	51.01 (16.0) [c]
2	de Oya et al, 2010 [28] [d]	Spain	CS	Adolescent boys	14	21.9	No	13	377	-	-	28.8 (15.2)	51.0 (34.6)		
3	Atlantis et al, 2009 [40] (1) [b]	Australia	CS	Men from the Florey Adelaide Male Ageing Study	53	-	No	445	737	12.2 (4.9)	15.3 (5.6)	30.8 (14.0)	37.1 (17.1)	-	-
	Atlantis et al, 2009 [40] (2) [b]	Australia	Cs	Men from the Florey Adelaide Male Ageing Study	53	-	No	498	691	12.1 (4.8)	15.5 (5.6)	31.2 (15.5)	37.2 (16.5)	-	-
4	Coviello et al, 2009 [41] (1) [d]	U.S.A.	CS	Fathers of women with PCOS	57	30.2	No	89	122	12.8 (5.2)	15.6 (4.3)	72 (40)	80 (37)	-	-
	Coviello et al, 2009 [41] (2) [d]	U.S.A.	CS	Brothers of women with PCOS	29	28.7	No	13	45	15.0 (4.6)	18.8 (7.0)	46 (22)	55 (26)	-	-
5	Demir et al, 2009 [42]	Turkey	CS	Men with lower urinary tract symptoms	60 [a]	27.4 [a]	No	60	130	14.0 (5.2)	16.0 (6.1)	-	-	-	-
6	Haring et al, 2009 [43]	Germany	LO	Men from the Study of Health in Pomerania	49	26.4	No	480	524	15.5 (4.8)	17.7 (5.3)	-	-	-	-
7	Chubb et al, 2008 [10]	Australia	CS	Nondiabetic men from the Health in Men Study	76 [a]	26.2 [a]	No	602	1900	14.0 (4.9)	16.7 (5.7)	36.8 (14.0)	45.5 (17.0)	274.6 (88.2)	291.2 (90.8)
8	Emmelot-Vonk et al, 2008 [44]	Netherlands	CS	Nondiabetic men with low normal testosterone levels	67	27.3	No	62	160	12.7 (2.3)	13.5 (2.4)	28.9 (8.9)	34.4 (10.5)	376.5 (104.0)	345.4 (128.5)
9	Goncharov et al, 2008 [45] (1) [b]	Russia	CS	Nondiabetic obese men	31	32.6	No	34	26	11.2 (4.0)	16.3 (6.8)	29.7 (21.5)	45.2 (32.0)	249.0 (94.0)	294.0 (129.0)
	Goncharov et al, 2008 [45] (2) [b]	Russia	CS	Nondiabetic obese men	31	32.6	No	23	37	10.9 (4.4)	15.0 (6.3)	33.9 (27.2)	37.4 (28.0)	230.0 (95.0)	295.0 (120.0)
	Goncharov et al, 2008 [45] (3) [b]	Russia	CS	Nondiabetic obese men	31	32.6	No	27	33	11.2 (4.6)	15.3 (6.3)	31.4 (22.3)	40.4 (30.7)	236.0 (85.0)	296.0 (125.0)
10	Laughlin et al, 2008 [31]	U.S.A.	CS	Men from the Rancho Bernardo Study	71	25.7	Yes	143	651	8.5 (2.8)	10.8 (3.4)	-	-	-	-
11	Suetomi et al, 2008 [46]	Japan	CS	Men with erectile dysfunction	60	23.9 [a]	No	25	108	15.3 (5.5)	16.0 (5.9)	-	-	33.7 (12.8)	36.1 (11.8)
12	Yeh et al, 2008 [47]	Taiwan	CS	Men with erectile dysfunction	58	24.9 [a]	No	38	65	12.4 (5.8)	16.2 (5.9)	-	-	-	-
13	Corona et al, 2007 [17] (1) [b]	Italy	CS	Male patients with sexual dysfunction	52	-	No	348	738	13.6 (6.0)	17.4 (7.2)	-	-	34.8 (14.0) [c]	40.8 (13.7) [c]
	Corona et al, 2007 [17] (2) [b]	Italy	CS	Male patients with sexual dysfunction	52	-	No	485	601	14.7 (7.4)	18.2 (6.0)	-	-	36.2 (14.1) [c]	42.5 (13.5) [c]
14	Guay et al, 2007 [33] (1) [b]	U.S.A.	CS	Men with erectile dysfunction	54	29.4	No	88	66	-	-	-	-	42.7 (18.4) [c]	49.3 (22.9) [c]
	Guay et al, 2007 [33] (2) [b]	U.S.A.	CS	Men with erectile dysfunction	54	29.4	No	54	100	-	-	-	-	39.6 (10.4) [c]	51.4 (22.9) [c]
15	Rodriguez et al, 2007 [48]	U.S.A.	CS [e]	Caucasian men from the Baltimore Longitudinal Study of Aging	63	26.0	Yes	113	505	12.8 (0.2)	14.9 (0.1)	62.9 (2.8)	82.1 (1.6)	-	-
16	Tang et al, 2007 [49]	Taiwan	CS	Men residing in a veterans' nursing home	79	23.8	No	101	280	13.3 (0.6)	16.2 (0.4)	39.9 (1.6)	53.9 (1.2)	194.5 (76.9)	205.4 (74.7)
17	Chen et al, 2006 [29]	Australia	CS	Nondiabetic men from the Australian Longitudinal Study of Aging	76	26.0	No	20	140	12.1 (3.6)	14.2 (4.7)	-	-	-	-
18	Gannagé-Yared et al, 2006 [50]	Lebanon	CS	Nondiabetic men	59	27.3	No	94	59	12.5 (3.8)	14.3 (4.0)	34.0 (13.7)	41.0 (15.5)	-	-
19	Kaplan et al, 2006 [51]	U.S.A.	CS	Men with dyslipidemia	52	27.4	No	265	597	14.0 (4.7)	16.1 (4.9)	-	-	-	-
20	Kupelian et al, 2006 [18]	U.S.A.	CS [e]	Men from the Massachusetts Male Aging Study	53 [a]	27.1 [a]	No	146	950	15.6 (6.4)	18.4 (5.9)	26.1 (11.8)	33.6 (16.1)	430.0 (190.0)	470.0 (180.0)
21	Maggio et al, 2006 [52]	Italy	CS	Men from the InCHIANTI study	75	26.6 [a]	No	73	389	13.8 (4.8)	15.0 (4.5)	83.6 (30.8)	104.0 (46.1)	145.7 (48.8)	131.9 (56.6)

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Table 1A Continued

Nr.	Source	Country	Study design	Participants	Mean age	Mean BMI	Adjusted for age	No.		Mean (SD), nmol/L Total Testosterone		Mean (SD), nmol/L SHBG		Mean (SD), pmol/L Free Testosterone	
								MetS$^+$	MetS$^-$	MetS$^+$	MetS$^-$	MetS$^+$	MetS$^-$	MetS$^+$	MetS$^-$
22	Mousavinasab et al, 2006 [53]	Finland	LO	Military service men on a high-caloric high-fat diet	17-28	24.3 [a]	No	11	169	-	-	15.1 (6.6)	19.1 (10.2)	-	-
23	Robeva et al, 2006 [54]	Bulgaria	CC	Nondiabetic, hyperinsulinaemic men with MetS and healthy-age matched controls	30	30.6	Yes	10	10	12.1 (3.7)	21.5 (7.5)	-	-	-	-
24	Kalme et al, 2005 [55]	Finland	CS	Men from the Finish part of the Seven Countries Study	70-89	-	Yes	94	241	16.4 (9.4)	23.2 (9.9)	54.4 (27.1)	74.4 (31.0)	-	-
25	Muller et al, 2005 [14]	Netherlands	CS	Independently living men	60	26.3	No	96	304	15.7 (4.5)	19.4 (5.3)	34.7 (12.4)	42.4 (14.6)	321.1 (90.7)	364.7 (98.2)
26	Nuver et al, 2005 [56]	Netherlands	CS	Testicular cancer patients treated with chemotherapy	38	25.4	No	22	62	18.3 (5.0)	20.0 (8.0)	20.0 (6.0)	26.0 (9.0)	442.0 (115.0)	495.0 (153.0)
27	Tong et al, 2005 [30] (1) [d]	China	CS	Men from the Hong Kong Diabetes Family Study without a family history of diabetes	44	24.7	Yes	30	98	15.8 (4.0)	18.4 (6.1)	27.1 (9.3)	30.8 (13.2)	-	-
	Tong et al, 2005 [30] (2) [d]	China	CS	Men from the Hong Kong Diabetes Family Study with a family history of diabetes	39	25.9	Yes	70	109	16.0 (3.7)	18.3 (5.6)	21.2 (8.6)	27.4 (14.4)	-	-
28	Laaksonen et al, 2003 [16]	Finland	CS	Nondiabetic men from the Kuopio Ischaemic Heart Disease Risk Factor Study	53 [b]	26.8 [b]	No	345	1551	17.6 (6.8)	21.6 (7.4)	31.2 (13.0)	38.1 (15.6)	273.0 (79.0)	307.0 (75.0)

BMI, body mass index; CC, case-control; CS, cross-sectional; LO, longitudinal; MetS, metabolic syndrome; MetS$^+$, subjects with the metabolic syndrome; MetS$^-$, subjects without the metabolic syndrome; No., number; SHBG, sex hormone-binding globulin; -, not applicable.

[a] mean age/BMI of study sample based on weighted means of age/BMI of subjects with and without MetS.

[b] studies using multiple criteria to define the metabolic syndrome (Atlantis et al, 2009: (1) NCEP ATP III, (2) IDF; Goncharov et al, 2008: (1) NCEP ATP III, (2) WHO, (3) IDF; Corona et al, 2007: (1) NCEP ATP III , (2) IDF; Guay et al, 2007: (1) NCEP ATP III modified, (2) WHO).

[c] free testosterone measured by radioimmunoassay.

[d] mean differences reported for two separate populations (1) and (2).

[e] longitudinal study providing data on hormonal levels in subjects with and without MetS at baseline.

SI conversion factors: to convert testosterone (total testosterone/free testosterone) to ng/dL divide by 0.0347. To convert SHBG to µg/mL divide by 8.896.

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Table 1B Characteristics of studies reporting on TT, SHBG and/or FT levels in women with and without MetS

Nr.	Source	Country	Study design	Participants	Mean age	Mean BMI	Adjusted for age	No.		Mean (SD), nmol/L Total Testosterone		Mean (SD), nmol/L SHBG		Mean (SD), pmol/L Free Testosterone	
								MetS$^+$	MetS$^-$	MetS$^+$	MetS$^-$	MetS$^+$	MetS$^-$	MetS$^+$	MetS$^-$
1	Alemzadeh et al, 2010 [57]	U.S.A.	CS	Obese adolescent girls with PCOS	16	36,2	No	35	68	-	-	-	-	48.6 (17.7)	38.5 (15.9)
2	Healy et al, 2010 [58]	Ireland	CS	Postmenopausal women with newly diagnosed breast cancer	68	28.3	No	42	63	1.14 (0.51)	1.07 (0.6)	49.4 (24.6)	57.0 (26.2)	-	-
3	de Oya et al, 2010 [28] [c]	Spain	CS	Adolescent girls	14	21.8	No	4	424	-	-	24.6 (11.2)	64.6 (34.9)	-	-
4	de Sousa et al, 2010 [60]	Germany	CS	Obese postmenarcheal adolescent girls	15	32.6	No	48	112	1.8 (0.7)	1.5 (0.7)	19.1 (7.9)	37.9 (8.5)	49.0 (7.0)	40.0 (15.0)
5	Ni et al, 2009 [59]	China	CS	Women with PCOS	27	21.9	No	97	481	2.1 (0.8)	2.2 (0.9)	27.8 (25.6)	55.4 (38.7)	152.7 (97.7)	111.1 (72.0)
6	Janssen et al, 2008 [61]	U.S.A.	CS	Women from the SWAN study at time of their final menstruation period	51	26.9	No	130	819	1.5 (0.6)	1.3 (0.6)	34.1 (19.4)	45.0 (24.2)	-	-
7	Maggio et al, 2007 [15]	Italy	CS	Women from the InCHIANTI Study 65 years and older	76	27.6 [a]	No	145	367	2.3 (1.1)	2.1 (0.9)	97.5 (51.6)	131.2 (66.9)	-	-
8	Park et al, 2007 [62]	Korea	CS	Women with PCOS	26	23.6	No	16	97	2.3 (0.9)	2.4 (1.1)	18.8 (8.9)	49.6 (40.6)	9.0 (2.8) [b]	5.9 (3.1) [b]
9	Coviello et al, 2006 [63]	U.S.A.	CS	Postmenarcheal adolescent girls with PCOS	17	32.0	No	18	31	2.8 (0.8)	2.5 (0.9)	33.0 (13.0)	77.0 (53.0)	-	-
10	Ehrmann et al, 2006 [12]	U.S.A.	CS	Nondiabetic PCOS women who participated in a large multicenter national trial	28 [a]	36.0 [a]	No	123	245	2.2 (1.2)	2.2 (1.1)	32.8 (15.5)	43.8 (21.9)	41.8 (17.7)	37.8 (20.4)
11	Leibel et al, 2006 [19]	U.S.A.	CS	Postmenarcheal adolescent girls with PCOS	16	32.4 [a]	No	10	26	-	-	8.4 (6.3)	15.4 (9.6)	90.2 (35.7)	67.0 (23.9)
12	Pasanisi et al, 2006 [64]	Italy	CS	Postmenopausal women operated for breast cancer	57	-	No	16	94	1.7 (0.5)	1.4 (0.5)	46.3 (28.1)	67.8 (29.8)	-	-
13	Weinberg et al, 2006 [32]	U.S.A.	CS	Postmenopausal women from the Women's Health Study (WHS)	65 [a]	26.2 [a]	Yes	108	104	0.8 (0.6)	0.6 (0.4)	32.6 (29.2)	55.8 (17.3)	-	-
14	Apridonidze et al, 2005 [65]	U.S.A.	CS	Women with PCOS	30 [a]	36.1 [a]	No	46	60	2.5 (1.0)	2.1 (1.0)	26.2 (31.5)	36.5 (19.8)	55.9 (26.3) [b]	37.1 (28.1) [b]
15	Dokras et al, 2005 [66]	U.S.A.	CS	Women with PCOS	28	-	No	45	84	1.9 (1.0)	1.9 (1.2)	20.0 (11.1)	32.0 (31.5)	27.4 (15.2)	27.1 (26.0)
16	Golden et al, 2004 [67] (1) [c]	U.S.A.	CS	Postmenopausal women from the ARIC study with minimal carotid atherosclerosis	62 [a]	27.4 [a]	No	60	121	0.9 (0.8)	0.7 (0.6)	-	-	-	-
	Golden et al, 2004 [67] (2) [c]	U.S.A.	CS	Postmenopausal women from the ARIC study with significant atherosclerosis	62 [a]	27.9 [a]	No	94	87	0.8 (0.6)	0.7 (0.5)	-	-	-	-
17	Korhonen et al, 2003 [20]	Finland	CC	Premenopausal women from a community-based study	43 [a]	28.3 [a]	Yes	63	88	1.4 (0.5)	1.3 (0.6)	37.4 (22.2)	52.9 (25.3)	21.5 (9.5)	16.8 (6.6)

ARIC, Atherosclerosis Risk in Communities Study; BMI, body mass index; CS, cross-sectional; LO, longitudinal; MetS, metabolic syndrome; MetS$^+$, subjects with the metabolic syndrome; MetS$^-$, subjects without the metabolic syndrome; No., number; PCOS, polycystic ovary syndrome; SHBG, sex hormone-binding globulin; SWAN, Study of Women's Health Across the Nation; -, not applicable.

[a] mean age/BMI of study sample based on weighted means of age/BMI of subjects with and without MetS;

[b] free testosterone measured by radioimmunoassay.

[c] mean differences reported for two separate populations (1) and (2).

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Table 2A Characteristics of studies presenting relative risk estimates for MetS according to TT, SHBG and/or FT levels, men

Nr.	Source	Country	Study design	Mean follow-up	Participants	N	Variables adjusted for	RR estimate TT [a] (95% CI)	RR estimate SHBG [a] (95% CI)	RR estimate FT [a] (95% CI)
1	Akishita et al, 2010 [27]	Japan	CS	-	Nondiabetic men	194	Age	OR 0.26 (0.11 - 0.59)	-	-
2	Li et al, 2010 [68]	U.S.A	CS	-	Men from the Third National Health and Nutrition Examination Survey (NHANES-III)	1226	Age, smoking, alcohol consumption, physical activity, race, CRP, LDL cholesterol, HOMA-IR	PR 0.52 (0.38 - 0.69)	PR 0.51 (0.34 - 0.79)	PR 0.87 (0.63 - 1.20)
3	Haring et al, 2009 [43 b]	Germany	LO	5.0 yr	Men from the Study of Health in Pomerania (SHIP) study	1004	-	RR 0.70 (0.59 - 0.83)	-	-
4	Schneider et al, 2009 [26]	Germany	CS	-	Men from the Diabetes Cardiovascular Risk-Evaluation: Targets and Essential DATA for Commitment of Treatment (DETECT)	2719	-	OR 0.26 (0.21 - 0.32)	-	-
5	Chubb et al, 2008 [10 b]	Australia	CS	-	Nondiabetic men from the Health in Men study	2052	-	OR 0.28 (0.22 - 0.36)	OR 0.21 (0.16 - 0.28)	-
6	Emmelot-Vonk et al, 2008 [44 b]	Netherlands	CS	-	Nondiabetic men with low normal testosterone levels	222	Age, smoking, alcohol consumption	OR 0.45 (0.21 - 0.95)	OR 0.25 (0.11 - 0.56)	OR 2.15 (1.00 - 4.57)
7	Kupelian et al, 2008 [69]	U.S.A.	CS	-	Men from the Boston Area Community Health (BACH) survey	1885	Age, smoking, alcohol consumption, physical activity, ethnicity	OR 0.16 (0.10 - 0.27)	OR 0.13 (0.08 - 0.23)	OR 0.22 (0.13 - 0.37)
8	Rodriguez et al, 2007 [48 b]	U.S.A.	LO	5.8 yr	Men from the Baltimore Longitudinal Study of Aging	417	Age, BMI	HR 0.46 (0.25 - 0.84)	HR 0.30 (0.17 - 0.58)	-
9	Kupelian et al, 2006 [18 b]	U.S.A.	LO	14.4 yr	Men from the Massachusetts Male Aging Study	950	-	RR 0.75 (0.55 - 0.97)	RR 0.50 (0.37 - 0.68)	RR 1.06 (0,81 - 1,41)
10	Muller et al, 2005 [14 b]	Netherlands	CS	-	Independently living men	400	Age, smoking, alcohol consumption, physical activity	OR 0.20 (0.10 - 0.38)	OR 0.17 (0.08 - 0.34)	OR 0.31 (0.15 - 0.63)
11	Tong et al, 2005 [30 b]	China	CS	-	Men from the Hong Kong Diabetes Family Study	307	Age, smoking, family history of diabetes, CRP (TT and SHBG), IGF-1 (SHBG only)	OR 0.25 (0.12 - 0.52)	OR 0.17 (0.08 - 0.38)	-
12	Laaksonen et al, 2004 [25]	Finland	LO	11.0 yr	Nondiabetic men from the Kuopio Ischaemic Heart Disease Risk Factor Study	702	Age, BMI, smoking, alcohol consumption, presence of CVD, socioeconomic status	OR 0.43 (0.25 - 0.76)	OR 0.37 (0.21 - 0.64)	OR 0.56 (0.31 - 0.99)
13	Laaksonen et al, 2003 [16 b]	Finland	CS	-	Nondiabetic men from the Kuopio Ischaemic Heart Disease Risk Factor Study	1896	Age, BMI, smoking, alcohol consumption, presence of CVD, socioeconomic status	OR 0.52 (0.36 - 0.75)	OR 0.54 (0.37 - 0.77)	OR 0.58 (0.41 - 0.83)

BMI, body mass index; CI, confidence interval; CS, cross-sectional; CVD, cardiovascular disease; CRP, C-reactive protein; FT, free testosterone; HR, hazard ratio; HOMA-IR, homeostasis model assessment of insulin resistance; IGF-1, insulin-like growth factor 1; LO, longitudinal; MetS, metabolic syndrome; OR, odds ratio; PR, prevalence ratio; RR, relative risk; SHBG, sex hormone-binding globulin; TT, total testosterone; yr, year; -, not applicable.
[a] relative risk estimates of MetS comparing highest versus lowest tertiles of TT, SHBG and FT.
[b] studies reporting both measures of association (relative risk estimates and mean differences).

Table 2B Characteristics of studies presenting relative risk estimates for MetS according to TT and/or SHBG levels, women

Nr.	Source	Country	Study design	Participants	N	Variables adjusted for	RR estimate TT [a] (95% CI)	RR estimate SHBG [a] (95% CI)	RR estimate FT [a] (95% CI)
1	Patel et al, 2009 [70]	U.S.A.	CS	Nondiabetic women 65 years and older from the Cardiovascular Health Study (CHS)	301	Age, race, estrogen use and number of ovaries removed	OR 2.49 (1.30 - 4.76)	-	OR 1.24 (0..67 – 2.32)
2	Maggio et al, 2007 [15 b]	Italy	CS	Women from the InCHIANTI Study 65 years and older	589	-	OR 1.40 (0.91 - 2.16)	OR 0.31 (0.19 - 0.49)	-
3	Chen et al, 2006 [71]	Taiwan	CS	Women with PCOS not undergoing treatment	106	Age	-	OR 0.10 (0.01 - 0.89)	-
4	Weinberg et al, 2006 [32 b]	U.S.A.	CS	Postmenopausal women from the Women's Health Study	212	Age, BMI, smoking, alcohol consumption, physical activity, and the presence of CVD at follow-up	OR 3.20 (1.40 - 7.30)	OR 0.14 (0.05 - 0.37)	-
5	Santoro et al, 2005 [72]	U.S.A.	CS	Nondiabetic women from the Study of Women's Health Across the Nation (SWAN)	2961	Age, smoking, ethnicity, site	OR 1.25 (1.12 - 1.40)	OR 0.36 (0.29 - 0.43)	-

BMI, body mass index; CI, confidence interval; CS, cross-sectional; CVD, cardiovascular disease; FT, free testosterone; MetS, metabolic syndrome; OR, odds ratio; PCOS, polycystic ovary syndrome; SHBG, sex hormone-binding globulin; SWAN, Study of Women's Health Across the Nation; TT, total testosterone; -, not applicable.
[a] relative risk estimates of MetS comparing highest versus lowest tertiles of TT, SHBG and FT.
[b] studies reporting both measures of association (relative risk estimates and mean differences).

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Table 3 Mean differences of TT, SHBG and FT between subjects with and without the MetS, men and women.

	Men			Women		
	Studies No.	**TT mean difference (95% CI) (nmol/L)**	I^2	**Studies No.**	**TT mean difference (95% CI) (nmol/L)**	I^2
Overall random effects	26	-2.64 (-2.95, -2.32)	89.1 % (p< 0.001)	15	0.14 (0.07, 0.20)	28.5 % (p = 0.14)
Age						
- Age < 55 years	12	-3.03 (-3.60, -2.45)	65.7% (p < 0.001)	9	0.10 (0.00, 0.21)	42.6% (p = 0.08)
- Age ≥ 55 years	14	-2.38 (-2.78, -1.99)	92.6% (p< 0.001)	6	0.19 (0.10, 0,25)	0.0% (p = 0.55)
BMI						
- BMI < 25 kg/m^2	4	-2.77 (-3.45, -2.08)	20.7% (p = 0.29)	2	-0.10 (-0.26, 0.07)	0.0% (p = 0.89)
- BMI ≥ 25 kg/m^2	19	-2.42 (-2.78, -2.06)	73.7% (p < 0.001)	11	0.16 (0.11, 0.22)	0.0% (p = 0.47)
PCOS status (women)						
- PCOS (women)	-	-	-	6	0.03 (-0.13, 0.18)	28.2% (p = 0.22)
- no PCOS (women)	-	-	-	9	0.17 (0.12, 0.23)	0.0 % (p = 0.66)
MetS criteria †						
- NCEP ATP III	16	-2.43 (-2.79, -2.08)		12	0.16 (0.10, 0.22)	
- Other (WHO, IDF, EGIR)	8	-3.90 (-4.83, -2.96)		0	-	
Control for age						
- adjusted for age	6	-2.87 (-3.68, -2.05)	79.5% (p < 0.001)	2	0.18 (0.05, 0.31)	36.3% (p = 0.21)
- not adjusted for age	20	-2.62 (-3.00, -2.23)	76.0% (p < 0.001)	13	0.13 (0.05, 0.20)	30.2% (p = 0.14)
Type 2 diabetes excluded						
- yes	7	-2,84 (-4.02, -1.66)	87.9% (p < 0.001)	2	0,10 (-0.09, 0.29)	54.8% (p = 0.14)
- no	19	-2,67 (-3.01, -2.32)	89.9% (p < 0.001)	13	0.15 (0.07, 0.22)	30.9% (p = 0.14)
Study design						
- cross-sectional	24	-2.64 (-.2.97, -2.32)	89.7% (p< 0.001)	14	0.14 (0.07, 0.21)	32.7% (p = 0.11)
- case-control	1	-9.43 (-14.59, -4.27)	-	1	0.10 (-0.07, 0.27)	-
- longitudinal	1	-2.20 (-2.82, -1.58)	-	-	-	-
	Studies No.	**SHBG mean difference (95% CI) (nmol/L)**	I^2	**Studies No.**	**SHBG mean difference (95% CI) (nmol/L)**	I^2
Overall random effects	19	-9.77 (-12.26, -7.29)	97.6% (p< 0.001)	15	-19.39 (-23.81, -14.98)	85.5% (p<0.001)
Age						
- Age < 55 years	10	-6.69 (-8,20, -5,19)	48.9% (p = 0.04)	11	-18.73 (-23.73, -13.73)	87.3% (p<0.001)
- Age ≥ 55 years	9	-12.00 (-15.13, -8.87)	98.2% (p< 0.001)	4	-21.42 (-31.76, -11.09)	76.5% (p = 0.01)
BMI						
- BMI < 25 kg/m^2	4	-10.36 (-17.50, 3.23)	93.7% (p<0.001)	3	-31.46 (-38.05, -24.86)	42.7% (p = 0.17)
- BMI ≥ 25 kg/m^2	13	-9.52 (-13.96, -5.08)	98.0% (p<0.001)	11	-16.07 (-20.64, -11.51)	83.2% (p < 0.001)
PCOS status (women)						
- PCOS (women)	-	-		7	-18.57 (-26.33, -10.82)	88.0% (p<0.001)
- no PCOS (women)	-	-		8	-20.41 (-26.15, -14.67)	83.9% (p < 0.001)
MetS criteria †						
- NCEP ATP IIII	10	-10.55 (-13.57, -7.53)		11	-17.94 (-23.01, -12.88)	
- Other (WHO, IDF, EGIR)	7	-7.17 (-10.25, -4.09)		0	-	
Control for age						
- adjusted for age	4	-12.19 (-21.34, -3.05)	97.1% (p<0.001)	2	-19.63 (-27.16, -12.11)	56.3% (p = 0.13)
- not adjusted for age	15	-9.02 (-11.70, -6.33)	95.2% (p<0.001)	13	-19.48 (-24,45, -14.51)	87.0% (p<0.001)
Type 2 diabetes excluded						
- yes	6	-7.04 (-8.59, -5.49)	46.7% (p = 0.10)	2	-10.97 (-13.65, -8.28)	0.0% (p = 0.98)
- no	13	-11.03 (-13.89, -8.17)	97.7% (p<0.001)	13	-21.19 (-26.32, -16.06)	83.6% (p<0.001)
Study design						
- cross-sectional	18	-10.11 (-12.65, -7.57)		14	-19.75 (-24.45, -15.05)	86.6% (p < 0.001)
- case-control	-	-	97.7% (p<0.001)	-	-	-
- longitudinal	1	-4.04 (-8.23, 0.15)	-	1	-15.50 (-23.12, -7.88)	-

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Table 3 Continued

	Studies No.	FT standardized mean difference (95% CI) (pmol/L)	I^2	Studies No.	FT standardized mean difference (95% CI) (pmol/L)	I^2
Overall random effects	13	-0.26 (-0.39; -0.13)	79.9% (p<0.001)	9	0.52 (0.33, 0.71)	61.1% (p=0.01)
Age						
- Age < 55 years	7	-0.41 (-0.51, -0.31)	32.9% (p = 0.18)	9	0.52 (0.33, 0.71)	61.1% (p = 0.01)
- Age ≥ 55 years	6	-0.09 (-0.29, 0.11)	79.3% (p<0.001)	0	-	
BMI						
- BMI < 25	3	-0.35 (-0.71, 0,02)	77.2% (p = 0.01)	2	0.71 (0.27, 1.15)	59.8% (p = 0.12)
- BMI ≥ 25 kg/m^2	9	-0.20 (-0.36, -0.04)	81.5% (p<0.001)	6	0.54 (0.33, 0.76)	51.2% (p = 0.07)
PCOS status (women)						
- PCOS (women)	-	-		7	0.49 (0.26, 0.73)	66.7% (p = 0.01)
- no PCOS (women)	-	-		2	0.64 (0.40, 0.88)	0% (p = 0.71)
MetS criteria †						
- NCEP ATP III	10	-0,19 (-0,34, -0,05)		6	0.50 (0.21, 0.79)	
- Other (WHO, IDF)	5	-0,47 (-0,55, -0,39)		0	-	
Control for age						
- adjusted for age	0	-	-	1	0.59 (0.26, 0.92)	-
- not adjusted for age	13	-0.26 (-0.39, -0.13)	79.9% (p< 0.001)	8	0.52 (0.31, 0.73)	64.9% (p = 0.01)
Type 2 diabetes excluded						
- yes	5	-0.29 (-0.53, 0.05)	87.8% (p<0.001)	3	0.50 (0.08, 0.91)	
- no	8	-0.23 (-0.40, -0.07)	74.1% (p<0.001)	2	0.31 (-0.26, 0.87)	
Study design						
- cross-sectional	13	-0.26 (-0.39; -0.13)	79.9% (p<0.001)	8	0.52 (0.31, 0.73)	64.9% (p = 0.01)
- case-control	0	-	-	1	0.59 (0.26, 0.92)	-
- longitudinal	0	-	-	0	-	-
Method of FT assessment						
- Direct measurement	3	-0.47 (-0.64, -0.30)	38.0% (p < 0.20)	6	0.57 (0.34, 0.80)	59.9% (p = 0.03)
- Algorithms	9	-0.18 (-0.34, -0.03)	81.7% (p< 0.001)	3	0.44 (-0.04, 0.84)	75.1% (p = 0.02)

CI, confidence interval; BMI, body mass index; FT, free testosterone; MetS, metabolic syndrome; No., number; PCOS, polycystic ovary syndrome; SHBG, sex hormone-binding globulin; TT, total testosterone; -, not applicable.

For Review Only

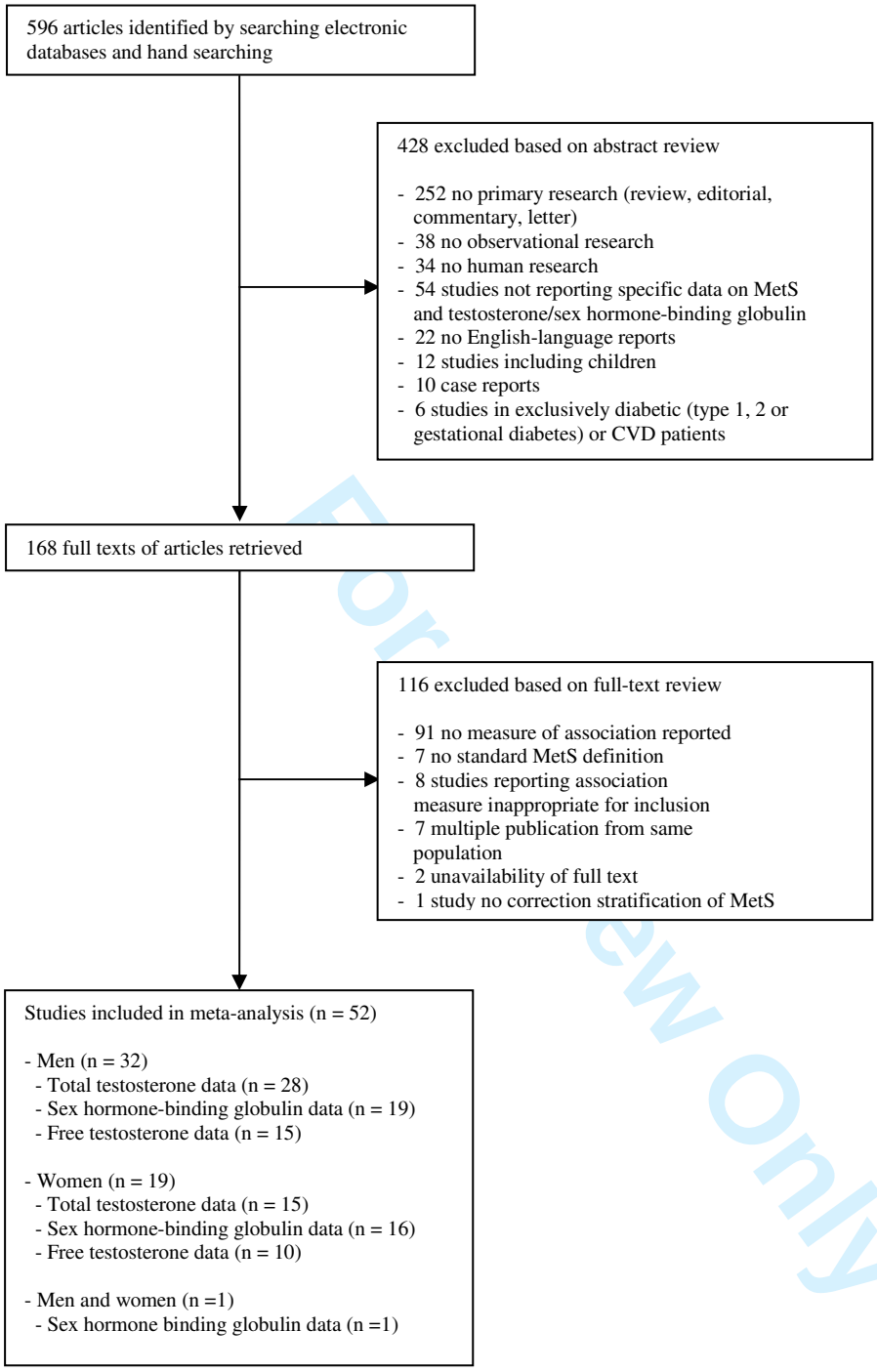

Figure 1 Flow diagram outlining the study selection process. MetS, metabolic syndrome; CVD, cardiovascular disease.

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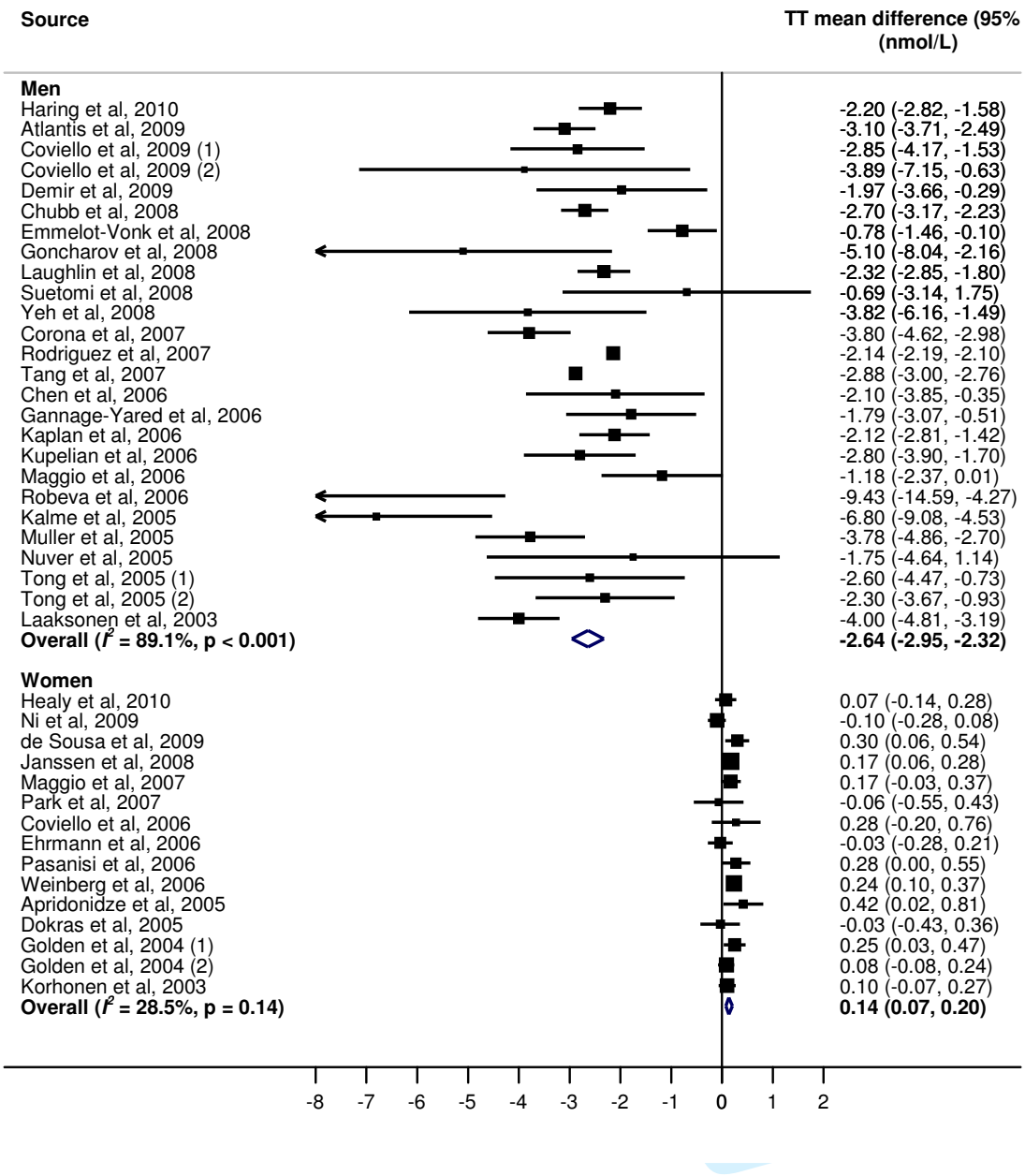

Figure 2A Random effects pooled mean difference of TT levels between subjects with and without MetS, men and women**.** CI, confidence interval; MetS, metabolic syndrome; TT, total testosterone. Negative values indicate lower TT levels in subjects with MetS; positive values indicate higher TT levels in subjects with MetS. Sizes of squares represent the weight of each study.

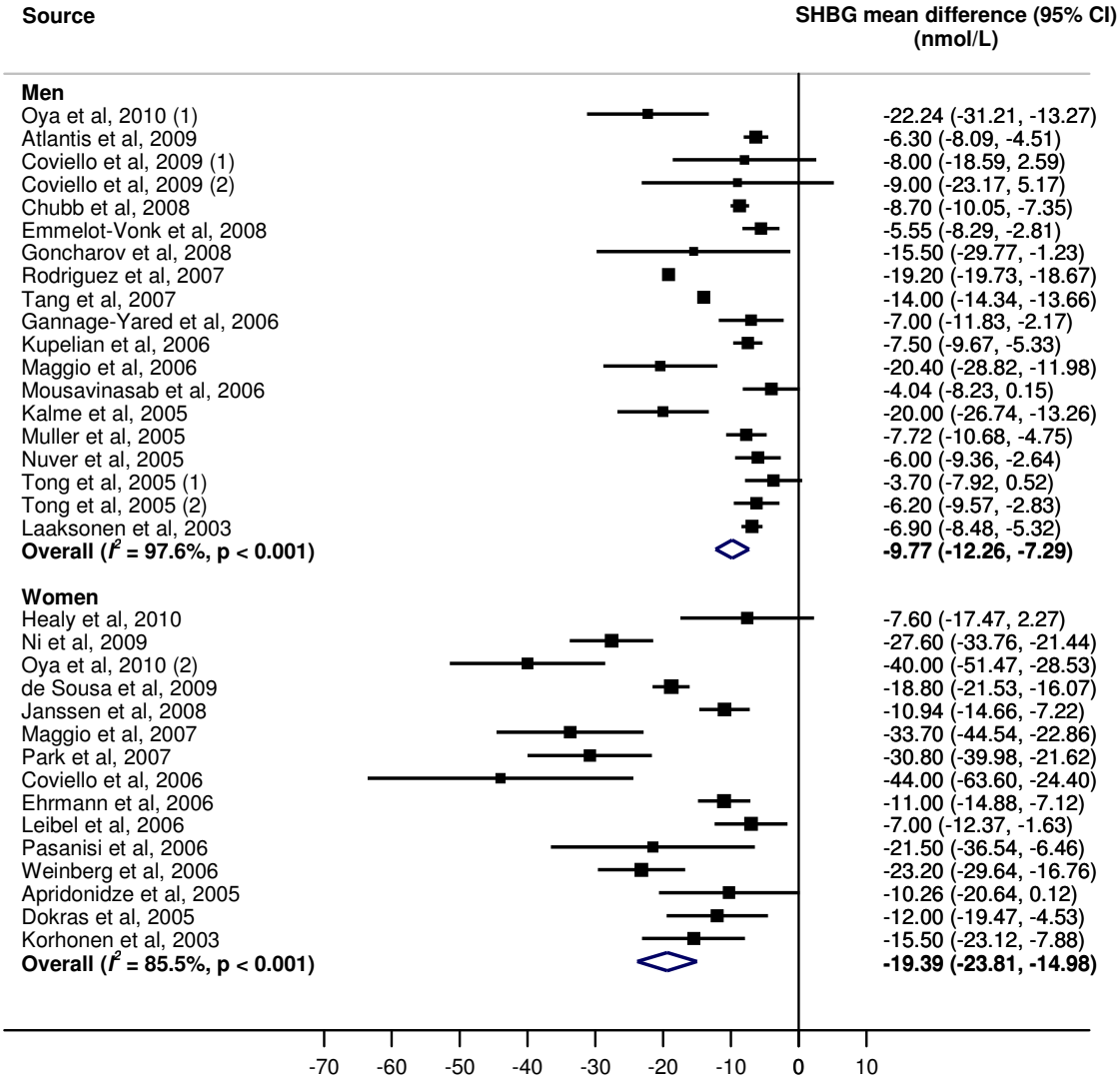

Figure 2B Random effects pooled mean difference of SHBG levels between subjects with and without MetS, men and women**.** CI, confidence interval; MetS, metabolic syndrome; SHBG, sex hormone-binding globulin. Negative values indicate lower SHBG levels in subjects with MetS; positive values indicate higher SHBG levels in subjects with MetS. Sizes of squares represent the weight of each study.

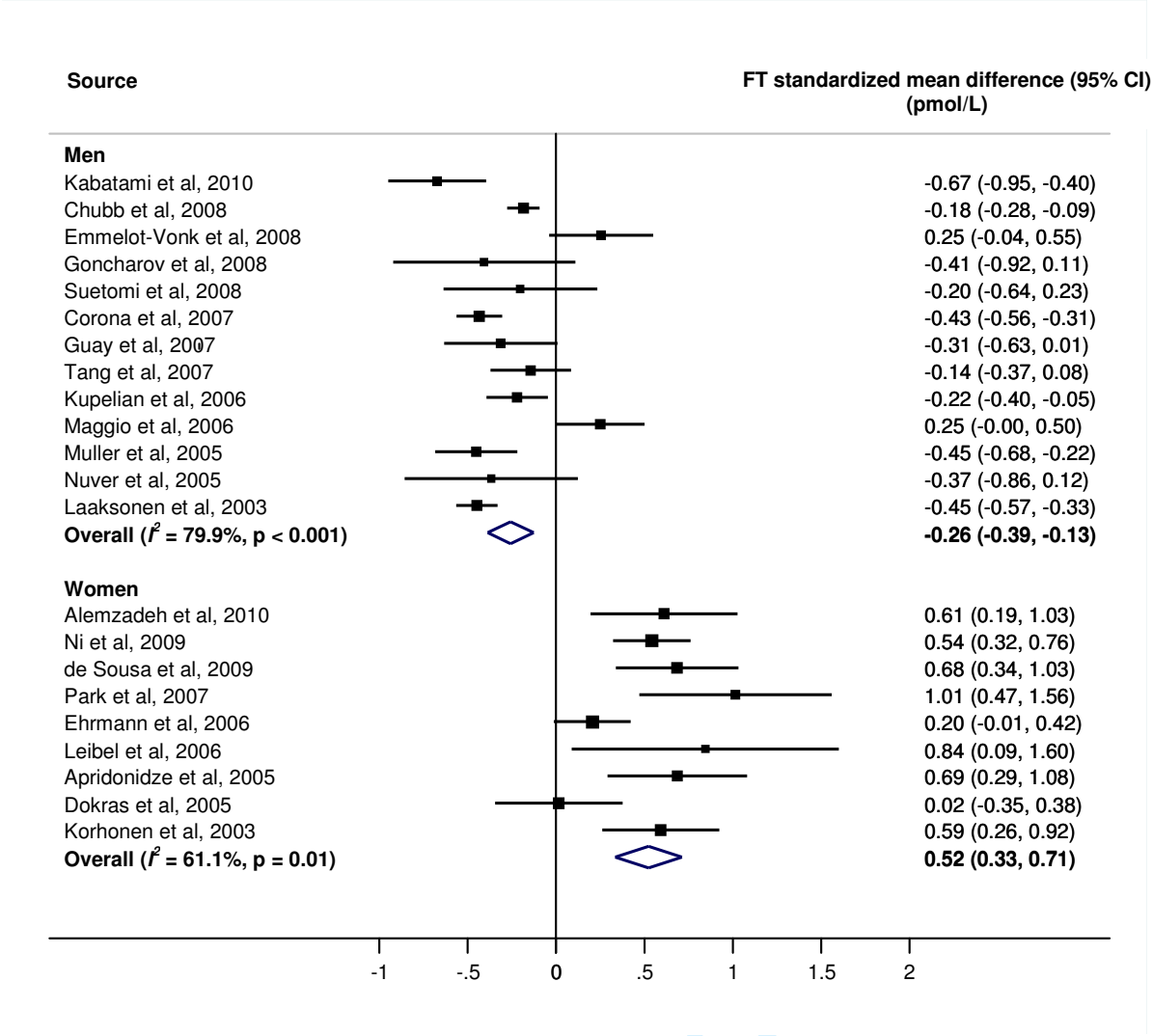

Figure 2C Random effects pooled mean difference of FT levels between subjects with and without MetS, men and women**.** CI, confidence interval; MetS, metabolic syndrome; FT, free testosterone. Negative values indicate lower FT levels in subjects with MetS; positive values indicate higher FT levels in subjects with MetS. Sizes of squares represent the weight of each study.

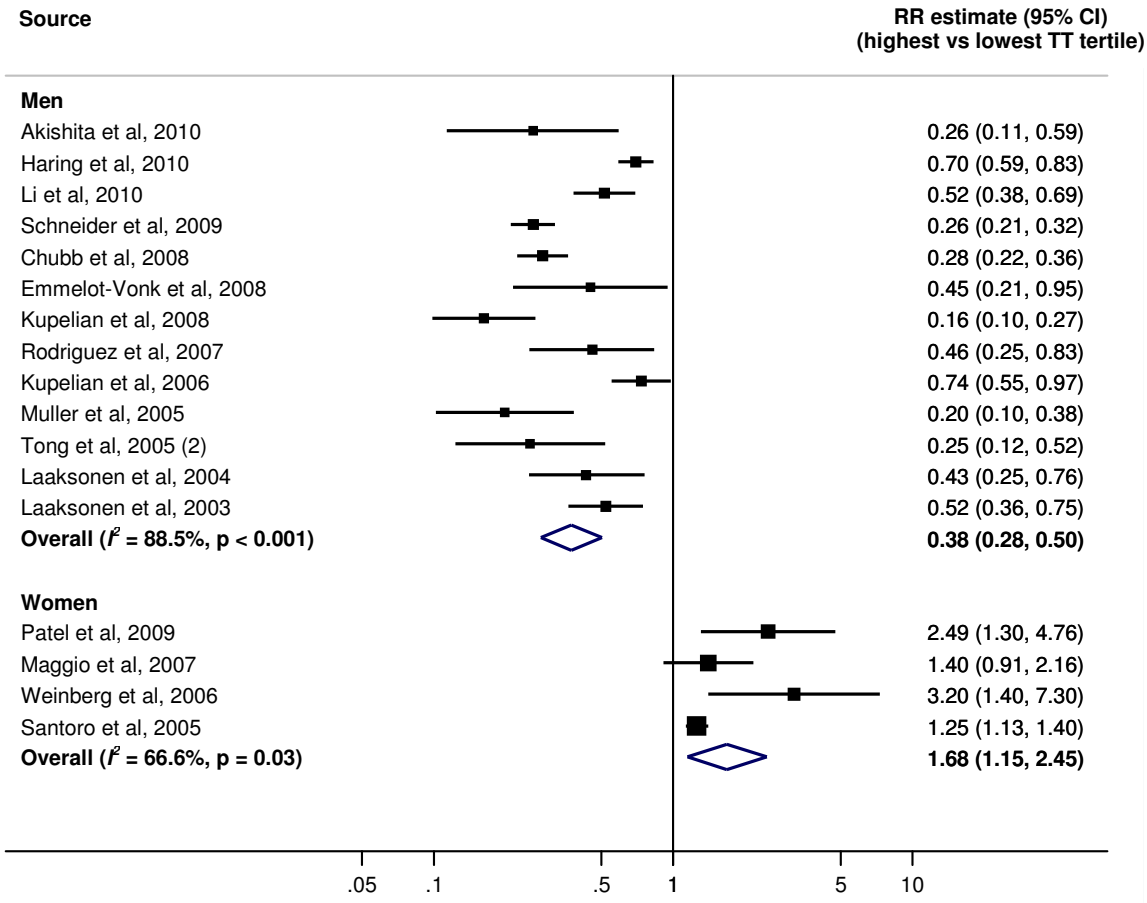

Figure 3A Random effects pooled relative risk estimate for MetS comparing highest versus lowest TT tertile, men and women. CI, confidence interval; MetS, metabolic syndrome; TT, total testosterone. Sizes of squares represent the weight of each study.

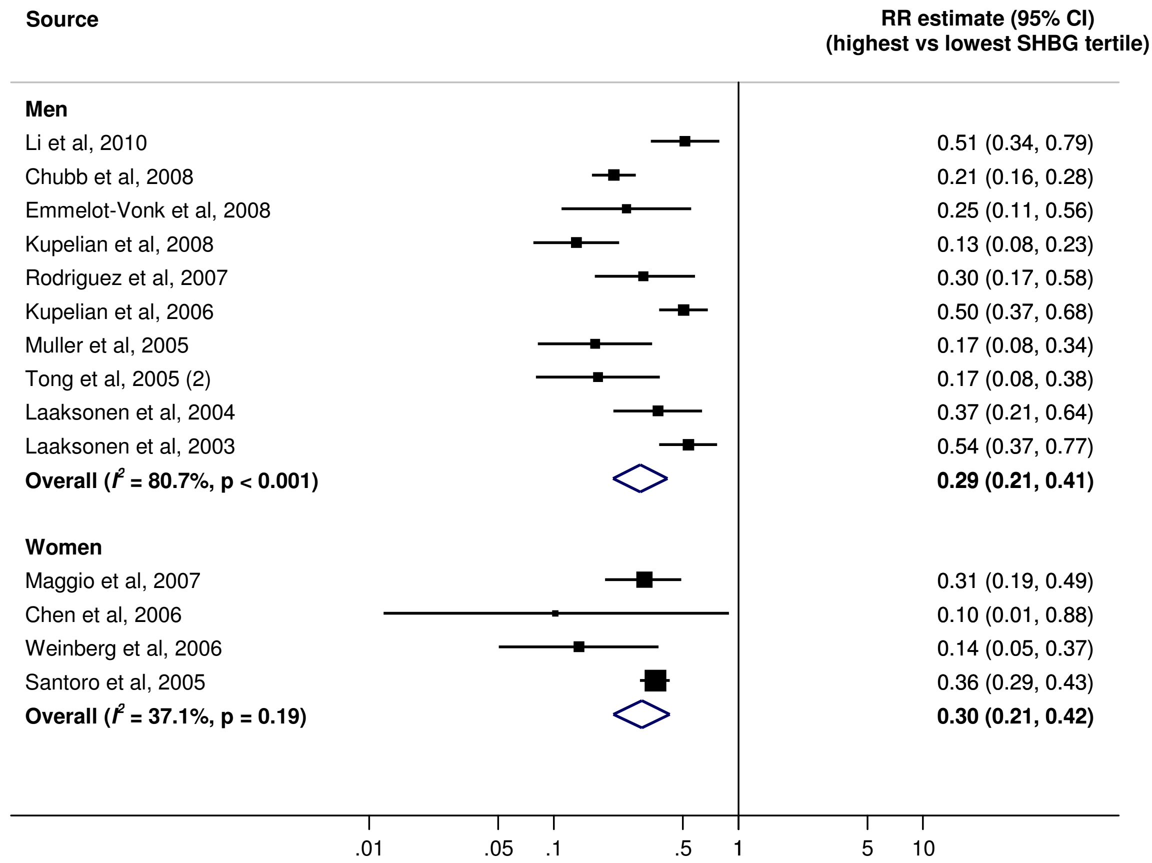

Figure 3B Random effects pooled relative risk estimate for MetS comparing highest versus lowest tertile of SHBG, men and women. CI, confidence interval; MetS, metabolic syndrome; SHBG, sex hormone-binding globulin. Sizes of squares represent the weight of each study.

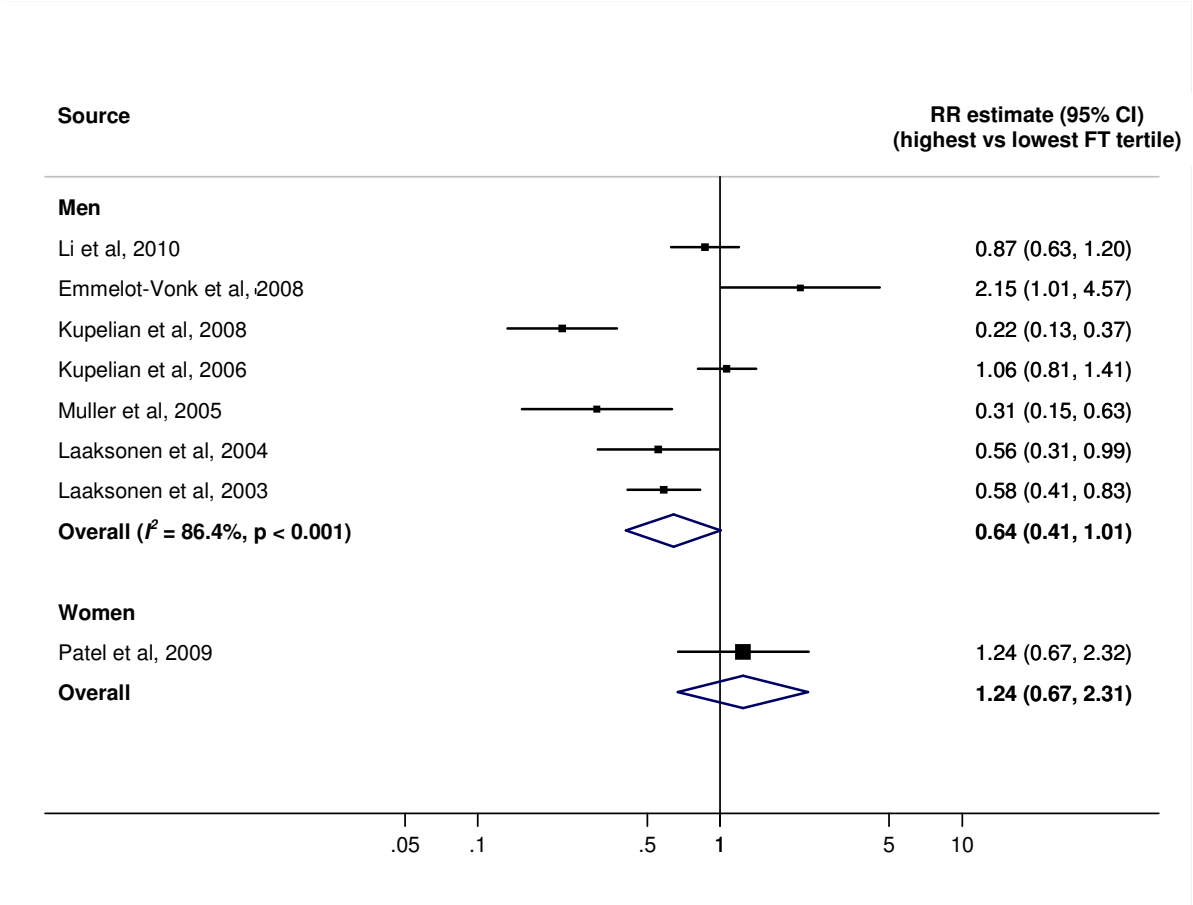

Figure 3B Random effects pooled relative risk estimate for FT comparing highest versus lowest tertile of FT, men and women. CI, confidence interval; MetS, metabolic syndrome; FT, free testosterone. Sizes of squares represent the weight of each study.