Testosterone, Sex Hormone-Binding Globulin and Nonalcoholic Fatty Liver Disease: A Systematic Review and Meta-Analysis

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ABSTRACT

Introduction and aim. Endogenous sex hormones are associated with the risk of diabetes and metabolic syndrome. Recent studies suggested the role of these hormones in nonalcoholic fatty liver disease (NAFLD). We conducted a systematic review and meta-analysis of observational studies investigating the association between sex hormones and NAFLD. **Material and methods.** A comprehensive search of the databases of the MEDLINE and EMBASE was performed from inception through April 2016. The inclusion criterion was the observational studies that assessed the association of serum total testosterone (TT) and sex-hormone binding globulin (SHBG) and NAFLD. We calculated pooled effect estimates of TT and SHBG with 95% confidence intervals (CI) comparing between subjects with and without NAFLD by using random-effects model. **Results.** Sixteen trials comprising 13,721 men and 5,840 women met the inclusion criteria. TT levels were lower in men with NAFLD (MD = -2.78 nmol/l, 95%CI -3.40 to -2.15, I^2 = 99%) than in those without. Men with higher TT levels had lower odds of NAFLD whereas higher TT levels increased the odds of NAFLD in women. In both sexes, SHBG levels were lower in patients with NAFLD than controls and this inverse association was stronger in women than men and higher SHBG levels were associated with reduced odds of NAFLD. **Conclusion.** Our meta-analysis demonstrated a sex-dependent association between TT and NAFLD. Lower TT levels are associated with men with NAFLD and inversely associated with women with NAFLD, whereas higher SHBG levels are associated with lower NAFLD odds in both men and women.

Key words. Meta-analysis. Non-Alcoholic Fatty Liver Disease. Sex hormone. Sex-hormone binding globulin. Testosterone.

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is characterized by hepatic triglyceride accumulation in the absence of excess alcohol intake. This condition ranges from simple steatosis to nonalcoholic steatohepatitis (NASH) that could progress to cirrhosis and hepatocellular carcinoma.[1-3] NAFLD is one of the most common causes of chronic liver disease and its prevalence has increased substantially throughout the world affecting 10 to 24 percent of general population from several countries[1,3] along with the growing epidemic of obesity and diabetes mellitus (DM). As NAFLD is known as the hepatic manifestation of metabolic syndrome, it is also associated with increased insulin resistance, visceral adipose tissue (VAT) and also the risk of cardiovascular disease.[4-7]

Previous studies have demonstrated the effect of endogenous total testosterone (TT) on the insulin resistance. In men, TT deficiency has been associated with increased accumulation of VAT and insulin resistance and the lower TT levels are associated with obesity[8-11] whereas hyperandrogenemia has been linked with increased VAT and insulin resistance in women.[9,12-15] In addition, sex hormone-binding globulin (SHBG), which is the circulating glycoprotein secreted from the liver and binds androgens to regulate their bioavailability, has been

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negatively associated with metabolic syndrome, insulin resistance and cardiovascular disease in both sexes.[16,17]

Both TT and SHBG were found to be associated with insulin resistance and adiposity, which are the contributing factors of metabolic syndrome. Several studies exploring the association between these hormones and NAFLD have demonstrated inconsistent results. Therefore, to better characterize this possible association, we conducted a systematic review and meta-analysis of all published observational studies relating TT, SHBG with NAFLD.

MATERIAL AND METHODS

Study selection and inclusion criteria

This systematic review and meta-analysis was conducted and reported according to the Meta-analysis Of Observational Studies in Epidemiology statement[18] and was registered in PROSPERO (registration number: CRD42016037921). Two authors (SU and VJ) independently searched published studies indexed in MEDLINE and EMBASE databases from date of inception to April 2016. The full search strategy was detailed in figure 1. A manual search of references of selected retrieved articles was also performed. To assess the quality of all studies, review articles, case reports, abstracts, and unpublished studies were excluded.

Our inclusion criteria were:

- Published observational studies including cross-sectional, cohort, and case-control studies assessing the association between the level of serum SHBG and/or TT and NAFLD.
- Participants aged 18 years or older.
- Odd ratios (OR), relative risks (RR) or hazard ratios (HR) were provided or sufficient raw data to calculate those ratios were provided.
- Participants without NAFLD were used as a reference group.

NAFLD was diagnosed using the definition by each study including liver function, imaging study or liver biopsy.

Two authors (SU and VJ) independently reviewed titles and abstracts of all citations that were identified. After all abstracts were reviewed, data comparisons between the two investigators were conducted to ensure completeness and reliability. The inclusion criteria were independently applied to all identified studies. Differing decisions were resolved by consensus between the two authors. Full-text versions of potentially relevant papers identified in the initial screening were retrieved. If multiple articles from the same study were found, only the article with the most complete data was included.
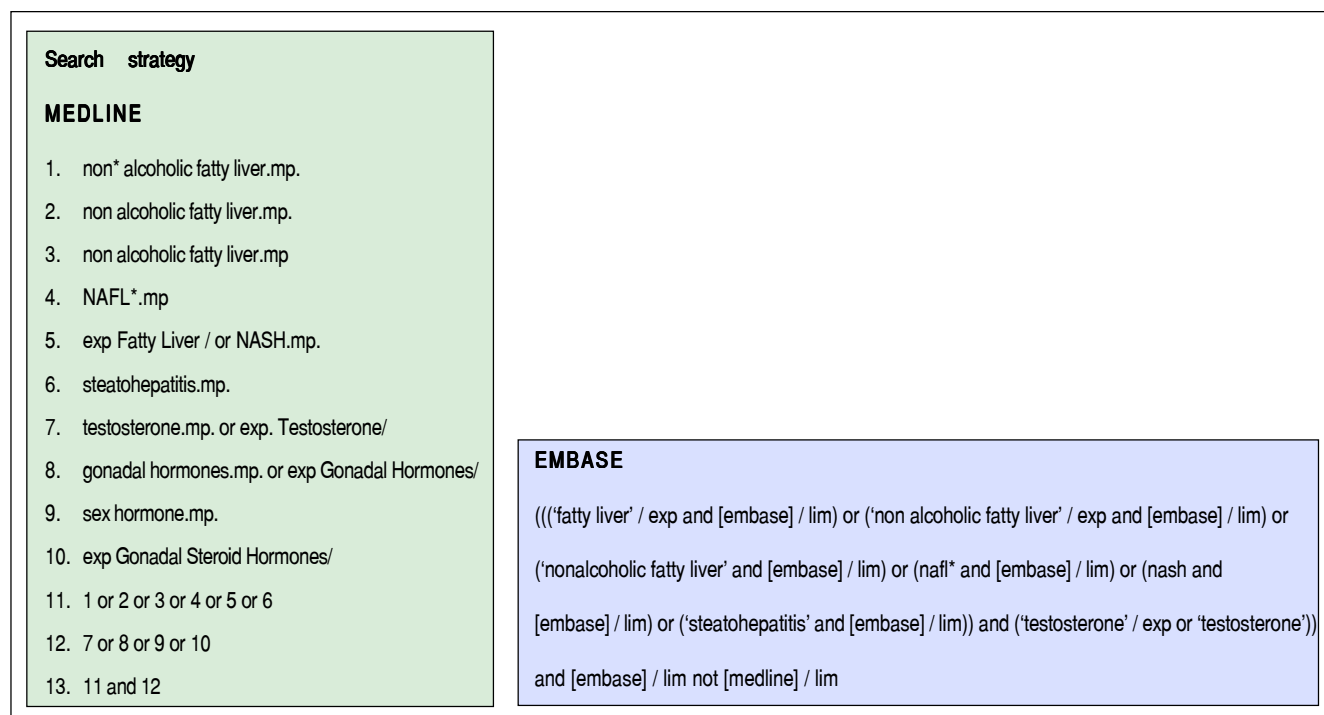
Search strategy

MEDLINE

1. non* alcoholic fatty liver.mp.
2. non alcoholic fatty liver.mp.
3. non alcoholic fatty liver.mp
4. NAFL*.mp
5. exp Fatty Liver / or NASH.mp.
6. steatohepatitis.mp.
7. testosterone.mp. or exp. Testosterone/
8. gonadal hormones.mp. or exp Gonadal Hormones/
9. sex hormone.mp.
10. exp Gonadal Steroid Hormones/
11. 1 or 2 or 3 or 4 or 5 or 6
12. 7 or 8 or 9 or 10
13. 11 and 12

EMBASE

((('fatty liver' / exp and [embase] / lim) or ('non alcoholic fatty liver' / exp and [embase] / lim) or ('nonalcoholic fatty liver' and [embase] / lim) or (nafl* and [embase] / lim) or (nash and [embase] / lim) or ('steatohepatitis' and [embase] / lim)) and ('testosterone' / exp or 'testosterone')) and [embase] / lim not [medline] / lim

Figure 1. Search strategy.

Data extraction and quality assessment

Using a standardized data extraction form, data concerning author, year of publication, study design, study location, participant characteristics, diagnosis ascertainment of NAFLD, effect estimates with 95%CI, and quality assessment were independently extracted by two authors. Discrepancies were resolved with group discussions. We contacted the authors of the primary reports to request any unpublished data. If the authors did not reply, we used the available data for our analyses.

A subjective assessment of methodological quality for observational studies was evaluated by two authors (SU and VJ) using the Newcastle-Ottawa Scale (NOS). The NOS is a quality assessment tool for non-randomized studies. A total score of 3 or less was considered poor, 4-6 was considered moderate, and 7-9 was deemed high quality.[19] We excluded poor quality studies in the sensitivity analysis.

Statistical analysis

Measures of association were analyzed for men and women, separately. To compare TT and SHBG levels between subjects with and without NAFLD, pooled analyses were performed using unstandardized mean difference (MD). The heterogeneity of effect size estimates across these studies was quantified using the Q statistic, its p-value, and I^2 (P < 0.10 was considered significant). A value of I^2 of 0-25% indicates insignificant heterogeneity, 26-50% low heterogeneity, 51-75% moderate heterogeneity and 76-100% high heterogeneity.[20] Clinical heterogeneity was assessed by grouping trials within each sex according to age of participants, BMI, menopausal status, study design and study quality. For these analyses, studies were stratified according to mean age (< 55 *vs.* ≥ 55), mean BMI (< 30 *vs.* ≥ 30), menopausal status (premenopausal *vs.* postmenopausal), study design (case-control *vs.* cross-sectional) and study quality (high *vs.* moderate). Furthermore, We used the techniques of meta-regression to quantitatively analyze which components of NAFLD best explained variance in sex hormone levels. If studies reported only median and interquartile range, we used the formula by Hozo, *et al.*[21] to estimate mean value and standard deviation. Adjusted point estimates were combined using a random-effects model because the heterogeneity was high. Publication bias was assessed using funnel plot, Egger's regression test and its implications with the trim and fill method.[22] Insufficient numbers of sex-stratified populations and inconsistent methods precluded the meta-analysis of free testosterone in this study. Test for interaction was applied to identify the difference between MD from subgroup analysis. All analyses were conducted using Comprehensive Meta-Analysis 3.3 software from Biostat, Inc.
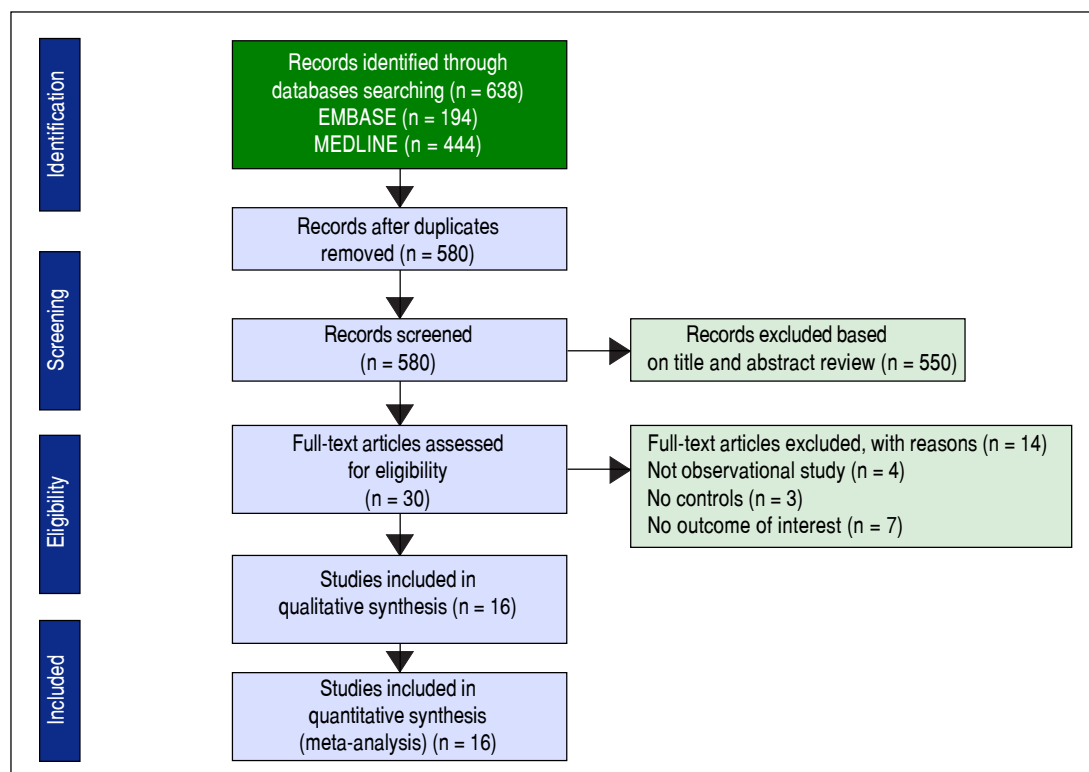

Figure 2. Search methodology and selection process.

Identification

- Records identified through databases searching (n = 638) EMBASE (n = 194) MEDLINE (n = 444)

Screening

- Records after duplicates removed (n = 580)
- Records screened (n = 580)
  - Records excluded based on title and abstract review (n = 550)

Eligibility

- Full-text articles assessed for eligibility (n = 30)
  - Full-text articles excluded, with reasons (n = 14) Not observational study (n = 4) No controls (n = 3) No outcome of interest (n = 7)

Included

- Studies included in qualitative synthesis (n = 16)
- Studies included in quantitative synthesis (meta-analysis) (n = 16)

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Table 1. Characteristics of studies included in the meta-analysis.

Study	Country	Study design	Participants	Diagnosis of NAFLD	Quality (NOS) (Selection, Comparability, outcome)	Sex	Total number	BMI	Mean age (years)	NAFLD (%)	TT (mean (SD), nmol/L)		SHBG (mean (SD), nmol/L)	
											Cases	Controls	Cases	Controls
Ayonrinde, et al. 2015[23]	Australia	CS	Adolescents from the Western Australian Pregnancy Cohort Study	USG	5,1,3	Women	167	F 30.3	15.3	15	1.06 (0.90)	1.16 (0.80)	53.6 (20.7)	57.3 (27.1)
Barbonetti, et al. 2016[24]	Italy	CS	Men with chronic spinal cord injury	USG	2,0,3	Men	55	M 26.8	52	49.1	261.6 (159.5)	505.7 (185.6)	16.4 (15.3)	18.3 (11.3)
Corbould, et al. 2014[25]	Australia	CS	Obese premenopausal women undergoing elective gastric band surgery	Biopsy	3,2,3	Women	22	F 44.9	36.3	54.5	1.0 (0.3)	0.9 (0.2)	30.7 (6.9)	32.7 (4.4)
Flechtner-Mors, et al. 2013[26]	Germany	CS	Population-based study from the EMIL Study (*Echinococcus Multilocularis* and other medical disorders in Leutkirch)	USG	4,0,5	Men	787	M 26.2	42.2	36.5	NA	NA	NA	NA
						Women	870	F 24.9	42.3	18.3	NA	NA	NA	NA
Hua, et al. 2014[36]	China	CC	Patients with type 2 diabetes	USG	3,0,4	Men	160	M and F 25.3	56.8	50	9.9 (3.4)	12.6 (5.5)	24.9 (10.4)	35.9 (13.0)
						Women	80			50	0.8 (0.2)	0.7 (0.1)	23.8 (12.1)	41.1 (16.5)
Kauffman, et al. 2010[27]	USA	CS	Healthy subjects with PCOS	USG	3,1,3	Women	53	37.9	30	39.6	NA	NA	17.0 (4.15)	24.0 (2.1)
Kim, et al. 2012[28]	Korea	CS	Healthy Korean men who visited hospital for routine health checkups	USG	4,1,1	Men	495	26.1	53.85	50.7	13.67 (1.01)	16.76 (1.28)	NA	NA
Lazo, et al. 2015[29]	USA	CS	Postmenopausal women and men from Multiethnic Study of Atherosclerosis (MESA)	CT	5,1,3	Men	2,899	M 31.9	58.7	5.3	12.7 (1.07)	14.2 (1.07)	32.7 (6.83)	40.9 (3.55)
						Women	2,835	F 32.9	61.2	5.6	1.1 (0.12)	0.9 (0.12)	40.8 (5.9)	59.8 (8.85)
Li, et al. 2015[30]	China	CS	Men undergoing routine assessment of their liver	USG	5,0,3	Men	196	M 23.6	35.4	52	13.0 (4.1)	17.4 (3.9)	18.3 (4.5)	29.7 (5.7)
Polyzos, et al. 2013[37]	Greece	CC	Postmenopausal women were recruited from an outpatient basis	USG	1,2,4	Women	40	F 32	55	55	0.85 (0.13)	1.02 (0.16)	30.8 (5.33)	57.7 (6.75)
Seo, et al. 2015[31]	Korea	CS	Voluntarily participated subjects in the Health Promotion Center	USG	3,1,3	Men	1,944	M 25.2	44	44.3	14.5 (1.03)	17.2 (1.17)	NA	NA

Study	Country	Design	Setting	Imaging	NOS	Sex	BMI	N	%	Age				
Shin, et al. 2011[32]	Korea	CS	Patients with type 2 DM were enrolled in an outpatient setting	USG	5,1,3	Men	M and F 26.8	87	81.6	57	10.76 (0.97)	13.19 (1.39)	1.04 (0.17)	1.04 (0.17)
						Women		61	83.6		32.0 (13.1)	47.9 (18.9)	38.0 (22.3)	64.9 (28.3)
Tian, et al. 2012[33]	China	CS	A population-based survey conducted among non-institutionalized Chinese men	USG	3,1,3	Men	M 23.6	1,882	30.4	46.4	20.51 (1.32)	20.99 (1.42)	43.14 (4.94)	36.73 (3.71)
Vassilatou, et al. 2010[38]	Greece	CC	Premenopausal patients who attended outpatient endocrine clinic	USG	3,1,3	Women	F 28.3	60	20	27.6	1.24 (0.48)	1.31 (0.48)	31.7 (14.0)	52.0 (21.2)
Volzke, et al. 2010[34]	Germany	CS	Population-based study in West Pomerania, Germany	USG	3,1,5	Men	M 29.1	1,912	37.9	56	14.2 (1.08)	17.2 (1.23)	NA	NA
Wang, et al. 2016[35]	China	CS	Population-based survey in East China	USG	5,0,3	Men	M 26.6	2,098	41.1	51	14.0 (4.7)	17.7 (6.1)	34.7 (17.7)	54.1 (27.1)
						Women	F 27.4	1,131	33.4	63	0.7 (0.6)	0.6 (0.4)	49.4 (21.1)	80.4 (35.2)

BMI: Body mass index. CC: Case control. CS: Cross-sectional. NA: Non available. NOS: Newcastle Ottawa scale. PCOS: Polycystic ovary syndrome. SHBG: Sex-hormone binding globulin. TT: Total testosterone. USG: Liver ultrasonography.

RESULTS

Description of included studies

The initial search yielded 580 articles; 550 articles were excluded based on title and abstract review. A total of 30 articles underwent full-length review. Fourteen articles were excluded (4 articles were not observational studies, 3 articles did not have control group, and 7 articles did not report outcome of interest). Data was extracted from 16 observational studies (13 cross-sectional studies[23-35] and 3 case-control studies[36-38]). Figure 2 outlines the search methodology and selection process. Table 1 describes the detailed characteristics and quality assessment of included studies. NAFLD was ascertained by either imaging studies or biopsy by all included studies. None of the studies used only liver function to diagnose NAFLD.

TT

Studies assessing TT levels in subjects with and without NAFLD involving 12,388 men (10 studies[24, 28-36]) and 4,855 women (7 studies[23,29,32,35-38]) were included in the meta-analysis. Men with NAFLD had significantly lower levels of TT with MD of -2.78 nmol/l (95%CI -3.40 to -2.15, $I^2 = 99\%$, $P_{heterogeneity} < 0.01$), whereas women with NAFLD did not show significant difference of TT levels with MD of 0.03 (-0.08 to 0.14, $I^2 = 93\%$, $P_{heterogeneity} = 0.01$) compared to those without NAFLD (Figure 3). Substantial between-study heterogeneity was observed in both men ($I^2 = 99\%$) and women ($I^2 = 93\%$). Subgroup analyses were performed based age of participants, BMI, menopausal status, study design and study quality. In men, the association tended to be more pronounced in subgroup of mean age < 55 years, BMI < 30 kg/m^2 and studies with moderate quality. The test for interaction showed significant results between study groups of the mean age of participants, BMI, and study design. These results indicated that the mean age of participants, BMI, and study design were partly the reason why there was high heterogeneity in the overall analysis (Table 2). However, meta-regression found no significant association between BMI and TT levels in both men (beta coefficient = 0.052, P-value = 0.72), and women (beta coefficient = -0.006, P-value = 0.80).

Analysis of OR estimates for TT levels in NAFLD comprised 4,715 men (5 studies[28,31,32,34,36]) and 1,581 women (3 studies[35-37]). Pooled analysis showed a reduced odds of NAFLD in men with higher TT levels with pooled OR of 0.56 (95%CI 0.39 to 0.80, $I^2 = 92\%$, $P_{heterogeneity} = 0.07$) whereas an opposite association was observed in women with pooled OR of 1.40 (95%CI 1.11 to 1.77, $I^2 = 21\%$, $P_{heterogeneity} < 0.01$) (Figure 4).
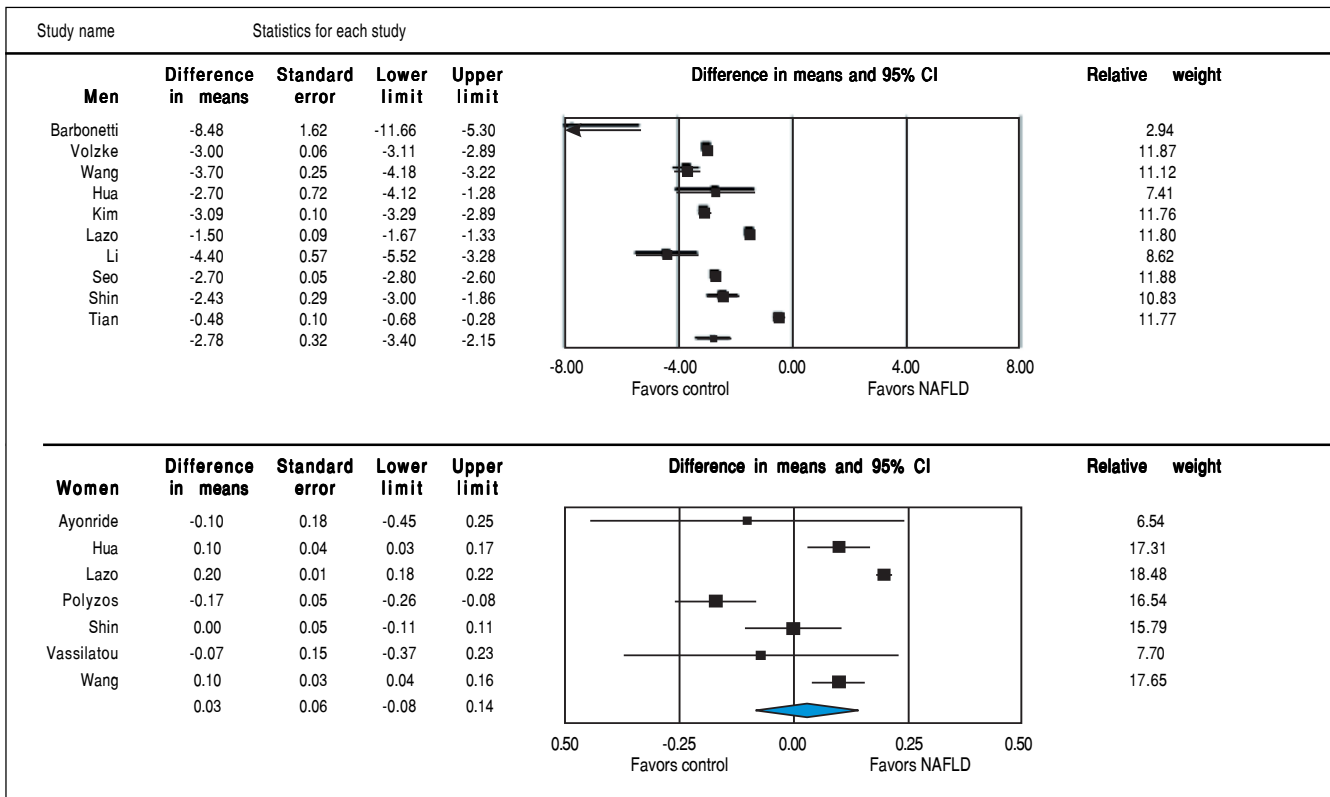
Study name	Statistics for each study				Difference in means and 95% CI	Relative weight
Men	Difference in means	Standard error	Lower limit	Upper limit		
Barbonetti	-8.48	1.62	-11.66	-5.30		2.94
Volzke	-3.00	0.06	-3.11	-2.89		11.87
Wang	-3.70	0.25	-4.18	-3.22		11.12
Hua	-2.70	0.72	-4.12	-1.28		7.41
Kim	-3.09	0.10	-3.29	-2.89		11.76
Lazo	-1.50	0.09	-1.67	-1.33		11.80
Li	-4.40	0.57	-5.52	-3.28		8.62
Seo	-2.70	0.05	-2.80	-2.60		11.88
Shin	-2.43	0.29	-3.00	-1.86		10.83
Tian	-0.48	0.10	-0.68	-0.28		11.77
	-2.78	0.32	-3.40	-2.15		

Study name					Difference in means and 95% CI	Relative weight
Women	Difference in means	Standard error	Lower limit	Upper limit		
Ayonride	-0.10	0.18	-0.45	0.25		6.54
Hua	0.10	0.04	0.03	0.17		17.31
Lazo	0.20	0.01	0.18	0.22		18.48
Polyzos	-0.17	0.05	-0.26	-0.08		16.54
Shin	0.00	0.05	-0.11	0.11		15.79
Vassilatou	-0.07	0.15	-0.37	0.23		7.70
Wang	0.10	0.03	0.04	0.16		17.65
	0.03	0.06	-0.08	0.14		

Figure 3. *Random effects pooled mean difference of TT in subjects with and without NAFLD in men and women.*

Study name	Odds ratio	Lower limit	Upper limit	Odds ratio and 95% CI	Relative weight
Men					
Hua	0.24	0.05	1.21		4.21
Kim	0.22	0.10	0.48		12.41
Seo	0.69	0.61	0.78		29.86
Shin	0.96	0.90	1.02		30.67
Volzke	0.40	0.27	0.59		22.85
	0.56	0.39	0.80		

Study name	Odds ratio	Lower limit	Upper limit	Odds ratio and 95% CI	Relative weight
Women					
Hua	1.04	0.16	6.72		1.58
Polyzos	1.25	1.01	1.55		62.27
Wang	1.72	1.23	2.40		36.15
	1.40	1.11	1.77		
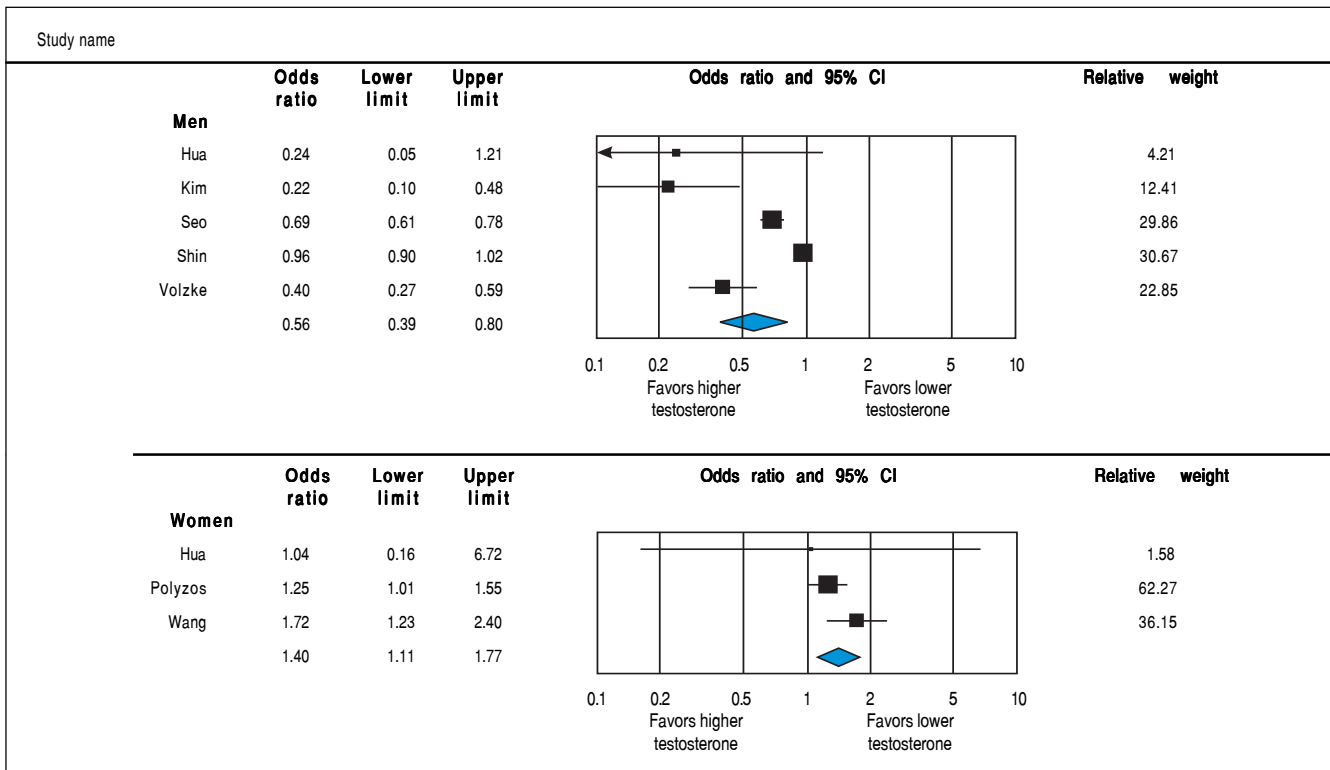
Figure 4. *Random effects pooled Odd Ratio of TT in subjects with and without NAFLD in men and women.*

Table 2. Subgroup analyses of included studies.

	Studies (n)	Men TT mean difference (95%CI) (nmol/l)	I² (%) and (P)	P-interaction	Studies (n)	Women TT mean difference (95%CI) (nmol/l)	I² (%) and (P)	P-interaction
Overall random effects	10	-2.78 (-3.40 to -2.15)	99 (< 0.01)		7	0.03 (-0.08 to 0.14)	93 (0.01)	
Age (years)								
<55	6	-2.88 (-3.77 to -2.00)	99 (< 0.01)	<0.01	3	0.06 (-0.17 to 0.29)	65 (0.05)	0.71
≥ 55	3	-2.81 (-3.23 to -2.39)	48 (0.15)		4	0.01 (-0.11 to 0.13)	89 (0.03)	
BMI (kg/m²)								
< 30	9	-2.94 (-3.60 to -2.28)	99 (< 0.01)	<0.01	4	0.08 (0.03 to 0.13)	22 (0.28)	0.57
≥ 30	1	-1.50 (-1.67 to 1.33)	NA		3	-0.01 (-0.32 to 0.29)	97 (0.08)	
Menopausal status								
Premenopause	NA	NA	NA	NA	1	-0.07 (-0.37 to 0.23)	NA	0.09
Postmenopause	NA	NA	NA		2	0.15 (0.06 to 0.25)	90 (< 0.01)	
Study design								
CS	9	-2.78 (-3.44 to -2.13)	99 (< 0.01)	0.79	4	0.09 (-0.01 to 0.20)	88 (0.02)	< 0.01
CC	1	-2.70 (-4.12 to 1.28)	NA		3	-0.04 (-0.26 to 0.18)	91 (0.01)	
Study quality								
High	8	-2.54 (-3.25 to -1.84)	99 (< 0.01)	<0.01	7	0.03 (-0.08 to 0.14)	93 (0.01)	< 0.01
Moderate	2	-5.54 (-10.80 to -0.28)	91 (< 0.01)		NA	NA	NA	

	Studies (n)	Men SHBG mean difference (95%CI) (nmol/l)	I² (%) and (P)	P-interaction	Studies (n)	Women SHBG mean difference (95%CI) (nmol/l)	I² (%) and (P)	P-interaction
Overall random effects	7	-8.72 (-16.70 to -0.75)	99 (< 0.01)		9	-17.05 (-23.58 to -10.53)	97 (< 0.01)	
Age (years)								
< 55	4	-6.63 (-20.65 to 7.40)	99 (< 0.01)	<0.01	4	-7.99 (-14.09 to -1.88)	80 (< 0.01)	< 0.01
≥ 55	3	-10.23 (-13.65 to -6.81)	66 (0.05)		5	-23.99 (-30.07 to -17.92)	91 (< 0.01)	
BMI (kg/m²)								
< 30	6	-8.83 (-20.11 to 2.44)	99.5 (< 0.01)	<0.01	4	-23.84 (-31.94 to -15.73)	81 (< 0.01)	< 0.01
≥ 30	1	-8.20 (-8.82 to -7.58)	NA		5	-12.25 (-20.48 to -4.02)	98 (< 0.01)	
Menopausal status								
Premenopause	NA	NA	NA	NA	4	-7.99 (-14.09 to 1.88)	80 (0.02)	< 0.01
Postmenopause	NA	NA	NA		3	-25.48 (-33.38 to -17.58)	95 (0.04)	
Study design								
CS	6	-8.34 (-17.01 to 0.33)	99 (< 0.01)	< 0.01	7	-15.45 (-23.03 to -7.88)	97 (< 0.01)	< 0.01
CC	1	-11.00 (-14.65 to -7.35)	NA		2	-22.46 (-31.84 to -13.07)	85 (0.01)	
Study quality								
High	6	-9.77 (-18.37 to -1.17)	99 (< 0.01)	0.98	9	-17.05 (-23.58 to -10.53)	97 (< 0.01)	
Moderate	1	-1.90 (-8.99 to 5.19)	NA		NA	NA	NA	

BMI: Body mass index. CC: Case-control. CS: Cross-sectional. NA: Not applicable. NAFLD: Non-alcoholic fatty liver disease. SHBG: Sex-hormone binding globulin. TT: Total testosterone.

To investigate potential publication bias, we examined the contour-enhanced funnel plot of the included studies that assessed mean difference of TT in both men and women (Figure 5). For both men and women, the plots exclude bias since there are symmetrical distributions of studies on both sides of the mean. Furthermore, the Egger's test was non-significant (P = 0.97 and 0.039, respectively). Using the trim and fill methods in the random-effects model, there was no difference of the imputed mean difference.

SHBG

Studies assessing SHBG levels in subjects with and without NAFLD involving 7,841 men (7 studies[24,29,30,32,33,35,36]) and 4,930 women (9 studies[23,25,27,29,32,35-38]) were included in
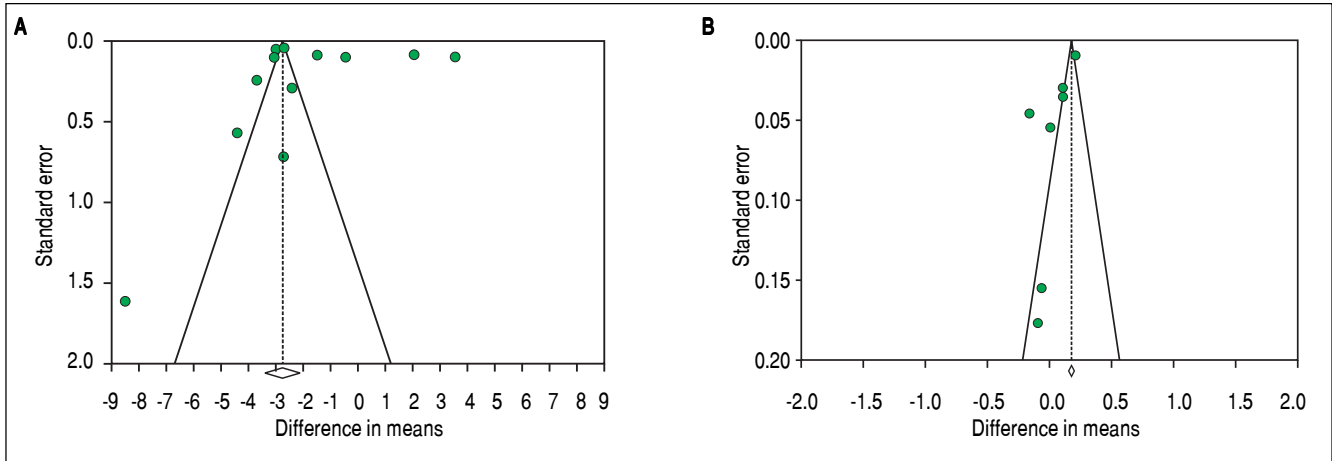
Figure 5. *Funnel plots of all included studies. Circles represent observed published studies. Funnel plots of standard error by diference in means.*

Study name		Statistics for each study				Difference in means and 95% CI	Relative weight
Men	Difference in means	Standard error	Lower limit	Upper limit			
Barbonetti	-1.90	3.62	-8.99	5.19		13.30
Hua	-11.00	1.86	-14.65	-7.35		14.42
Lazo	-8.20	0.32	-8.82	-7.58		14.85
Li	-11.40	0.73	-12.83	-9.97		14.80
Shin	-15.90	3.94	-23.63	-8.17		13.05
Tian	6.41	0.28	5.85	6.97		14.86
Wang	-19.40	1.05	-21.46	-17.34		14.72
	-8.72	4.07	-16.70	-0.75		

Study name		Statistics for each study				Difference in means and 95% CI	Relative weight
Women	Difference in means	Standard error	Lower limit	Upper limit			
Ayonride	-3.70	5.70	-14.87	7.47		9.24
Corbould	-2.00	2.63	-7.15	3.15		11.74
Hua	-17.30	3.24	-23.64	-10.96		11.31
Kauffman	-7.00	0.86	-8.69	-5.31		12.55
Lazo	-19.00	0.71	-20.39	-17.61		12.59
Polyzos	-26.90	1.91	-30.64	-23.16		12.15
Shin	-26.90	7.58	-41.76	-12.04		7.64
Vassilatou	-20.30	4.05	-28.25	-12.35		10.66
Wang	-31.00	1.97	-34.86	-27.14		12.12
	-17.05	3.33	-23.58	-10.53		

Figure 6. *Random effects pooled mean difference of SHBG in subjects with and without NAFLD in men and women.*
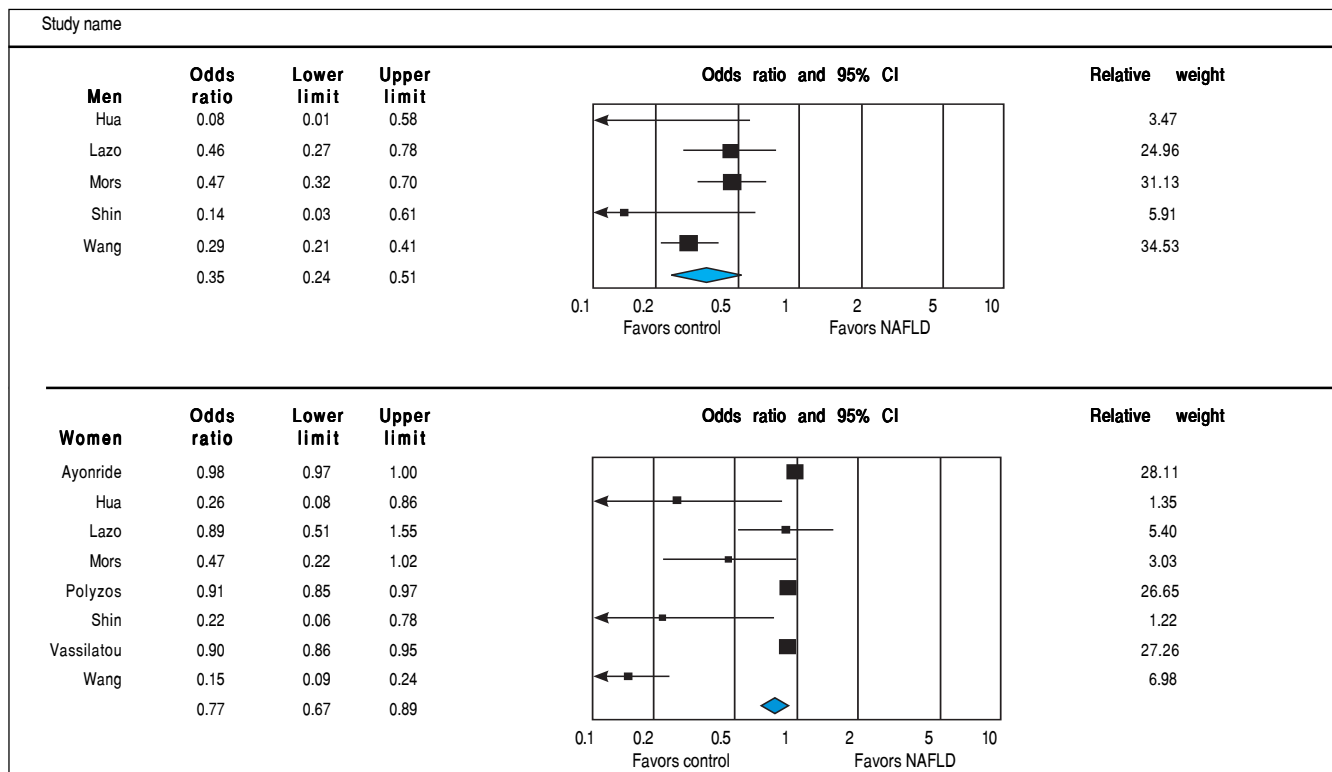
Figure 7. *Random effects pooled odd ratio of SHBG in subjects with and without NAFLD in men and women.*

the meta-analysis. In both sexes, SHBG was lower in subjects with NAFLD (men: MD = -8.72 nmol/l, 95%CI -16.70 to -0.75, I^2 = 99%, $P_{heterogeneity}$ < 0.01; women: MD = -17.05, -23.58 to -10.53, I^2 = 97%, $P_{heterogeneity}$ < 0.01) compared to those without NAFLD (Figure 6). This inverse association between SHBG levels and NAFLD was stronger in women than men ($P_{sex\ difference}$ < 0.01). Substantial between-study heterogeneity was observed in both men (I^2 = 99%) and women (I^2 = 97%). In men, this significant heterogeneity could be partly explained by differences in age, BMI and study design (all P-interaction < 0.01). In women, the association between SHBG levels and NAFLD was stronger in those with age ≥ 55 years, BMI < 30, post-menopause, and case-control studies. So the differences in age, BMI, menopausal status and study design could partly contribute to the high between-study heterogeneity (all P-interaction < 0.01) (Table 2). By meta-regression, we show a significant association between BMI and SHBG in women such that patients with increased BMI will have higher SHBG levels (beta coefficient = 1.20, 95% CI 0.56-1.85, P-value < 0.001) whereas BMI did not significantly impact on the change in SHBG in men (beta coefficient = -0.67, P-value = 0.72).

Analysis of OR estimates for SHBG levels in NAFLD comprised 6,691 men (5 studies[26,29,32,35,36]) and 5,725 women (8 studies[23,26,29,32,35-38]). Pooled analysis showed a reduced

risk of NAFLD with higher SHBG levels in both men (pooled OR of 0.35, 95%CI 0.25 to 0.45, I^2 = 50%, $P_{heterogeneity}$ = 0.09) and women (pooled OR of 0.77, 95%CI 0.67 to 0.89, I^2 = 92%, $P_{heterogeneity}$ = 0.02) (Figure 7).

To investigate potential publication bias, we examined the contour-enhanced funnel plot of the included studies that assessed mean difference of SHBG in both men and women (Figure 5). For both men and women, the plots exclude bias since there are symmetrical distributions of studies on both sides of the mean. Furthermore, the Egger's test was non-significant (P = 0.36 and 0.74, respectively). Using the trim and fill methods in the random-effects model, there was no difference of the imputed mean difference.

DISCUSSION

The association between TT and SHBG and NAFLD has been investigated in several studies. This meta-analysis aims to comprehensively review and combine all available evidence with an attempt to provide the definitive conclusion of this potential association. Our meta-analysis demonstrated the sex differences for the association between endogenous TT and NAFLD. TT was lower in men with NAFLD compared with non-NAFLD controls but did not show any associations in women. However, Higher

TT levels were found to decrease odds of NAFLD in men and increase odds of NAFLD in women. There was no sex-specific association between SHBG level and NAFLD. In both men and women, lower SHBG levels were associated with NAFLD. By meta-regression, BMI appeared to be the effect modifier of the association between NAFLD and SHBG in women.

Our results were in line with the previous meta-analysis from Brand, *et al.*,[39] which demonstrated the presence of sex-dependent association between testosterone and metabolic syndrome: TT and FT were lower in men with metabolic syndrome but they were higher in women with metabolic syndrome and no sex difference for the association between SHBG and metabolic syndrome, which showed higher risk of metabolic syndrome in subjects with lower SHBG levels. Another meta-analysis from Ding, *et al.*[40] also indicated that TT differently modulated the risk of diabetes in men and women. High testosterone levels are associated with higher risk of diabetes in women but with lower risk in men; the inverse association of SHBG and NAFLD was observed in both sexes with the stronger relationship in women than in men.

The exact mechanisms underlying the sex-specific associations between testosterone and NAFLD remain poorly understood. The association between low testosterone and NAFLD in men could be explained by hypogonadal-obesity-adipocytokine hypothesis.[41,42] Visceral adiposity positively correlates with insulin resistance and NAFLD.[43] Increasing visceral adipose tissue leads to increased aromatase enzyme activity that functions to convert testosterone to estrogen leading to decrease testosterone level. The low testosterone level further increases lipoprotein lipase activity, which in turn causes increased triglyceride uptake into the adipocytes resulting in increasing visceral adiposity. This exacerbates insulin resistance and causes vicious cycle by further decreasing testosterone. Moreover, the pro-inflammatory adipocytokines including tumor necrosis factor-alpha, interleukin-1 and interleukin-6 releasing from the adipose tissue could potentially inhibit pituitary axis resulting in lower testosterone levels.[44,45] In women, the opposite association between TT and NAFLD was clearly observed but the mechanism still remains uncertain. Previous study showed that higher TT levels were associated with increased visceral adiposity and insulin resistance in women.[46] These differences could be driven by several factors. First, it could be from relative androgen excess to estrogen level, which is known to have protective effect against fatty liver, during the menopausal transition period.[47] Second, the liver showed considerable sexual dimorphism in gene expression likely due to the differences in metabolic needs for reproduction including androgen receptors. The liver also displays marked changes in androgen sensitivity.[48]

Genetic studies in animal models compared global gene expression on testosterone between sexes showed substantial sex differences in transcription response to testosterone, which suggests that males and females may employ different pathways when responding to elevated testosterone.[49] However, it should be noted that liver fat plays a key role in development of metabolic syndrome. Changes in hormone levels might be merely a consequence of fatty liver leading to visceral adiposity.

The lack of a sex-specific association between SHBG and NAFLD is not fully understood. Recent evidence suggests the correlation between plasma level of several cytokines and plasma SHBG levels. SHBG was found to be upregulated by proinflammatory cytokines (tumor necrosis factor alpha and interleukin 1 beta) and downregulated by anti-inflammatory cytokines (adiponectin).[17] The imbalance of those cytokines seems to have a major role in the development of NAFLD as they are involved in various aspects of its pathogenesis.[50]

In consideration of the increasing trend of NAFLD incidence and being one of the most common indications of liver transplantation, effective prevention and treatment strategies are urgently needed. The currently mainstay treatments are lifestyle modifications including physical activity and healthy diet, which are difficult to maintain. It is of great significance to find novel treatment strategies. TT and SHBG levels have shown to be related to the risk of NAFLD and insulin resistance. Previous meta-analysis summarizing the effects of testosterone replacement therapy from RCTs have shown the significant improvements of insulin resistance, glucose control, body composition and lipid metabolism.[51] Few recent randomized controlled trials have addressed this issue relating to hepatic steatosis, but reported conflicting results. Hoyos, *et al.*[52] conducted 18-week testosterone treatment in obese men demonstrating significantly reduced liver fat, whereas Huang, *et al.*[53] reported that 6-month testosterone administration in older men with mobility limitation was not associated with a reduction in hepatic fat. Determining whether improving SHBG level or testosterone administration prevents or improves NAFLD requires further research. However, caution must be taken for the role of testosterone therapy, since it was found to increase risks of cardiovascular-related events and hepatocellular carcinoma.[54-58]

As with any meta-analysis, we acknowledge that there are some limitations. First, a significant heterogeneity was observed in this study. The possible sources of these heterogeneities include the differences in the study design, methodology and population as demonstrated by subgroup analyses. Second, this is a meta-analysis of observational studies, which can only demonstrate an association, not causality. Therefore, we cannot make a conclusion those endogenous sex hormones themselves versus other

potential confounders cause the increased NAFLD risk. Third, most of the included studies used ultrasonography to detect hepatic steatosis. Although this imaging technique is widely accepted as the diagnostic tool of choice for screening NAFLD due to its low cost, safety and accessibility, it has limited accuracy in detecting mild steatosis and operator dependency.[59,60] Liver biopsy is regarded as a gold standard for detecting hepatic steatosis.[1]

In summary, our meta-analysis demonstrated a sex-dependent association between TT and NAFLD. Higher TT levels are associated with men with NAFLD and inversely associated with women with NAFLD, whereas no sex-specific association was identified between SHBG and NAFLD. Higher SHBG levels are associated with lower NAFLD odds in both men and women.

POTENTIAL COMPETING INTERESTS

None.

ETHICAL APPROVAL

This article does not contain any studies with human participants or animals performed by any of the authors.

INFORMED CONSENT

No informed consent.

DISCLOSURE STATEMENT

The authors have nothing to disclose.

AUTHOR CONTRIBUTIONS

VJ conceived of the study, assessed the quality of the studies, and drafted the manuscript. SU searched the literature, assessed the quality of the studies, performed the statistical analysis, and drafted the manuscript. AS and TR participated in the statistical analysis and drafted the manuscript. All authors read and approved the final manuscript.

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REFERENCES

1. Angulo P. Nonalcoholic fatty liver disease. *New Engl J* 2002; 346: 1221-31.
2. Rinella ME. Nonalcoholic fatty liver disease: a systematic review. *JAMA* 2015; 313: 2263-73.
3. Vernon G, Baranova A, Younossi ZM. Systematic review: the epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in adults. *Alimentary pharmacology & therapeutics* 2011; 34: 274-85.
4. Jaruvongvanich V, Sanguankeo A, Upala S. Significant Association Between Gallstone Disease and Nonalcoholic Fatty Liver Disease: A Systematic Review and Meta-Analysis. *Digestive Diseases And Sciences* 2016.
5. Marchesini G, Bugianesi E, Forlani G, Cerrelli F, Lenzi M, Manini R, Natale S, et al. Nonalcoholic fatty liver, steatohepatitis, and the metabolic syndrome. *Hepatology* 2003; 37: 917-23.
6. Targher G, Day CP, Bonora E. Risk of cardiovascular disease in patients with nonalcoholic fatty liver disease. *New Engl J* 2010; 363: 1341-50.
7. van der Poorten D, Milner KL, Hui J, Hodge A, Trenell MI, Kench JG, London R, et al. Visceral fat: a key mediator of steatohepatitis in metabolic liver disease. *Hepatology* 2008; 48: 449-57.
8. Endre T, Mattiasson I, Berglund G, Hulthen UL. Low testosterone and insulin resistance in hypertension-prone men. *J Human Hypertension* 1996; 10: 755-61.
9. Oh JY, Barrett-Connor E, Wedick NM, Wingard DL, Rancho Bernardo S. Endogenous sex hormones and the development of type 2 diabetes in older men and women: the Rancho Bernardo study. *Diab Car* 2002; 25: 55-60.
10. Pitteloud N, Mootha VK, Dwyer AA, Hardin M, Lee H, Eriksson KF, Tripathy D, et al. Relationship between testosterone levels, insulin sensitivity, and mitochondrial function in men. *Diab Car* 2005; 28: 1636-42.
11. Kelly DM, Jones TH. Testosterone and obesity. *Obesity Reviews* 2015; 16: 581-606.
12. Tok EC, Ertunc D, Evruke C, Dilek S. The androgenic profile of women with non-insulin-dependent diabetes mellitus. *The Journal of reproductive medicine* 2004; 49: 746-52.
13. Toprak S, Yonem A, Cakir B, Guler S, Azal O, Ozata M, Corakci A. Insulin resistance in nonobese patients with polycystic ovary syndrome. *Hormone Research* 2001; 55: 65-70.
14. Kalish GM, Barrett-Connor E, Laughlin GA, Gulanski BI. Postmenopausal Estrogen/Progestin Intervention T. Association of endogenous sex hormones and insulin resistance among postmenopausal women: results from the Postmenopausal Estrogen/Progestin Intervention Trial. *The Journal Of Clinical Endocrinology And Metabolism* 2003; 88: 1646-52.
15. Andersson B, Marin P, Lissner L, Vermeulen A, Bjorntorp P. Testosterone concentrations in women and men with NIDDM. *Diab Car* 1994; 17: 405-11.
16. Pugeat M, Nader N, Hogeveen K, Raverot G, Dechaud H, Grenot C. Sex hormone-binding globulin gene expression in the liver: drugs and the metabolic syndrome. *Molecular and cellular Endocrinology* 2010; 316: 53-9.
17. Simo R, Saez-Lopez C, Barbosa-Desongles A, Hernandez C, Selva DM. Novel insights in SHBG regulation and clinical implications. *TEM* 2015; 26: 376-83.
18. Stroup DF, Berlin JA, Morton SC, Olkin I, Williamson GD, Rennie D, Moher D, et al. Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. *JAMA* 2000; 283: 2008-12.
19. Stang A. Critical evaluation of the Newcastle-Ottawa scale for the assessment of the quality of nonrandomized studies in meta-analyses. *Eur J Epidemiol* 2010; 25: 603-5.
20. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. *BMJ* 2003; 327: 557-60.
21. Hozo SP, Djulbegovic B, Hozo I. Estimating the mean and variance from the median, range, and the size of a sample. *BMC Medical Research Methodology* 2005; 5: 13.

22. Sterne JA, Egger M. Funnel plots for detecting bias in meta-analysis: guidelines on choice of axis. *Journal Of Clinical Epidemiology* 2001; 54: 1046-55.
23. Ayonrinde OT, Adams LA, Doherty DA, Mori TA, Beilin LJ, Oddy WH, Hickey M, et al. Adverse metabolic phenotype of adolescent girls with non-alcoholic fatty liver disease plus polycystic ovary syndrome compared with other girls and boys. *J Gastroenter Hepatol* 2016; 31: 980-7.
24. Barbonetti A, Caterina Vassallo MR, Cotugno M, Felzani G, Francavilla S, Francavilla F. Low testosterone and non-alcoholic fatty liver disease: Evidence for their independent association in men with chronic spinal cord injury. *The Journal Of Spinal Cord Medicine* 2016: 1-7.
25. Corbould A, Bhathal PS, Dixon JB, O'Brien PE. Interrelationships of serum androgens, omental adipose tissue metabolism, and nonalcoholic fatty liver disease in obese premenopausal women. *Metabolic syndrome and related disorders* 2014; 12: 311-9.
26. Flechtner-Mors M, Schick A, Oeztuerk S, Haenle MM, Wilhelm M, Koenig W, Imhof A, et al. Associations of fatty liver disease and other factors affecting serum SHBG concentrations: a population based study on 1657 subjects. *Hormone and metabolic research [Hormon- und Stoffwechselforschung = Hormones et metabolisme]* 2014; 46: 287-93.
27. Kauffman RP, Baker TE, Baker V, Kauffman MM, Castracane VD. Endocrine factors associated with non-alcoholic fatty liver disease in women with polycystic ovary syndrome: do androgens play a role? *Gynecological Endocrinology* 2010; 26: 39-46.
28. Kim S, Kwon H, Park JH, Cho B, Kim D, Oh SW, Lee CM, et al. A low level of serum total testosterone is independently associated with nonalcoholic fatty liver disease. *BMC Gastroenterology* 2012; 12: 69.
29. Lazo M, Zeb I, Nasir K, Tracy RP, Budoff MJ, Ouyang P, Vaidya D. Association Between Endogenous Sex Hormones and Liver Fat in a Multiethnic Study of Atherosclerosis. *Clin Gastroenterol Hepatol* 2015; 13: 1686-93 e2.
30. Li Y, Liu L, Wang B, Chen D, Wang J. Nonalcoholic fatty liver disease and alteration in semen quality and reproductive hormones. *Eur J Gastroenterol Hepatol* 2015; 27: 1069-73.
31. Seo NK, Koo HS, Haam JH, Kim HY, Kim MJ, Park KC, Park KS, et al. Prediction of prevalent but not incident non-alcoholic fatty liver disease by levels of serum testosterone. *J Gastroenterol Hepatol* 2015; 30: 1211-6.
32. Shin JY, Kim SK, Lee MY, Kim HS, Ye BI, Shin YG, Baik SK, et al. Serum sex hormone-binding globulin levels are independently associated with nonalcoholic fatty liver disease in people with type 2 diabetes. *Diab Research Clin Pract* 2011; 94: 156-62.
33. Tian GX, Sun Y, Pang CJ, Tan AH, Gao Y, Zhang HY, Yang XB, et al. Oestradiol is a protective factor for non-alcoholic fatty liver disease in healthy men. *Obesity reviews* 2012; 13: 381-7.
34. Volzke H, Aumann N, Krebs A, Nauck M, Steveling A, Lerch MM, Rosskopf D, et al. Hepatic steatosis is associated with low serum testosterone and high serum DHEAS levels in men. *Int J Androl* 2010; 33: 45-53.
35. Wang N, Zhai H, Zhu C, Li Q, Han B, Chen Y, Zhu C, et al. Combined Association of Vitamin D and Sex Hormone Binding Globulin With Nonalcoholic Fatty Liver Disease in Men and Postmenopausal Women: A Cross-Sectional Study. *Medicine* 2016; 95: e2621.
36. Hua X, Sun Y, Zhong Y, Feng W, Huang H, Wang W, Zhang T, et al. Low serum sex hormone-binding globulin is associated with nonalcoholic fatty liver disease in type 2 diabetic patients. *Clin Endocrinol* 2014; 80: 877-83.
37. Polyzos SA, Kountouras J, Tsatsoulis A, Zafeiriadou E, Katsiki E, Patsiaoura K, Zavos C, et al. Sex steroids and sex hormone-binding globulin in postmenopausal women with nonalcoholic fatty liver disease. *Hormones* 2013; 12: 405-16.
38. Vassilatou E, Lafoyianni S, Vryonidou A, Ioannidis D, Kosma L, Katsoulis K, Papavassiliou E, et al. Increased androgen bioavailability is associated with non-alcoholic fatty liver disease in women with polycystic ovary syndrome. *Human reproduction* 2010; 25: 212-20.
39. Brand JS, van der Tweel I, Grobbee DE, Emmelot-Vonk MH, van der Schouw YT. Testosterone, sex hormone-binding globulin and the metabolic syndrome: a systematic review and meta-analysis of observational studies. *Int J Epidemiol* 2011; 40: 189-207.
40. Ding EL, Song Y, Malik VS, Liu S. Sex differences of endogenous sex hormones and risk of type 2 diabetes: a systematic review and meta-analysis. *JAMA* 2006; 295: 1288-99.
41. Cohen PG. The hypogonadal-obesity cycle: role of aromatase in modulating the testosterone-estradiol shunt—a major factor in the genesis of morbid obesity. *Medical Hypotheses* 1999; 52: 49-51.
42. Rao PM, Kelly DM, Jones TH. Testosterone and insulin resistance in the metabolic syndrome and T2DM in men. *Nat Rev Endocrinol* 2013; 9: 479-93.
43. Mirza MS. Obesity, Visceral Fat, and NAFLD: Querying the Role of Adipokines in the Progression of Nonalcoholic Fatty Liver Disease. *ISRN Gastroenterology* 2011; 2011: 592404.
44. van der Poll T, Romijn JA, Endert E, Sauerwein HP. Effects of tumor necrosis factor on the hypothalamic-pituitary-testicular axis in healthy men. *Metabolism* 1993; 42: 303-7.
45. Tsigos C, Papanicolaou DA, Kyrou I, Raptis SA, Chrousos GP. Dose-dependent effects of recombinant human interleukin-6 on the pituitary-testicular axis. *Journal Of Interferon & Cytokine Research* 1999; 19: 1271-6.
46. Phillips GB, Jing T, Heymsfield SB. Does insulin resistance, visceral adiposity, or a sex hormone alteration underlie the metabolic syndrome? Studies in women. *Metabolism* 2008; 57: 838-44.
47. Liu Y, Ding J, Bush TL, Longenecker JC, Nieto FJ, Golden SH, Szklo M. Relative androgen excess and increased cardiovascular risk after menopause: a hypothesized relation. *Am J Epidemiology* 2001; 154: 489-94.
48. Rando G, Wahli W. Sex differences in nuclear receptor-regulated liver metabolic pathways. *Biochimica et biophysica acta* 2011; 1812: 964-73.
49. Peterson MP, Rosvall KA, Taylor CA, Lopez JA, Choi JH, Ziegenfus C, Tang H, et al. Potential for sexual conflict assessed via testosterone-mediated transcriptional changes in liver and muscle of a songbird. *J Experimental Biol* 2014; 217: 507-17.
50. Stojsavljevic S, Gomercic Palcic M, Virovic Jukic L, Smircic Duvnjak L, Duvnjak M. Adipokines and proinflammatory cytokines, the key mediators in the pathogenesis of nonalcoholic fatty liver disease. *World J Gastroenterol* 2014; 20: 18070-91.
51. Corona G, Monami M, Rastrelli G, Aversa A, Tishova Y, Saad F, Lenzi A, et al. Testosterone and metabolic syndrome: a meta-analysis study. *The Journal Of Sexual Medicine* 2011; 8: 272-83.
52. Hoyos CM, Yee BJ, Phillips CL, Machan EA, Grunstein RR, Liu PY. Body compositional and cardiometabolic effects of testosterone therapy in obese men with severe obstructive sleep apnoea: a randomised placebo-controlled trial. *European Journal Of Endocrinology / European Federation Of Endocrine Societies* 2012; 167: 531-41.

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53. Huang G, Bhasin S, Tang ER, Aakil A, Anderson SW, Jara H, Davda M, et al. Effect of testosterone administration on liver fat in older men with mobility limitation: results from a randomized controlled trial. *The Journals Of Gerontology Series A, Biological Sciences And Medical Sciences* 2013; 68: 954-9.

54. Basaria S, Coviello AD, Travison TG, Storer TW, Farwell WR, Jette AM, Eder R, et al. Adverse events associated with testosterone administration. *N Engl J* 2010; 363: 109-22.

55. Kosaka A, Takahashi H, Yajima Y, Tanaka M, Okamura K, Mizumoto R, Katsuta K. Hepatocellular carcinoma associated with anabolic steroid therapy: report of a case and review of the Japanese literature. *J Gastroenterol* 1996; 31: 450-4.

56. Socas L, Zumbado M, Perez-Luzardo O, Ramos A, Perez C, Hernandez JR, Boada LD. Hepatocellular adenomas associated with anabolic androgenic steroid abuse in bodybuilders: a report of two cases and a review of the literature. *Brit J Sports Med* 2005; 39: e27.

57. Solbach P, Potthoff A, Raatschen HJ, Soudah B, Lehmann U, Schneider A, Gebel MJ, et al. Testosterone-receptor positive hepatocellular carcinoma in a 29-year old bodybuilder with a history of anabolic androgenic steroid abuse: a case report. *BMC Gastroenterology* 2015; 15: 60.

58. Xu L, Freeman G, Cowling BJ, Schooling CM. Testosterone therapy and cardiovascular events among men: a systematic review and meta-analysis of placebo-controlled randomized trials. *BMC Medicine* 2013; 11: 108.

59. Lee SS, Park SH. Radiologic evaluation of nonalcoholic fatty liver disease. *World J Gastroenterol* 2014; 20: 7392-402.

60. Hernaez R, Lazo M, Bonekamp S, Kamel I, Brancati FL, Guallar E, Clark JM. Diagnostic accuracy and reliability of ultrasonography for the detection of fatty liver: a meta-analysis. *Hepatology* 2011; 54: 1082-90.

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