Sex Differences of Endogenous Sex Hormones and Risk of Type 2 Diabetes

A Systematic Review and Meta-analysis

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Context Inconsistent data suggest that endogenous sex hormones may have a role in sex-dependent etiologies of type 2 diabetes, such that hyperandrogenism may increase risk in women while decreasing risk in men.

Objective To systematically assess studies evaluating the association of plasma levels of testosterone, sex hormone–binding globulin (SHBG), and estradiol with risk of type 2 diabetes.

Data Sources Systematic search of EMBASE and MEDLINE (1966-June 2005) for English-language articles using the keywords *diabetes*, *testosterone*, *sex-hormone-binding-globulin*, and *estradiol*; references of retrieved articles; and direct author contact.

Study Selection From 80 retrieved articles, 43 prospective and cross-sectional studies were identified, comprising 6974 women and 6427 men and presenting relative risks (RRs) or hormone levels for cases and controls.

Data Extraction Information on study design, participant characteristics, hormone levels, and risk estimates were independently extracted by 2 investigators using a standardized protocol.

Data Synthesis Results were pooled using random effects and meta-regressions. Cross-sectional studies indicated that testosterone level was significantly lower in men with type 2 diabetes (mean difference, –76.6 ng/dL; 95% confidence interval [CI], –99.4 to –53.6) and higher in women with type 2 diabetes compared with controls (mean difference, 6.1 ng/dL; 95% CI, 2.3 to 10.1) ($P<.001$ for sex difference). Similarly, prospective studies showed that men with higher testosterone levels (range, 449.6-605.2 ng/dL) had a 42% lower risk of type 2 diabetes (RR, 0.58; 95% CI, 0.39 to 0.87), while there was suggestion that testosterone increased risk in women ($P=.06$ for sex difference). Cross-sectional and prospective studies both found that SHBG was more protective in women than in men ($P\leq.01$ for sex difference for both), with prospective studies indicating that women with higher SHBG levels (>60 vs ≤60 nmol/L) had an 80% lower risk of type 2 diabetes (RR, 0.20; 95% CI, 0.12 to 0.30), while men with higher SHBG levels (>28.3 vs ≤28.3 nmol/L) had a 52% lower risk (RR, 0.48; 95% CI, 0.33 to 0.69). Estradiol levels were elevated among men and postmenopausal women with diabetes compared with controls ($P=.007$).

Conclusions This systematic review indicates that endogenous sex hormones may differentially modulate glycemic status and risk of type 2 diabetes in men and women. High testosterone levels are associated with higher risk of type 2 diabetes in women but with lower risk in men; the inverse association of SHBG with risk was stronger in women than in men.

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DIABETES MELLITUS CURrently afflicts approximately 21 million Americans, 90% to 95% of whom have type 2 diabetes.[1] An important sex difference is recognized in which type 2 diabetes is a more powerful risk factor for coronary heart disease mortality in women than in men, ie, diabetes increases the risk of coronary heart disease mortality by approximately 3.5-fold in women but only 2-fold in men ($P=.008$ for sex difference).[2] Furthermore, evidence suggests a sex difference such that the positive association between adiposity and risk of type 2 diabetes is stronger for women compared with men.[3]

Previous studies have suggested the role of endogenous sex hormones in the development of type 2 diabetes.[4] Hyperandrogenic conditions, such as polycystic ovarian syndrome (PCOS), have been strongly associated with glucose intolerance and insulin resistance in women,[5-14] while hypoandrogenism has been linked with insulin resistance[13-26] and adiposity[14,15,18,23,25,27-31] in men. Sex-dependent relationships may also exist for estradiol and risk of diabetes. Several studies have observed positive associations between estra-

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diol and insulin resistance in women[5,10,14,32] but not in men,[14,15,26,32,33] while results from other studies were conflicting.[24,34-36] Moreover, sex hormone–binding globulin (SHBG), a serum protein that affects free circulating hormone levels, has been inversely associated with insulin resistance and type 2 diabetes in both sexes,[20,23,26,34,37,38] though multiple studies have reported no inverse associations in men.[39-42]

To comprehensively assess the relations between levels of endogenous sex hormones and risk of type 2 diabetes and potential heterogeneity by sex, we conducted a systematic review and meta-analysis of available prospective and cross-sectional studies relating testosterone, SHBG, and estradiol levels with risk of type 2 diabetes.

METHODS

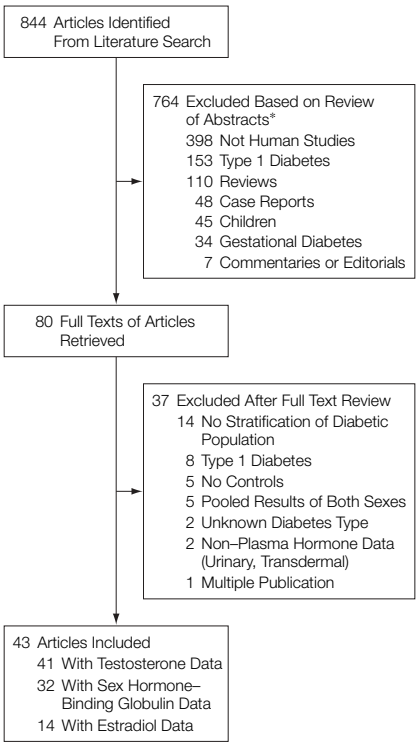
Study Selection

We conducted a comprehensive literature search of EMBASE and MEDLINE from 1966 through June 2005 using the keywords and Medical Subject Headings *diabetes* combined with *testosterone*, *sex-hormone-binding-globulin,* and *estradiol*. Additional studies were retrieved via references of articles and direct contact of authors deemed to possess relevant data. The search was restricted to English-language articles and studies of human participants. In the first round of screening (n=844), 764 articles were excluded for at least 1 of the following criteria: nonhuman study (398 articles), type 1 diabetes (153 articles), gestational diabetes (34 articles), children (45 articles), reviews (110 articles), editorials (7 articles), or case reports (48 articles) (FIGURE 1). In a second round of screening retrieving full texts (n=80), 37 articles were excluded: type 1 diabetes (8 articles), unknown diabetes type (2 articles), nonserum hormone data (2 articles), no controls (5 articles), results of combined sexes (5 articles), no results stratified for diabetes (14 articles), or multiple publication (1 article). Attempts to contact authors of relevant articles published after 1995 for additional information yielded 3 unanswered, 2 successful but authors unable to provide de novo analysis results, and 1 successful in providing stratified data for participants with type 2 diabetes. Our search strategy and inclusion criteria resulted in a total of 43 articles being included in the meta-analysis. Of these, 41 were available for examining sex differences in the effects of testosterone on type 2 diabetes, 32 for examining sex differences in the effects of SHBG, and 14 for examining sex differences in the effects of estradiol. In total, these studies included 6427 men and 6974 women (TABLE 1 and TABLE 2).

Data Extraction

Using a standardized data extraction form, 2 independent investigators (E.L.D. and V.S.M) extracted and tabulated all data. Discrepancies were resolved via referencing the original article and via group discussions. Information extracted included lead author; publication year; population; sex; menopausal status; study design; length of follow-up if prospective; race (proportion white); diagnostic criteria of diabetes categorized by differential definitions of fasting blood glucose level (National Diabetes Data Group 1979 or World Health Organization 1985 cut point of ≥140 mg/dL [7.8 mmol/L] vs American Diabetes Association 1997 or World Health Organization 1999 cut point of ≥126 mg/dL [7.0 mmol/L]); number of cases and controls; age; body mass index (BMI); waist-hip ratio (WHR); internal adjustment or matching (internal control) for age, BMI, or WHR between cases and controls; mean hormone/SHBG levels in cases and controls; unit of measurement; SD of hormone/SHBG levels (derived if SEs were reported); relative risks (RRs) of type 2 diabetes comparing various levels of hormone/SHBG if available; and confidence intervals (CIs) or *P* values, from which variances were derived. In the very few studies reporting only interquartile ranges, an approximate SD was derived. For free testosterone, additional data were collected on the method of free hormone assessment, excluding studies that used the less reliable method of direct radioimmunoassay[71-77] and attempting to pool only those studies that used the same methods of free hormone measurement. Due to the high degree of natural premenopausal fluctuations, estradiol analysis was restricted to men and postmenopausal women. Results for men and women were extracted as separate populations.

Figure 1. Summary of Article Selection Process



*Sum of specific excluded studies greater than total due to overlapping classifications of excluded studies.

Statistical Analyses

For prospective studies, RRs were used as measures of association between hormone levels and incident type 2 diabetes. For 1 prospective study reporting no incident cases of type 2 diabetes in a study group, the conventional half-integer correction was applied to calculate RRs and variances. For studies reporting RR for per-unit change in hormone levels, the equivalent RR of an upper-half vs lower-

half dichotomized comparison was derived from the trend RR comparison of the 75th vs 25th percentile. Relative risks of the reported dichotomy in each study were pooled via DerSimonian and Laird random-effects models.[78]

For cross-sectional studies, the primary summary measure of association was the overall random-effects mean difference in hormone/SHBG levels between cases and controls. To minimize chance findings from individual studies, conservative 99% CIs were expressed for individual studies, while 95% CIs were presented for summary estimates. Additionally, meta-regressions[79] using conservative robust SEs were conducted to examine whether differences in age, BMI, race, and diabetes diagnosis criteria influenced associations and explained heterogeneity across studies. Results from unadjusted meta-regressions were checked to ensure unity with primary DerSimonian and Laird results. *P* values for sex differences were calculated using 2-sample *z* tests. As determined a priori, men and women were analyzed separately unless results clearly showed no sex dimorphism. Insufficient numbers of sex-stratified populations and inconsistent methods precluded the meta-analysis of free hormones in this study. To assess the presence of publication bias, we assessed relative symmetry of individual study estimates around the overall estimate using Begg funnel plots[80] in which log RRs and mean differences were plotted against their corresponding SEs, stratified by sex. All analyses were conducted using STATA 8.2 (StataCorp, College Station, Tex); $P<.05$ was considered statistically significant.

RESULTS

The descriptive characteristics of included studies are presented in Tables 1 and 2.*

*References 5, 8, 11, 13, 14, 23, 28, 32, 34, 35, 39-70.

Testosterone

The body of testosterone studies included 3825 men and 4795 women from 36 cross-sectional study populations and 368 cases from 7 prospective study populations. In cross-sectional studies, women with type 2 diabetes had significantly higher levels of testosterone compared with controls (mean difference, 6.1 ng/dL; 95% CI, 2.3 to 10.1 [0.21 nmol/L; 95% CI, 0.08 to 0.35]), whereas men with type 2 diabetes had significantly lower levels of plasma total testosterone (mean difference, –76.6 ng/dL; 95% CI, –99.4 to –53.6 [–2.66 nmol/L; 95% CI, –3.45 to –1.86]) (FIGURE 2). This sex difference for testosterone was highly significant ($P<.001$ for interaction). These sex-dependent findings remained significant even with adjustment for study-level differences in age, race, and diabetes diagnosis criteria, as well as internal control for BMI and WHR. While statistical power was limited in stratified analyses, no significant

Table 1. Characteristics of Prospective Studies of Testosterone, Sex Hormone–Binding Globulin, and Estradiol

Source	Location	Sex	Follow up, y	Diabetes, No.		Mean Age, y	Mean BMI*	Mean WHR	Mean, nmol/L					
									Testosterone		SHBG		Estradiol	
				Yes	No				Cases	Controls	Cases	Controls	Cases	Controls
Lindstedt et al,[43] 1991	Gothenburg, Sweden (1969)	Women†‡	12	43	1381	46.8					55.0	88.0		
Haffner et al,[44] 1993	San Antonio, Tex	Men	8	20	36	48.4	29.0				42.8	38.6		
		Women‡	8	19	42	52.7	29.5				66.4	78.5		
		Women†	8	19	29	38.5	25.9				41.6	74.4		
Haffner et al,[35] 1996	United States-MRFIT (national)	Men	5	176	176	44.8	29.4		17.3	18.0	37.0	41.5	136.6	129.2
Tibblin et al,[45] 1996	Sweden (men born in 1913)	Men	13	35	411	67.0	25.2	102	13.6§	17.3§	39.9	51.5		
Okubo et al,[46] 2000	United States (Japanese Americans)	Men	3	20	183	68.3	23.7	88.1			45.7	45.1		
		Women‡	3	23	257	65.4	23.0	83.2			56.5	69.7		
Stellato et al,[47] 2000	Massachusetts	Men	8.9	54	976	53.9	27.1		15.2§	18.3§	24.4	32.3		
Oh et al,[14] 2002	Rancho Bernardo, Calif	Men	8	26	268	66.8	26.0	91.0	NA‖	NA‖			NA‖	NA‖
		Women‡	8	17	216	72.4	24.2	80.1	NA‖	NA‖			NA‖	NA‖
Rosmond et al,[48] 2003	Gothenburg, Sweden	Men	5	3	132	51.0	26.6		NA‖	NA‖				
Laaksonen et al,[49] 2004	Kuopio, Finland	Men	11	57	645	51.3	26.2	93.0	18.0	20.6	26.2	35.6		

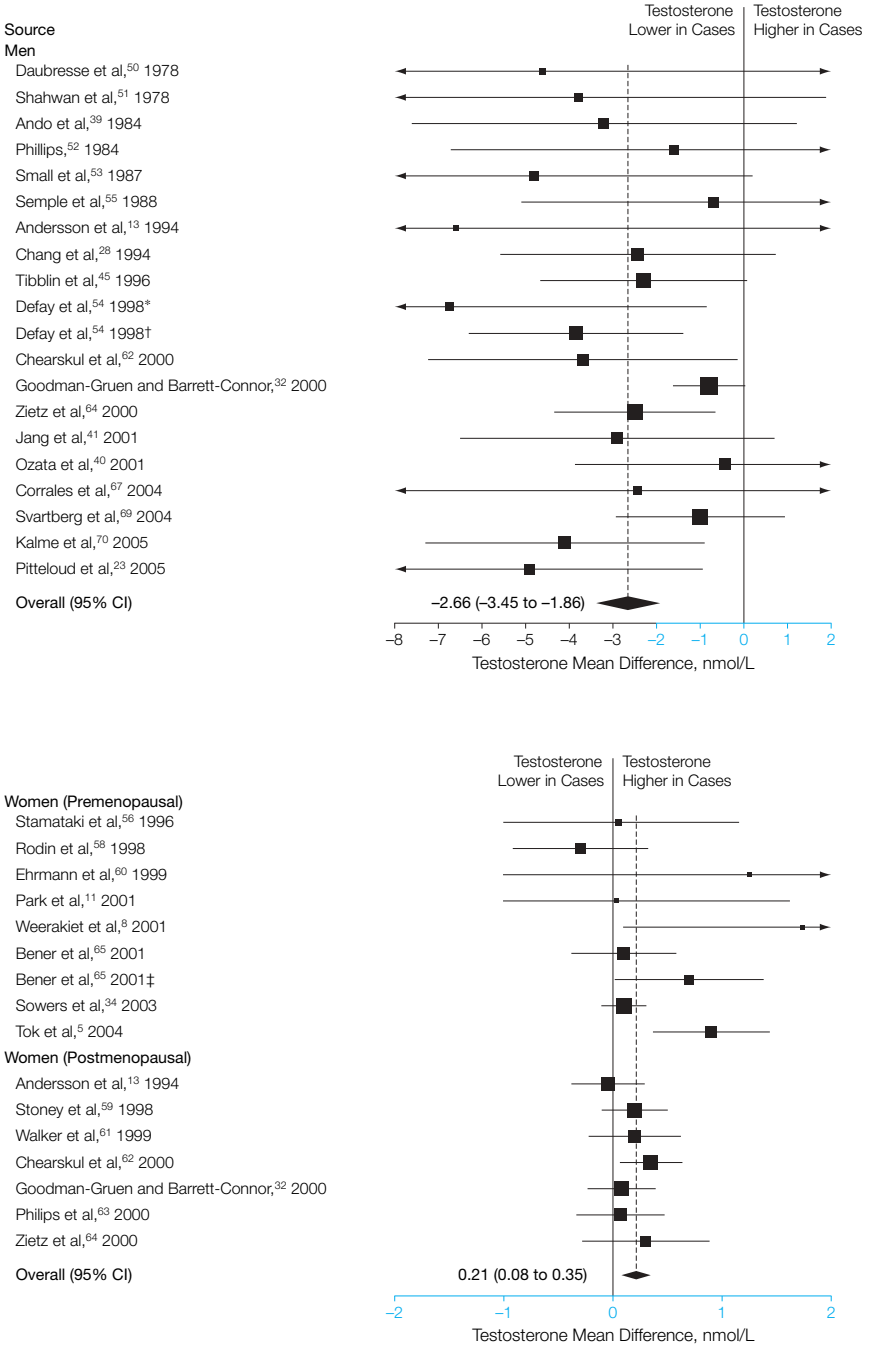
Abbreviations: blank cells, data not provided; BMI, body mass index; MRFIT, Multiple Risk Factor Intervention Trial; NA, not available; SHBG, sex hormone–binding globulin; WHR, waist-hip ratio.
SI conversion factors: To convert testosterone values to ng/dL, divide by 0.0347; to convert estradiol values to pg/mL, divide by 3.671.
*Calculated as weight in kilograms divided by the square of height in meters.
†Premenopausal.
‡Postmenopausal.
§Denotes prospective study with mean hormone levels at baseline between subsequent incident cases and noncases but no relative risk data.
‖Relative risk data only.

Table 2. Characteristics of Cross-Sectional Studies of Testosterone, Sex Hormone–Binding Globulin, and Estradiol

Source	Location	Sex	Diabetes, No.		Mean	Mean	Mean	Mean, nmol/L					
								Testosterone		SHBG		Estradiol	
			Yes	No	Age, y	BMI*	WHR	Cases	Controls	Cases	Controls	Cases	Controls
Daubresse et al,[50] 1978	Leige, Belgium	Men	8	7	45.0			23.4	28.0				
Shahwan et al,[51] 1978	Surrey, United Kingdom		39	10	52.1			15.2	19.0				
Ando et al,[39] 1984	Belgium	Men	30	47	37.8†			16.9	20.1	63.0	52.0		
Phillips,[52] 1984	New York	Men	16	19	43.7†	25.9‡		17.7	19.3			145.7	116.4
Small et al,[53] 1987	Scotland	Men	28	15	56.2	26.4‡		12.9	17.7			121.0	76.0
Semple et al,[55] 1988	Scotland	Men	15	15	52.4†	26.3		14.2	14.9				
Andersson et al,[13] 1994	Goteborg, Sweden	Men	46	11	56.5†	26.5‡	97.6	16.0	22.6	25.0	41.3		
		Women§	39	17	61.4†	27.8‡#	88.8‖	0.9	0.9	55.9	131.0	55.1	63.1
Chang et al,[28] 1994	Taiwan, China	Men	20	40	64.3†	24.2	92.3	17.0	19.4				
Stamataki et al,[56] 1996	Greece	Women¶	31	23	34.6†	31.9		1.7	1.7			261.8	333.8
Tibblin et al,[45] 1996	Sweden	Men	40	411	67.0**	25.1‡#	101.4‖	15.0	17.3	41.1	51.5		
Goodman-Gruen et al,[57] 1997	Rancho Bernardo, Calif	Women§	111	358	72.5	24.4	79.9			47.0	67.0		
Defay et al,[54] 1998	French Polynesia (Europeans)	Men	16	16	46.9†	29.0	98.0	13.8	20.5				
	French Polynesia (Melanesians)	Men	77	77	46.7†	28.9	95.5	16.4	20.2				
		Women§	35	35	54.3†	29.4‡	94.5‖			26.1	44.6		
		Women¶	69	69	41.8†	30.5	92.0			25.7	42.2		
Rodin et al,[58] 1998	United Kingdom	Women¶	13	13	38.2†	28.0‡	80.5	1.5	1.8	31.8	51.1		
Stoney et al,[59] 1998	Melbourne, Australia	Women§	42	42	63.5†	28.9‡	86.0	1.1	0.9	27.8	40.3		
Ehrmann et al,[60] 1999	Chicago, Ill	Women¶	12	67	25.5†	34.6		4.0	2.7				
Walker et al,[61] 1999	Victoria, Australia	Women§	11	20	56.7†	29.9‡#		1.3	1.1	28.5	39.9		
Chearskul et al,[62] 2000	Bangkok, Thailand	Men	43	39	65.7	23.5		12.4	16.1			139.9	146.8
		Women§	60	60	63.2	24.4		1.4	1.1			116.8	84.5
Goodman-Gruen and Barrett-Connor,[32] 2000	Rancho Bernardo, Calif	Men	112	397	71.9**	25.9††	91.4	10.5	11.3			77.6	74.4
		Women§	107	326	74.9**	24.8††	80.0	0.6	0.5			25.4	20.6
Phillips et al,[63] 2000	Manhattan, NY	Women§	20	29	66.3†	28.9‡	88.7	1.0	0.9	97.0	138.0	101.0	80.8
Zietz et al,[64] 2000	Germany	Men	155	155	58.0†	26.7‡		11.8	14.3				
		Women§	126	126	60.7†	26.5‡		1.7	1.4				
Bener et al,[65] 2001	United Arab Emirates 1	Women¶	36	56	32.6†	28.3		1.9	1.8				
	United Arab Emirates 2‡‡	Women¶	36	56	29.9†	30.3		2.8	2.1				
Jang et al,[41] 2001	South Korea	Men	26	64	53.5†	24.5‡	92.9	15.5	18.4	48.1	52.8		
Ozata et al,[40] 2001	Turkey	Men	20	20	41.5†	35.7‡	87.5	12.3	12.7	26.4	15.5		
Park et al,[11] 2001	South Korea	Women¶	6	5	34.3†	26.4‡	97.4‖	1.1	1.1	35.8	89.1	194.9	198.2
Weerakiet et al,[8] 2001	Bangkok, Thailand	Women¶	14	47	28.1†	26.1	82.7‖	5.2	3.5				
van der Merwe et al,[66] 2001	Johannesburg, S Africa	Women¶	10	10	38.2†	36.3‡	83.0‖			23.5	42.4		
Abate et al,[42] 2002	Texas	Men	33	24	50.8	27.9‡#	95.7‖			19.1	19.1	87.1	81.8
Sowers et al,[34] 2003	United States-SWAN	Women¶	94	3200	46.0†	26.7	80.2	1.5	1.4	29.2	41.4	176.9	194.9
Corrales et al,[67] 2004	Salamanca, Spain	Men	55	8	63.7†	27.5‡		15.6	18.0				
Jayagopal et al,[68] 2004	Hull, United Kingdom	Women§	12	11	59.1	31.8‡				38.8	42.2		
Svartberg et al,[69] 2004	Tromsø, Norway	Men	55	1364	60.5**	26.1	95.5‖	12.2	13.2	43.7	51.8		
Tok et al,[5] 2004	Turkey	Women¶	31	30	35.5	26.2‡	71.0‖	1.9	1.0	62.8	73.1	354.9	206.8
Kalme et al,[70] 2005	Finland	Men	109	152	79.5**	27.6		19.2	23.3	62.4	74.4		
Pitteloud et al,[23] 2005	Malmö, Sweden; Massachusetts	Men	21	27	60.6	26.6		13.1	18	31.5	49.4		

Abbreviations: blank cells, data not provided; BMI, body mass index; SHBG, sex hormone–binding globulin; SWAN, Study of Women Across the Nation; WHR, waist-hip ratio. SI conversion factors: To convert testosterone values to ng/dL, divide by 0.0347; to convert estradiol values to pg/mL, divide by 3.671.
*Calculated as weight in kilograms divided by the square of height in meters.
†Mean hormone levels between cases and controls matched on age.
‡Mean hormone levels between cases and controls matched on BMI.
§Postmenopausal.
‖Mean hormone levels between cases and controls matched on WHR.
¶Premenopausal.
#Mean hormone levels between cases and controls matched on waist circumference.
**Direct internal adjustment for age.
††Direct internal adjustment for BMI.
‡‡Independent population of type 2 diabetes cases and controls among women with acanthosis nigricans.

Figure 2. Random-Effects Pooled Mean Difference of Testosterone Levels Between Type 2 Diabetes Cases and Controls, Men and Women



Negative values represent lower levels among type 2 diabetes cases. Sizes of data markers represent the statistical weight that each study contributed to the overall random-effects estimate. To convert nmol/L to ng/dL, divide by 0.0347. CI indicates confidence interval.
*European population.
†Melanesian population.
‡Independent population of type 2 diabetes cases and controls among women with acanthosis nigricans.

heterogeneity by age, menopause, diabetes criteria, or white race was found (TABLE 3).

Analysis of mean difference in prospective studies of men also found lower testosterone levels among incident cases (mean difference, –71.5 ng/dL; 95% CI, –116.4 to –26.8 [–2.48 nmol/L; 95% CI, –4.04 to –0.93]). Moreover, those men in the upper dichotomy of total testosterone concentration (range, 449.6-605.2 ng/dL [15.6-21.0 nmol/L]) had a 42% lower risk of type 2 diabetes (RR, 0.58; 95% CI, 0.39 to 0.87) compared with those with lower concentrations (range, 213.2-446.7 ng/dL [7.4-15.5 nmol/L]) (FIGURE 3). In contrast, the 1 prospective study among women found that individuals in the top quartile of total testosterone (range, 51.9-92.2 ng/dL [1.8-3.2 nmol/L]) had a nonsignificant 60% higher risk of type 2 diabetes compared with women in the bottom 3 quartiles (range, 1.2-49.0 ng/dL [0.04-1.7 nmol/L]).[14] Similarly in the same study, women in the highest quartile of bioavailable testosterone had a significant 3-fold higher risk of type 2 diabetes compared with women in the bottom 3 quartiles.[14] Despite limited prospective studies in women, available data suggest a sex dimorphism for the association between total testosterone and type 2 diabetes ($P = .06$ for sex difference). No significant publication biases were detected among sex-stratified prospective and cross-sectional analyses.

Sex Hormone–Binding Globulin

The body of SHBG studies included 2500 men and 4765 women from 23 cross-sectional study populations and 466 cases from 10 prospective study populations. In cross-sectional studies, a significant sex difference was found ($P = .006$ for interaction), such that women with type 2 diabetes had significantly lower plasma levels of SHBG than did controls (mean difference, –16.2 nmol/L; 95% CI, –20.2 to –12.2), while men with type 2 diabetes had marginally lower levels of SHBG than controls, albeit not statistically significant (mean difference, –5.07

nmol/L; 95% CI, –11.9 to 1.77) (Table 3 and FIGURE 4). These sex-dependent associations generally remained consistent after adjusting for study-level differences in age, race, diabetes diagnosis criteria, and internal control for BMI. Although statistical power was limited, no significant heterogeneity by these characteristics in stratified analyses were found, except for a suggestive weaker sex difference among older men and women.

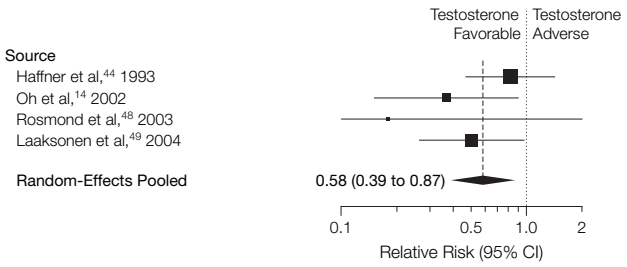
A stronger inverse association for SHBG among women was also seen from the pooled RR estimates in prospective studies. Women with higher levels of SHBG had an 80% lower risk of type 2 diabetes (RR, 0.20; 95% CI, 0.12 to 0.32) (>60 vs ≤60 nmol/L), while men with higher levels of SHBG had a 52% lower risk of type 2 diabetes (RR, 0.48; 95% CI, 0.34 to 0.69) (>28.3 vs ≤28.3 nmol/L) ($P = .003$ for sex difference) (FIGURE 5). Furthermore, a consistent sex difference was also observed in prospective studies using measures of mean differences in levels of SHBG, indicating a stronger inverse association for women (mean difference, –24.3 nmol/L; 95% CI, –37.3

Table 3. Mean Differences of Testosterone and Sex Hormone–Binding Globulin Between Type 2 Diabetes Cases and Controls*

	Women			Men			
	Studies, No.	Mean Difference (95% CI)	*P* Value	Studies, No.	Mean Difference (95% CI)	*P* Value	*P* Value for Sex Difference
Testosterone, nmol/L							
Prospective studies†	NA	NA	NA	4	–2.48 (–4.04 to –0.93)	.002	NA
Cross-sectional studies							
Overall random-effects†	16	0.21 (0.08 to 0.35)	.002	20	–2.66 (–3.45 to –1.86)	<.001	<.001
Model 1‡	16	0.23 (0.09 to 0.38)	.002	20	–1.84 (–2.83 to –0.84)	<.001	<.001
Model 2§	16	0.24 (0.09 to 0.38)	.001	20	–2.68 (–3.44 to –1.92)	<.001	<.001
Model 2b (BMI matched)‖	8	0.22 (0.05 to 0.40)	.02	9	–2.21 (–3.19 to –1.23)	<.001	<.001
Model 2c (WHR matched)‖	4	0.46 (–0.15 to 1.06)	.14	2	–1.61 (–2.56 to –0.65)	.001	<.001
Cross-sectional: stratified‡							
Menopausal status (women) or age (men)							
Premenopause or age <55 y	9	0.32 (–0.01 to 0.65)	.43	9	–2.82 (–3.98 to –1.67)	.73	<.001
Postmenopause or age ≥55 y	7	0.18 (0.09 to 0.27)		11	–2.56 (–3.49 to –1.64)		<.001
Diabetes criteria							
ADA-1997 or WHO-1999	10	0.29 (0.08 to 0.51)	.10	5	–1.98 (–3.24 to –0.71)	.12	<.001
NDDG-1979 or WHO-1985	6	0.12 (–0.02 to 0.27)		15	–3.03 (–3.77 to –2.29)		<.001
Race							
≥50% white	7	0.18 (0.08 to 0.27)	.45	15	–2.54 (–3.61 to –1.48)	.82	<.001
<50% white	9	0.27 (0.03 to 0.51)		5	–2.72 (–3.83 to –1.62)		<.001
Sex Hormone–Binding Globulin, nmol/L							
Prospective studies†	4	–24.3 (–37.3 to –11.3)	<.001	6	–7.16 (–9.99 to –4.33)	<.001	.01
Cross-sectional studies							
Overall random-effects†	14	–16.2 (–20.2 to –12.2)	<.001	9	–5.07 (–11.9 to 1.77)	.15	.006
Model 1‡	14	–16.4 (–20.0 to –12.8)	<.001	9	–6.21 (–13.8 to 1.33)	.11	.02
Model 2§	14	–16.9 (–19.9 to –14.0)	<.001	9	–6.10 (–13.5 to 1.35)	.10	.004
Model 2b (BMI matched)‖	10	–17.1 (–21.8 to –12.5)	<.001	5	–6.19 (–14.6 to 2.25)	.15	.02
Model 2c (WHR matched)‖	5	–19.0 (–28.7 to –9.37)	<.001	3	–12.2 (–17.5 to –7.05)	<.001	.22
Cross-sectional: stratified‡							
Menopausal status (women) or age (men)							
Premenopause or age <55 y	6	–14.8 (–18.9 to –10.72)	.23	4	2.14 (–8.10 to 12.4)	.006	.003
Postmenopause or age ≥55 y	8	–18.1 (–22.4 to –13.8)		5	–13.3 (–17.6 to –8.92)		.12
Diabetes Criteria							
ADA-1997 or WHO-1999	7	–14.4 (–19.2 to –9.68)	.17	3	–8.83 (–12.9 to –4.72)	.47	.08
NDDG-1979 or WHO-1985	7	–18.5 (–22.3 to –14.8)		6	–4.80 (–15.6 to 5.99)		.02
Race							
≥50% white	6	–17.5 (–22.7 to –12.2)	.53	7	–8.61 (–17.1 to –0.17)	.11	.08
<50% white	8	–15.5 (–19.5 to –11.5)		2	3.00 (–9.20 to 15.2)		.005

Abbreviations: ADA, American Diabetes Association; BMI, body mass index; CI, confidence interval; NA, not applicable; NDDG, National Diabetes Data Group; WHO, World Health Organization; WHR, waist-hip ratio.
SI conversion factors: To convert testosterone values to ng/dL, divide by 0.0347; to convert estradiol values to pg/mL, divide by 3.671.
*Positive values denote higher hormone levels, while negative values denote lower hormone levels in type 2 diabetes cases compared with nondiabetic controls. Prospective studies compared incident cases and controls, while cross-sectional studies compared prevalent cases and controls.
†DerSimonian and Laird random effects.
‡Adjusted for age.
§Adjusted for age, race, and diagnostic criteria.
‖Respective model, among studies matched on BMI between cases and controls.

Figure 3. Random-Effects Pooled Relative Risks of Testosterone and Type 2 Diabetes from Prospective Studies in Men



Relative risks reported for upper dichotomy (449.6-605.2 ng/dL [15.6-21.0 nmol/L]) vs lower dichotomy (213.2-449.5 ng/dL [7.4-15.5 nmol/L]). Sizes of data markers represent the statistical weight that each study contributed to the overall random-effects estimate. Only 1 prospective study among women ($P=.06$ for sex dimorphism). CI indicates confidence interval.

to −11.3) than for men (mean difference, −7.16; 95% CI, −9.99 to −4.33) ($P=.01$ for sex difference). We did not observe any significant publication bias.

Estradiol

The body of estradiol studies included 726 men and 658 postmenopausal women from 9 cross-sectional study populations and 219 cases from 3 prospective study populations. In cross-sectional studies, elevated levels of estradiol in type 2 diabetes were suggested among both men and postmenopausal women (mean difference, 3.5 pg/mL; 95% CI, 0.94 to 6.0 [12.8 pmol/L; 95% CI, 3.44 to 22.2]), with no apparent sex dimorphism ($P=.87$) (TABLE 4 and FIGURE 6). Studies internally controlled for BMI showed that estradiol was still associated with type 2 diabetes. Due to insufficient prospective data (only 1 study of each sex reporting RR), RR estimates could not be pooled. No apparent publication bias was observed.

COMMENT

We observed significant sex differences for the associations between endogenous testosterone and risk of type 2 diabetes. High testosterone levels were associated with higher risk of type 2 diabetes among women, while simultaneously associated with decreased risk of type 2 diabetes among men. Our results also indicate that low plasma levels of SHBG were weakly associated with type 2 diabetes in men but strongly associated with type 2 diabetes in women. In contrast, endogenous estradiol levels may be elevated both in men and in postmenopausal women with type 2 diabetes. Inconsistencies from previous studies may be due to small sample sizes and lack of attention to sex-specific associations, as we found that much of the heterogeneity across individual studies could be explained by stratifying on sex. Further adjustment for many other potential differences in population characteristics across individual studies, including age and BMI, did not materially change the pooled estimates for the associations of testosterone and SHBG with risk of type 2 diabetes.

These findings are also corroborated by 2 other prospective studies that did not provide RRs for total testosterone levels but reported significantly higher incidence of type 2 diabetes among men with lower testosterone levels.[45,47] Additionally, studies suggest that levels of testosterone are also associated with the metabolic syndrome in a similarly sex-divergent manner.[26,49,81,82] The observed sex-dimorphic associations between these hormonal biomarkers and type 2 diabetes may be explained by multiple pathways. First, endogenous hormones may influence insulin sensitivity via their effects on adiposity. Testosterone is inversely associated with adiposity in men† but positively associated with adiposity in women.[10,14] More importantly, clinical trials show that androgen deprivation increases adiposity and insulin resistance in men,[86,87] while testosterone therapy decreases adiposity[21,88-94] and improves insulin sensitivity[21,22,95] in obese men and in men with low testosterone levels. Specifically, a short-term trial in men showed that 250 to 500 mg/wk of intravenous testosterone decreased fat mass by 1 kg in 3 weeks,[93] while a long-term trial found that a low-dose (6 mg/d) scrotal testosterone patch decreased fat mass by 3 kg in 3 years compared with placebo.[88] However, exact opposite effects have been reported in women, in whom androgen therapy increases adiposity[96] and antiandrogen therapy decreases adiposity.[97] Moreover, the persistence of the significant associations after accounting for BMI suggests additional mechanisms apart from adiposity. This finding is consistent with emerging studies in both men and women showing that SHBG, bioavailable testosterone, and estradiol are all associated with insulin resistance and glucose levels independent of adiposity.[10,98,99] Further, several clinical trials show that androgen deprivation improves insulin sensitivity in women, independent of changes in BMI,[100] while testosterone therapy decreases insulin resistance in men, independent of changes in either BMI or WHR.[22,95]

The exact biological mechanisms for sex-dimorphic associations between testosterone and risk of type 2 diabetes remain to be determined, however. Laboratory experiments confirm that testosterone treatment impairs insulin-mediated glucose uptake in female rats,[101] while androgen receptor knockout rapidly causes significant insulin resistance in male mice.[102] Testosterone has also been shown to increase lipogenesis in visceral fat depots in women,[103] while, in contrast, stimulating lipolysis of visceral fat depots[103] and inhibiting adipocyte uptake of triglycerides in men,[91,104] via possible effects on lipoprotein lipase activity. Testosterone may also influence glucose con-

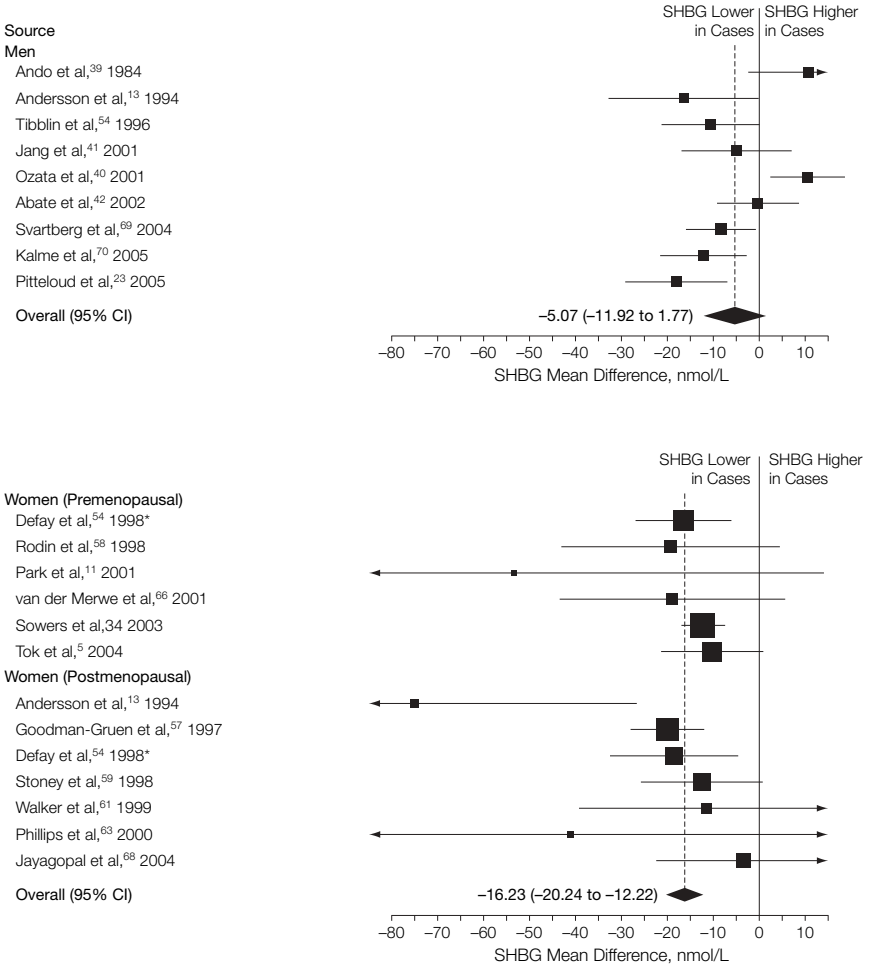
†References 14, 15, 18, 23, 25, 27-31, 83-85.

trol through increasing lean body mass in men.[90,105-109] Further, testosterone treatment in men may reduce levels of tumor necrosis factor α,[110,111] an inflammatory cytokine capable of inducing insulin resistance in both adipose and muscle tissues[112-114] and associated with higher risk of type 2 diabetes, either dependent[115,116] or independent of adiposity.[116] Sex dimorphisms in production of inflammatory cytokines[117,118] and inflammatory cytokines predicting type 2 diabetes risk[115,116] have also been suggested. Taken together, multiple lines of evidence from animal experiments, observational studies, and randomized trials in humans all appear to support a likely causal sex-dimorphic association between testosterone and risk of type 2 diabetes.

Increased risk of type 2 diabetes with low SHBG levels may represent the stronger effects of more bioavailable testosterone and estradiol, and thus, the sex-dependent associations of SHBG can be explained by its interactions with the sex hormones. While SHBG binds both testosterone and estradiol, it has a more than 2-fold higher affinity for binding testosterone than for binding estradiol,[119] thus making SHBG a stronger marker of androgens.[44] Because higher levels of SHBG lead to a decrease in levels of free testosterone, high SHBG levels are likely protective against the adverse effects of testosterone in women, whereas less free testosterone due to high SHBG levels is not protective against type 2 diabetes in men, explaining the sex-dependent association between SHBG and risk of type 2 diabetes.

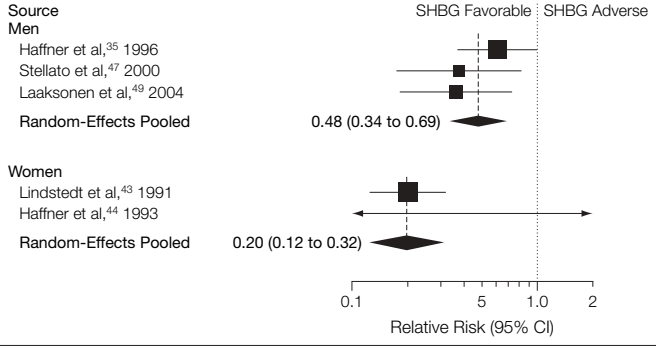
The question of whether there is a direct relationship between plasma estradiol and risk of type 2 diabetes is less certain, however. Although adipose tissue is a major source of estrogen both in men and in postmenopausal women, analysis restricted to studies internally matched for BMI found that the estradiol association remained statistically significant. Nevertheless, there were no apparent sex differences in these limited studies. However, insufficient data precluded further

Figure 4. Random-Effects Pooled Mean Difference of Sex Hormone–Binding Globulin Levels Between Type 2 Diabetes Cases and Controls, Men and Women



Sizes of data markers represent the statistical weight that each study contributed to the overall random-effects estimate. Significant sex dimorphism ($P<.001$ for effect modification by sex). CI indicates confidence interval. *Melanesian population.

Figure 5. Random-Effects Pooled Relative Risks of Sex Hormone–Binding Globulin (SHBG) and Type 2 Diabetes from Prospective Studies



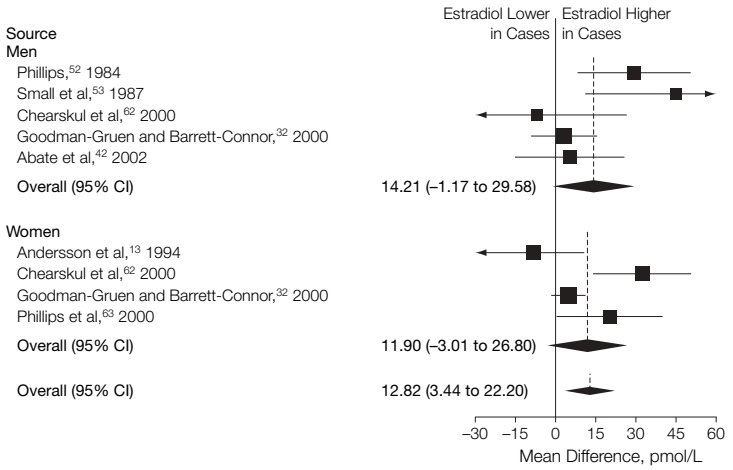
Relative risks reported for upper dichotomy (>60 nmol/L for women and >28.3 nmol/L for men) vs lower dichotomy. Sizes of data markers represent the statistical weight that each study contributed to the overall random-effects estimate. Significant sex difference ($P=.003$ for interaction). CI indicates confidence interval.

Table 4. Mean Differences of Estradiol Between Type 2 Diabetes Cases and Controls in Cross-Sectional Studies*

Model	Studies, No.	Mean Difference (95% CI), nmol/L: Postmenopausal Women	Mean Difference (95% CI), nmol/L: Men	Studies, No.	Men + Women, Mean Difference (95% CI), nmol/L	*P* Value	*P* Value for Sex Difference
Overall random-effects†	4 + 5	11.9 (–3.01 to 26.8)	14.2 (–1.17 to 29.6)	9	12.8 (3.44 to 22.2)	.007	.87
Model 1‡	4 + 5	11.5 (–2.22 to 22.8)	16.7 (–4.02 to 37.4)	9	15.7 (2.92 to 28.6)	.02	.67
Model 1b (BMI-matched)§	3 + 4	6.33 (–3.04 to 15.7)	24.9 (2.15 to 47.8)	7	17.9 (3.30 to 32.6)	.02	.14
Model 2‖	4 + 5	11.5 (–3.33 to 23.4)	13.6 (–2.45 to 29.6)	9	15.8 (4.04 to 27.5)	.01	.64

Abbreviations: BMI, body mass index; CI, confidence interval.
SI conversion factors: To convert testosterone values to ng/dL, divide by 0.0347; to convert estradiol values to pg/mL, divide by 3.671.
*Insufficient number of estradiol studies to further stratify and match on WHR. Positive values denote higher hormone levels, while negative values denote lower hormone levels in type 2 diabetes cases compared with nondiabetic controls. Cross-sectional studies compared prevalent cases and controls.
†DerSimonian and Laird random effects.
‡Adjusted for age.
§Respective model, among studies matched on BMI between cases and controls.
‖Adjusted for age, race, and diagnostic criteria.

Figure 6. Random-Effects Pooled Mean Difference of Estradiol Levels Between Type 2 Diabetes Cases and Controls, Men and Postmenopausal Women



Sizes of data markers represent the statistical weight that each study contributed to the overall random-effects estimate. *P* = .87 for sex difference. To convert pmol/L to pg/mL, divide by 3.671. CI indicates confidence interval.

assessment of the influence of age, menopausal timing, and different formulations of postmenopausal hormones[120-122] on the relation between plasma estradiol and risk of type 2 diabetes.

From a clinical perspective, sex hormones may offer a new realm for prediction of diabetes risk that has long been relatively ignored, as they were thought to be only markers of adiposity, which is already well established for predicting diabetes. The consistent findings among both men and women of significant associations for testosterone, SHBG, and estradiol, even after accounting for BMI, suggest possible clinical applications of sex hormone biomarkers in potentially adding predictive risk information above and beyond obesity. Although more prospective investigations are needed to better define risk levels, the approximate dichotomy of adverse levels of testosterone and SHBG presented in the results may be important in guiding clinical risk stratification. Furthermore, the potential adverse clinical diabetes risk associated with antiandrogen therapy for men and testosterone therapy for women should also be carefully considered.

As with any meta-analysis, our study has limitations. First, all the studies included are observational, and potential selection biases and confounding may exist. Therefore, to the extent possible, studies were adjusted for traditional diabetes risk factors, and our analysis accounted for age, race, menopausal status, diabetes diagnosis criteria, BMI, and WHR. While residual confounding remains theoretically possible, the robustness of the sex-specific associations observed in multiple sensitivity analyses indicates that it is unlikely that any unknown confounder can revert highly divergent sex-specific associations. Corroboration of sex differences in multiple study designs, including randomized trials, also suggests minimal residual confounding.

Second, due to the lack of reliable data on levels of free hormones, we were unable to examine whether total or free hormone fractions were more important for estimating risk. However, recent experiments indicate that SHBG-bound hormones may also be biologically active,[123,124] and thus, total concentration of plasma sex hormones may be a more robust index[10] and better reflect aggregate physiological effects. Moreover, previous studies of free hormones were not pooled due to heterogeneous or unreliable methods, as laboratory experiments indicate that the free androgen index (ie, molar ratio between testosterone and SHBG) is a less than optimal index of free testosterone level,[73,75] as is free testosterone level measured via direct radioimmunoassay, regarded by many experts as an invalid assay of free testosterone.[71-77] However, future research and clinical practice can improve on the reliability and efficiency

of assessment of free sex hormones by relying on the calculation-based methods of Vermuelen et al[73] and Sodergard et al,[125] which have been validated in multiple populations of men and women.[73,75,76,125-127]

Finally, characterized by chronic anovulation and hyperandrogenism, PCOS may also be partly responsible for the observed positive testosterone association in women, as there is increasing evidence that insulin resistance with compensatory hyperinsulinemia plays a role in the onset of hyperandrogenism by stimulating ovarian androgen production and by reducing levels of SHBG.[128,129] Thus, women of reproductive age with PCOS may be prone to metabolic disorders and type 2 diabetes. However, no prospective studies directly link PCOS with risk of incident type 2 diabetes, and PCOS is unlikely to explain the testosterone association among postmenopausal women and populations of diabetic cases and controls both with PCOS.[6,8,10,13,14,60] Furthermore, reverse causation explanation of diabetes-related hyperinsulinemia is also unlikely, as cohort studies and clinical trials establish temporality for causation. In addition, previous prospective studies have shown that testosterone, estradiol, and SHBG were all uncorrelated with baseline fasting insulin levels[14,57] and that SHBG remained correlated with insulin sensitivity independent of levels of both insulin and C-peptide.[37] In our analysis, the pooled mean testosterone difference of –76.6 ng/dL (95% CI, –99.4 to –53.6 [–2.66 nmol/L; 95% CI, –3.45 to –1.86]) from cross-sectional studies was highly consistent with results from prospective studies (–71.5 ng/dL; 95% CI, –116.4 to –26.8 [–2.48 nmol/L; 95% CI, –4.04 to –0.93). Consistent sex-specific SHBG results were also observed from prospective studies in men (mean difference, –7.16 nmol/L; 95% CI, –9.99 to –4.33) and women (mean difference, –24.3 nmol/L; 95% CI, –37.3 to –11.3), indicating temporality and consistency of these associations.

In conclusion, our systematic review of 43 studies comprising 6427 men and 6974 women suggests that endogenous levels of testosterone and SHBG each exhibit sex-dependent relations with risk of type 2 diabetes, such that high testosterone levels were associated with greater type 2 diabetes risk in women but lower risk in men, and also suggests that the inverse association of SHBG was stronger in women than in men. These findings highlight the importance of investigating the sex-specific etiologies of type 2 diabetes and its associated complications. Large prospective studies that comprehensively assess levels of these endogenous sex hormones are needed to further clarify their clinical predictive roles in the development of type 2 diabetes in both men and women.

Author Contributions: Mr Ding and Dr Liu had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.
Study concept and design: Ding, Liu.
Acquisition of data: Ding, Malik, Liu.
Analysis and interpretation of data: Ding, Song, Malik, Liu.
Drafting of the manuscript: Ding, Song, Malik, Liu.
Critical revision of the manuscript for important intellectual content: Ding, Song, Liu.
Statistical analysis: Ding, Song, Liu.
Obtained funding: Liu.
Administrative, technical, or material support: Liu.
Study supervision: Song, Liu.
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Role of the Sponsor: The NIH had no role in the design and conduct of the study; the collection, management, analysis, and interpretation of the data; or the preparation, review, or approval of the manuscript.
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