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Plasma sex steroid hormones and risk of developing type 2 diabetes in women: a prospective study

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Abstract

Aims/hypothesis Prospective data directly investigating the role of endogenous sex hormones in diabetes risk have been scant, particularly in women. We aimed to examine comprehensively plasma sex hormones in connection with risk of developing type 2 diabetes in postmenopausal women.

Methods We conducted a prospective, nested case–control study of plasma oestradiol, testosterone and dehydroepiandrosterone sulfate and risk of type 2 diabetes in a cohort of women health professionals with a mean age of 60.3 and 12.2 years since menopause. Among women not using hormone therapy and free of baseline cardiovascular disease, cancer and diabetes, 359 incident cases of type 2 diabetes were matched with 359 controls during an average follow-up of 10 years.

Results Oestradiol and testosterone were each strongly and positively associated with risk of type 2 diabetes. After adjustment for BMI, family history, lifestyle and reproductive variables, the multivariable relative risks (95% CI) comparing the highest vs lowest quintile were 12.6 (2.83–56.3) for total oestradiol ($p=0.002$ for trend), 13.1 (4.18–40.8) for free oestradiol ($p<0.001$ for trend), 4.15 (1.21–14.2) for total testosterone ($p=0.019$ for trend) and 14.8 (4.44–49.2) for free testosterone ($p<0.001$ for trend). These associations remained robust after adjusting and accounting for other metabolic syndrome components and baseline HbA_{1c} levels.

Conclusions/interpretation In postmenopausal women, higher plasma levels of oestradiol and testosterone were strongly and prospectively related to increased risk of developing type 2 diabetes. These prospective data indicate that endogenous levels of sex hormones may play important roles in the pathogenesis of type 2 diabetes.

ClinicalTrials.gov ID no.: NCT00000479.

Keywords Androgen · Dehydroepiandrosterone sulphate · Estradiol · Estrogen · Oestradiol · Oestrogen · Sex hormones · Steroid hormones · Testosterone · Type 2 diabetes · Women

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Abbreviations

CRP	C-reactive protein
DHEA	dehydroepiandrosterone
DHEAS	dehydroepiandrosterone sulphate
HRT	hormone replacement therapy
PEPI	Postmenopausal Estrogen/Progestin Intervention trial
RR	relative risk

Introduction

A large body of clinical data suggests the important roles of endogenous sex hormones in the pathogenesis of type 2 diabetes [1]. In short-term randomised trials and cross-sectional observational studies in women, plasma oestradiol and testosterone have been positively associated with glucose intolerance and insulin resistance, independently of adiposity [1, 2]. Similar associations of free testosterone and C-reactive protein (CRP; a systemic inflammatory marker) have also been reported in women [3, 4]. In contrast, little is known about the roles of dehydroepiandrosterone (DHEA; a sex hormone precursor) and its relatively more stable circulating form dehydroepiandrosterone sulfate (DHEAS) in the development of type 2 diabetes. Favourable effects of DHEA on glycaemic control have been reported in some short-term trials of women [5, 6], but not in others [7, 8]. Overall, no large prospective study has yet comprehensively investigated plasma sex steroid hormones and long-term risk of type 2 diabetes in women.

To provide such information, we prospectively investigated the relationships of the plasma steroid sex hormones total and free testosterone, total and free oestradiol, and DHEAS, as well as the testosterone:oestradiol ratio, with risk of developing type 2 diabetes. We also comprehensively examined each hormone found associated with diabetes risk in the context of established traditional risk factors, reproductive factors and established biochemical markers of inflammation and glycaemic control.

Methods

Study population The Women's Health Study, begun in 1993, is a randomised, double-blind, placebo-controlled, 2×2 factorial trial of low-dose aspirin and vitamin E for the primary prevention of cardiovascular disease and cancer among 39,876 US female health professionals aged 45 years and older, and free of baseline diabetes, cancer (other than non-melanoma skin cancer) and cardiovascular disease [9]. Of the 39,876 participants, 28,345 (71%) provided baseline blood samples. We restricted our study to 6,574 postmenopausal women drawn from the 28,345 and not using hormone replacement therapy (HRT) at the time of blood collection. By February 2005 with 10 years average follow-up, 366 of these initially healthy women had developed incident diabetes. Using the principle of risk-set sampling to randomly select controls from the source population [10], volunteers serving as controls were matched in 1:1 ratio to cases by age (within 1 year), duration of follow-up (within 1 month), race and fasting status at time of blood draw (72% provided fasting blood, defined as ≥10 h since last meal). Based on these criteria, 359 cases and 359 controls were selected. Written informed consent was obtained from all participants; this study was conducted according to the ethical guidelines of Brigham and Women's Hospital and Harvard Medical School.

Ascertainment of diabetes After excluding prevalent cases at baseline, all eligible participants were asked every year whether and when they had been diagnosed with diabetes since completing the previous questionnaire. Women self-reporting incident diabetes in annual follow-up were mailed supplementary questionnaires to confirm diabetes symptoms and provide results from fasting glucose and oral glucose tolerance tests, as well as details of treatments. Diabetes was confirmed according to the American Diabetes Association criteria of 1997. Previous Women's Health Study diabetes validation via physician-led telephone interviews and supplementary questionnaires yielded positive predictive values >91%, while confirmation of diabetes via combined supplementary questionnaire and medical records was 99% [9, 11]. Only confirmed diabetes cases were included in this study.

Laboratory procedures Blood samples were centrifuged and stored in liquid nitrogen freezers until the time of laboratory analysis. Long-term stability of plasma hormones has been previously documented [12–14]. Matched case–control pairs were handled identically and assayed in random order in the same analytical run. Laboratory personnel were blinded to case–control status during all assays. Plasma concentrations of biomarkers were measured using chemiluminescent immunoassays (Elecsys Autoanalyzer 2010; Roche Diagnostics, Indianapolis, IN, USA), validated for measurement of plasma sex hormones [15, 16]. Among eight different oestradiol assays tested and compared, the Elecsys 2010 assay yielded the smallest coefficient of variation and had the best sensitivity for measuring low oestradiol concentrations [16]. HbA_{1c} was measured using an assay approved by the US National Glycohemoglobin Standardization Program (Hitachi 911; Roche Diagnostics), as previously described [17]. Levels of CRP were measured using a high-sensitivity assay, as previously described [18]. For the hormone levels in this study, the coefficients of variation from blinded quality control samples were 5.2% for oestradiol, 7.4% for testosterone, 2.8% for DHEAS and 2.8% for sex hormone binding globulin. Free oestradiol and free testosterone were calculated via the Sodergard method [19], which has been previously validated among postmenopausal women [20, 21].

Statistical analysis Baseline characteristics of participants were compared using mixed-effects regression for clustered data and conditional logistic regression. Age-adjusted par-

Table 1 Comparison of characteristics between cases and controls at baseline

Characteristics	Cases	Controls	p value[a]
n	359	359	
Age (years)	60.3±6.1	60.3±6.1	(matched)
Race (% white)	92.5	92.5	(matched)
BMI (kg/m^2)	30.9±6.1	26.0±4.9	<0.001
Alcohol consumption (g/day)	2.62±7.4	4.19±8.3	0.007
Smoking (% current)	14.2	13.7	0.83
Strenuous physical activity (once or more per week)	30.7	38.7	0.06
Past postmenopausal hormone use (%)	32.0	27.9	0.22
Ever oral contraceptive use (%)	49.9	47.1	0.23
Age at menopause	48.0±6.1	48.0±5.7	0.92
Years since menopause	12.3±8.2	12.1±7.9	0.88
Cause of menopause (% natural)	63.0	69.4	0.08
Age at menarche <12 (%)	25.4	21.7	0.46
Age at first pregnancy of ≥6 months, <25 (%)	56.8	48.8	0.05
Family history of diabetes (%)	48.5	24.0	<0.001
History of hypertension (%)	50.1	30.4	<0.001
Pregnancies ≥5 (%)	30.1	34.0	0.10
Marital status (% currently married)	63.5	65.2	0.15
Education (% college graduate)	30.4	38.7	0.05
Sex hormones			
Oestradiol (pmol/l)[b]	90.3±58.2	75.3±41.3	<0.001
Free oestradiol (pmol/l)[c]	1.79±1.13	1.27±0.67	<0.001
Testosterone (nmol/l)[b]	1.03±0.66	1.00±0.66	0.49
Free testosterone (pmol/l)[c]	19.0±13.9	13.4±9.8	<0.001
DHEAS (nmol/l)[b]	2,453±1,653	2,496±1,446	0.67

Unless otherwise indicated, values are means ± SD

[a] Mixed-effects models used to test mean difference between cases and controls; if categorical, test for homogeneity across levels from conditional logistic regression

[b] Conversion of units: oestradiol pmol/l to pg/ml, divide by 3.671; testosterone nmol/l to ng/dl, divide by 0.0347; DHEAS nmol/l to ug/dl, divide by 26.95

[c] Free hormones were calculated via the Sodergard formula [19] using total hormone and sex hormone-binding globulin

tial correlations were used to evaluate associations between plasma biomarkers among controls. Because of the risk-set sampling approach for selection of cases and controls from the cohort [10], hazard ratios (referred to here as relative risks [RR]) and 95% CIs were directly computed using conditional logistic regressions. We divided the distributions of the biomarkers among controls into quintiles to calculate the RR of type 2 diabetes for each quintile versus the lowest quintile as referent category, and estimated the significance of trend across increasing quintiles. We also adopted a systematic approach in our analyses following the current understanding of sex hormone biology. For example, we controlled for upstream precursor hormones, which may be confounders of a causal relationship, while we did not adjust for downstream hormone metabolites that may be causal intermediates. Thus, the sex hormone precursor DHEAS was adjusted for in all models that included either testosterone or oestradiol, whereas testosterone, a precursor of oestradiol in aromatase conversion, was controlled for in all models that included oestradiol. In

multivariable analyses, we first adjusted for BMI (continuous), smoking (current, former, never), alcohol consumption (continuous), exercise (rarely/never, 1, 2–3, 4–6 and ≥7 times per week), systolic blood pressure (10 mmHg increments), past HRT use (yes, no), years of oral contraceptive use (<0.5, 0.5–2, >2 years), use of multivitamins (current, former, never), marital status (current, former, never married), education (high school, associates, bachelors, masters, doctoral) and family history of diabetes (yes, no) in all hormone models. Next, we fitted expanded multivariable models to include reproductive covariates: years since menopause (<5, 5–10, >10), age at menarche (<12, 12, 13, >13), total pregnancies (0, 1–2, 3–4, ≥5), pregnancies lasting ≥6 months and age at first pregnancy lasting ≥6 months (nulliparous, <25, ≥25) and finally cause of menopause (natural vs surgical, radiation or chemotherapy). We also estimated the RR per SD increase in biomarkers and assessed potential effect modification of associations by BMI (<25, 25–29.9, ≥30), past HRT use, years since menopause (<5, 5–10, >10), hypercholester-

Table 2 Age-adjusted partial correlations between plasma sex hormones in controls

Hormones	Oestradiol	Free oestradiol	Testosterone	Free testosterone	DHEAS	BMI	HbA$_{1c}$
Oestradiol	1.00						
Free oestradiol	0.91($p<0.001$)	1.00					
Testosterone	0.41($p<0.001$)	0.37($p<0.001$)	1.00				
Free testosterone	0.29($p<0.001$)	0.44($p<0.001$)	0.86($p<0.001$)	1.00			
DHEAS	0.23($p<0.001$)	0.22($p<0.001$)	0.63($p<0.001$)	0.59($p<0.001$) 1.00	1.00		
BMI	0.17($p=0.001$)	0.33($p<0.001$)	0.11($p=0.03$)	0.28($p<0.001$)	0.03 0.58	1.00	
HbA$_{1c}$[a]	0.12($p=0.02$)	0.15($p=0.004$)	0.05($p=0.32$)	0.09($p=0.09$)	0.02($p=0.71$)	0.11($p=0.03$)	1.00

[a] Baseline HbA$_{1c}$ (all participants non-diabetic at baseline)

olaemia and family history of diabetes. We conducted further sensitivity analyses excluding cases that developed during the first 3 years of follow-up and further accounting for waist circumference, baseline lipids, CRP and HbA$_{1c}$. Analyses were conducted using STATA 9.2 (StataCorp LP, College Station, TX, USA).

Results

As an indication of successful matching, diabetes cases and controls had identical mean age (60.3 years, range 45.7–76.2), race (92.5% white) and fasting status (72.1%). Overall, diabetes cases had higher BMI, lower frequency of physical activity, lower consumption of alcohol, more prevalent hypertension and a greater likelihood of family history of diabetes than controls. Additionally, diabetic women had significantly higher levels of total oestradiol, free oestradiol, free testosterone, CRP and HbA$_{1c}$ than controls and no difference in DHEAS levels compared with controls (Table 1). Among controls, oestradiol was positively correlated with all hormone biomarkers, CRP and HbA$_{1c}$, while testosterone was significantly correlated with DHEAS only (Table 2).

Conditioning on matching factors only, we found that elevated levels of total oestradiol, free oestradiol and free testosterone were each strongly associated with higher risk ($p<0.001$ for trend for each). In multivariable models adjusted for BMI, smoking, exercise, alcohol, blood pressure, family history of diabetes, education, marital status, past HRT use, multivitamins and respective hormones, all of the aforementioned associations remained significant ($p\leq0.01$ for trend for all). Additionally, total testosterone emerged as a significant risk factor in multivariable models. Controlling for reproductive variables likely to be associated with past sex hormone exposure either strengthened or did not materially affect any associations. Specifically, the multivariable-adjusted RRs (95% CI) comparing the highest vs lowest quintile were 12.6 (2.83–56.3) for total oestradiol ($p=0.002$ for trend), 13.1 (4.18–40.8) for free

oestradiol ($p<0.001$ for trend), 4.15 (1.21–14.2) for total testosterone ($p=0.019$ for trend) and 14.8 (4.44–49.2) for free testosterone ($p<0.001$ for trend). While an inverse association was suggested, DHEAS was not statistically significantly associated with lower risk. The ratio of total testosterone:oestradiol was also not associated with risk (Table 3). Because associations of continuous hormone variables appeared log-linear, relative risks in stratified and sensitivity analyses were expressed as per 1 log-SD increase in hormone biomarkers. Associations were only minimally attenuated and remained significant and robust in sensitivity analyses controlling for CRP and waist circumference. Moreover, among participants with baseline HbA$_{1c}$ <6%, increased total oestradiol, free oestradiol and free testosterone were still strongly associated with risk of developing diabetes, these associations being independent of waist circumference, CRP and HbA$_{1c}$. None of the sensitivity analyses materially affected these results (Table 4). Furthermore, assessment of potential effect modification of sex hormone associations revealed no significant interactions with BMI, past hormone use, years since menopause, hypercholesterolaemia and family history of diabetes, with the exception of total oestradiol with family history of diabetes (Table 5).

Discussion

In this prospective study of postmenopausal women followed for an average of 10 years, elevated levels of total and free oestradiol and testosterone at baseline were statistically significantly associated with increased risk of developing type 2 diabetes. These positive associations were independent of traditional risk factors including BMI, waist circumference, smoking, exercise, alcohol, blood pressure, past hormone use, family history of diabetes, as well as more recently established biomarkers such as CRP and HbA$_{1c}$. These prospective data indicate that these sex hormones are strong and robust risk markers of type 2 diabetes in women.

Table 3 Sex steroid hormones and relative risk (95% CI) of developing type 2 diabetes in women

Hormones	Quintiles					
	1 (lowest)	2	3	4	5 (highest)	p value for trend
Oestradiol (pmol/l)						
Median	41.9	55.2	65.5	79.4	100.6	
n (cases/controls)	21/72	54/72	66/72	95/72	123/71	
Model 1[a]	1.00	2.80 (1.53–5.13)	3.13 (1.67–5.86)	5.18 (2.79–9.63)	7.61 (4.02–14.4)	<0.001
Model 2[b]	1.00	2.66 (0.82–8.70)	3.00 (1.08–8.37)	4.56 (1.51–13.7)	9.69 (2.54–37.0)	0.001
Model 3[c]	1.00	3.20 (0.77–13.2)	3.91 (1.11–13.8)	5.39 (1.43–20.3)	12.6 (2.83–56.3)	0.002
Free oestradiol (pmol/l)						
Median	0.66	0.92	1.13	1.43	2.07	
n (cases/controls)	15/72	25/71	49/71	102/72	168/71	
Model 1[a]	1.00	1.44 (0.68–3.04)	3.35 (1.61–6.97)	7.31 (3.57–15.0)	14.2 (7.10–28.4)	<0.001
Model 2[b]	1.00	0.86 (0.32–2.31)	2.95 (1.20–7.28)	6.27 (2.52–15.6)	10.1 (3.68–27.9)	<0.001
Model 3[c]	1.00	0.71 (0.23–2.16)	2.85 (1.14–7.11)	7.62 (2.73–21.3)	13.1 (4.18–40.8)	<0.001
Testosterone (nmol/l)						
Median	0.35	0.62	0.87	1.15	1.84	
n (cases/controls)	68/79	68/68	70/77	76/65	77/70	
Model 1[a]	1.00	1.18 (0.73–1.90)	1.08 (0.67–1.74)	1.41 (0.86–2.29)	1.35 (0.81–2.25)	0.18
Model 2[b]	1.00	1.45 (0.55–3.77)	2.05 (0.81–5.19)	2.84 (1.17–6.88)	2.68 (1.00–7.19)	0.027
Model 3[c]	1.00	1.51 (0.49–4.66)	3.34 (0.98–11.4)	2.85 (1.07–7.57)	4.15 (1.21–14.2)	0.019
Free testosterone (pmol/l)						
Median	4.60	7.39	10.9	16.2	27.6	
n (cases/controls)	38/72	37/70	64/72	87/72	133/71	
Model 1[a]	1.00	1.11 (0.63–1.96)	1.69 (1.02–2.81)	2.49 (1.47–4.19)	5.04 (2.84–8.93)	<0.001
Model 2[b]	1.00	2.16 (0.85–5.45)	3.32 (1.40–7.86)	6.04 (2.20–16.6)	13.2 (4.54–38.3)	<0.001
Model 3[c]	1.00	3.11 (1.05–9.19)	5.26 (1.80–15.4)	7.92 (2.40–26.1)	14.8 (4.44–49.2)	<0.001
DHEAS (nmol/l)						
Median	981	1,586	2,184	2,933	4,472	
n (cases/controls)	89/72	66/73	69/70	69/72	66/71	
Model 1[a]	1.00	0.70 (0.43–1.13)	0.74 (0.47–1.19)	0.75 (0.47–1.20)	0.71 (0.44–1.17)	0.24
Model 2[b]	1.00	0.74 (0.37–1.45)	0.89 (0.45–1.72)	0.95 (0.51–1.77)	0.68 (0.32–1.41)	0.50
Model 3[c]	1.00	0.84 (0.42–1.70)	0.83 (0.41–1.69)	0.66 (0.34–1.26)	0.59 (0.28–1.24)	0.13
Ratio testosterone:oestradiol						
Median	5.67	9.48	13.1	16.3	23.8	
n (cases/controls)	102/72	75/73	61/72	70/72	51/71	
Model 1[a]	1.00	0.75 (0.49–1.15)	0.59 (0.37–0.94)	0.70 (0.44–1.10)	0.51 (0.31–0.82)	p=0.007
Model 2[b]	1.00	0.66 (0.29–1.50)	0.60 (0.25–1.42)	1.26 (0.51–3.09)	0.88 (0.36–2.17)	p=0.72
Model 3[c]	1.00	0.50 (0.19–1.28)	0.53 (0.22–1.31)	1.06 (0.33–2.96)	0.76 (0.26–2.23)	p=0.89

[a] Model 1 conditioned on matching factors: age, race, fasting status

[b] Model 2 adjusted for: matching factors, BMI, smoking, alcohol use, exercise, blood pressure, family history of diabetes, years of oral contraceptive use, past HRT use, multivitamin use, marital status and education in all models, as well as for total testosterone (for oestradiol), DHEAS (for oestradiol, testosterone and testosterone:oestradiol ratio) and sex hormone binding globulin (for total testosterone, total oestradiol and testosterone:oestradiol ratio)

[c] Model 3 adjusted variables in model 2 plus reproductive covariates: age at menarche, number of pregnancies, pregnancies lasting >6 months, age at first pregnancy of >6 months, years since menopause and cause of menopause

Testosterone We could identify only one previous study that reported a positive association between testosterone and diabetes risk in women; it included only 17 cases in a small California community [22]. Because of the large numbers of incident cases with comprehensive assessment of hormones, our findings on the independent association of testosterone with diabetes risk appear much stronger and more precise than that study. Experiments in female rats show that testosterone impairs insulin-mediated glucose uptake and increases lipogenesis [23], providing mechanistic explanations for our observation of increased diabetes risk associated with elevated levels of testosterone [24]. Further, testosterone therapy has been shown to induce insulin resistance in female-to-male transsexual [25] and increase visceral adiposity in postmenopausal women [26], whereas anti-androgen therapy decreases visceral adiposity in obese women with polycystic ovarian syndrome [27] and improves insulin sensitivity in non-obese women [28].

Table 4 Sensitivity analyses of sex hormones and type 2 diabetes risk, accounting for duration of follow-up, HDL, triacylglycerol, waist circumference, C-reactive protein and baseline HbA$_{1c}$

Analysis	Oestradiol[a] RR (95% CI)	p value for trend	Free oestradiol[b] RR (95% CI)	p value for trend	Testosterone[c] RR (95% CI)	p value for trend	Free testosterone[d] RR (95% CI)	p value for trend
Multivariate	1.97 (1.28–3.04)	0.002	2.78 (1.89–4.10)	<0.001	1.48 (1.01–2.17)	0.05	2.30 (1.60–3.30)	<0.001
Multivariate (excluding first 3 years follow-up)	1.88 (1.09–3.25)	0.02	2.97 (1.82–4.85)	<0.001	1.46 (0.92–2.34)	0.11	2.60 (1.63–4.14)	<0.001
Multivariate +HDL + TG[e]	2.08 (0.93–4.65)	0.07	2.91 (1.47–5.73)	0.002	1.72 (0.92–3.20)	0.09	2.38 (1.14–4.97)	0.02
Multivariate + waist circumference	1.80 (1.15–2.83)	0.01	2.55 (1.72–3.78)	<0.001	1.43 (0.93–2.20)	0.11	2.14 (1.48–3.10)	<0.001
Multivariate + C-reactive protein	2.25 (1.39–3.66)	0.001	2.70 (1.78–4.10)	<0.001	1.33 (0.88–2.02)	0.18	1.99 (1.31–3.03)	0.001
Multivariate (among those with HbA$_{1c}$ <6%)	2.00 (1.18–3.36)	0.009	3.07 (1.85–5.09)	<0.001	1.39 (0.86–2.24)	0.17	2.40 (1.49–3.88)	<0.001

Multivariate models adjusted for variables in model 3 of Table 3. Values are +1 log SD. [a] per 50% increase, [b] per 60% increase, [c] per 90% increase and [d] per 100% increase
[e] HDL and TG-adjusted analyses among those with fasting samples TG, triacylglycerol

Table 5 Sex hormone analyses of type 2 diabetes, stratified by BMI, past postmenopausal hormone use, years since menopause, hypercholesterolaemia and family history of diabetes

Analysis	Oestradiol[a] RR (95% CI)	p value for interaction[e]	Free oestradiol[b] RR (95% CI)	p value for interaction[e]	Testosterone[c] RR (95% CI)	p value for interaction[e]	Free testosterone[d] RR (95% CI)	p value for interaction[e]
BMI (kg/m^2)[f]								
<25	2.22 (1.10–4.50)	0.71	3.15 (1.80–5.50)	0.54	1.48 (0.82–2.64)	0.54	2.48 (1.39–4.40)	0.38
25–29.9	2.30 (0.98–5.37)		3.14 (1.74–5.65)		1.70 (0.95–3.02)		2.54 (1.53–4.20)	
≥30	1.55 (0.82–2.96)		2.03 (1.01–4.06)		1.24 (0.76–2.03)		1.83 (1.19–2.80)	
Postmenopausal hormone use								
Never	1.72 (0.97–3.04)	0.17	2.46 (1.65–3.69)	0.15	1.50 (0.98–2.30)	0.82	2.27 (1.49–3.46)	0.91
Past	2.78 (1.53–5.04)		3.74 (2.12–6.59)		1.41 (0.82–2.41)		2.34 (1.48–3.71)	
Time since menopause (years)								
<5	2.70 (1.05–6.96)	0.29	3.01 (1.72–5.27)	0.90	0.93 (0.38–2.27)	0.14	2.09 (1.07–4.07)	0.39
5–10	1.09 (0.49–2.41)		2.59 (1.40–4.80)		2.41 (1.21–4.82)		3.28 (1.74–6.20)	
>10	2.15 (1.11–4.16)		2.74 (1.67–4.50)		1.36 (0.84–2.18)		2.18 (1.46–3.27)	
Hypercholesterolaemia								
No	2.53 (1.52–4.21)	0.11	2.84 (1.88–4.28)	0.84	1.51 (0.93–2.45)	0.78	2.57 (1.66–3.99)	0.51
Yes	1.44 (0.80–2.62)		2.66 (1.51–4.69)		1.43 (0.92–2.21)		2.19 (1.45–3.29)	
Family history of diabetes								
No	2.76 (1.68–4.54)	0.004	2.98 (2.00–4.45)	0.45	1.34 (0.83–2.17)	0.50	2.01 (1.33–3.04)	0.24
Yes	1.10 (0.64–1.87)		2.41 (1.39–4.16)		1.65 (0.99–2.75)		2.70 (1.69–4.34)	

Results are adjusted for variables in model 3 of Table 3. RR per 1 logSD increase; SD determined from control distribution. Values are +1 logSD increase, [a] per 50% increase, [b] per 60% increase, [c] per 90% increase and [d] per 100% increase
[e] p values are for the overall modification effect across each group
[f] To control for confounding by BMI within BMI categories, stratified analyses controlled for within-stratum BMI

DHEAS Previous short-term trials have indicated that DHEA can improve glucose control and insulin sensitivity in women [5, 6], although conflicting results have also been reported [7, 8]. Villareal et al. [6] found that treatment with DHEA for 6 months decreased visceral adiposity and improved insulin action, while Nair et al., in a 2-year trial, [7] did not observe any benefits of DHEA. The inverse association between DHEAS and diabetes risk in our study was only of borderline significance, indicating that the long-term effects of DHEAS, if any, may be small.

Oestradiol Our prospective data also provided supporting evidence for the independent role of plasma oestradiol in the development of type 2 diabetes. Previous cross-sectional analyses indicate that testosterone and oestradiol are both associated with glucose levels and insulin resistance independently of adiposity [2, 29]. The effects of oestradiol on diabetes risk remained significant in our prospective data, even after control for testosterone and measures of adiposity, supporting the notion that oestradiol may be more than just a marker of increased aromatase conversion. Our findings for the positive relationship between plasma oestradiol and diabetes risk were in contrast with results from previous trials of oral oestrogen therapy, which showed a lower risk of type 2 diabetes among post-menopausal women who used oestrogen treatment [30–32].

However, it has been reported that exogenous oestrogen may have different physiological effects depending on dose, duration and mode of exposure [33–38]. Notably in two randomised trials [33, 34] simultaneously comparing oral oestrogen versus transdermal oestradiol, the latter consistently elevated plasma glucose to a greater degree than oral oestrogen [39]. Furthermore, even among oral oestrogen trials, strikingly divergent effects of oestrogen therapy on fasting glucose vs glucose tolerance have been seen, e.g. in the Postmenopausal Estrogen/Progestin Intervention (PEPI) Study [40]. Specifically, although active oestrogen regimens reduced fasting insulin and fasting glucose levels compared with placebo, oestrogen treatments in the PEPI trial simultaneously increased glucose intolerance measured via 2 h OGTT.

The exact mechanisms for these various divergent effects of oestrogen have not been precisely delineated, although many lines of evidence indicate potential explanations for the different physiological effects. For route of administration, transdermal oestradiol treatment does not appear to affect endothelial dysfunction [37, 41], a strong risk factor for type 2 diabetes [42], while, in contrast, oral oestrogen therapy favourably improved endothelial function [37]. For fasting vs post-challenge glucose differences, oestrogen on the one hand can suppress hepatic glucose production [43, 44], thereby resulting in lower fasting glucose; on the other hand, it can also inhibit and delay insulin release in response to rising glucose levels [45, 46], thereby promoting glucose intolerance.

In addition, bimodal relationships of oestrogen dose may exist, as one clinical trial in postmenopausal women found that lower dose (0.625 mg/day) oestrogen therapy, similar to that used in the WHI trial, increased insulin sensitivity, but higher dose (1.25 mg/day) oestrogen had the opposite effect, namely of significantly decreasing insulin sensitivity [47]. Similarly, an adverse effect of oestrogen on decreasing insulin sensitivity has also been reported among healthy pre-menopausal women randomised to ethinyl oestradiol oral contraceptives [48]. Moreover, duration-dependent effects have been observed in animal studies, where oestradiol and oestradiol-agonists transiently decreased short-term glucose levels in short-term exposure, while extended exposure had remarkably opposite effects, inducing insulin resistance and glucose intolerance during oestradiol treatment of longer duration [49].

Thus, available experimental and trial evidence indicates that higher and extended oestradiol exposure may have adverse effects on glucose levels, supporting a causal relationship between the observed strong associations of oestradiol with long-term risk of type 2 diabetes. Additional studies to evaluate the effects of oestrogen by means of administration, dose and duration on diabetes risk are warranted.

Strengths and limitations We adjusted for a wide range of lifestyle variables, reproductive factors and traditional clinical risk factors, including two different measures of adiposity, and also accounted for components of the metabolic syndrome in sensitivity analyses. It has been shown in postmenopausal women that there is unlikely to be any residual confounding by adiposity after controlling for BMI, as all associations between sex hormones biomarkers and measures of adiposity tissue, as assessed by dual energy X-ray absorptiometry, have been shown to be eliminated after BMI adjustment [50]. Additionally, results remained robust even after controlling for metabolic syndrome components. The prospective design of this study of apparently healthy participants also establishes the temporal relationship between these hormone biomarkers and incident diabetes. Robust and consistent results for all hormones in analyses excluding cases that developed during the first 3 years of follow-up also indicate that reverse causation by subclinical disease was not an explanatory factor for our findings. As with any observational study, residual confounding remains a possibility. However, the strength and robustness of the compelling associations found by us, in conjunction with the consistent and supportive body of short-term randomised trials and other experimental evidence, indicate that confounding is unlikely to explain the strong associations observed.

Conclusion

In this prospective study of apparently healthy postmenopausal women, higher endogenous levels of plasma oestradiol and testosterone were strongly and independently associated with increased risk of developing type 2 diabetes. These prospective data indicate that endogenous levels of sex hormones may play crucial roles in the pathogenesis of type 2 diabetes. This may have important implications for preventive interventions.

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