

The Association of Endogenous Sex Hormones, Adiposity, and Insulin Resistance with Incident Diabetes in Postmenopausal Women

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Context: In postmenopausal women, endogenous bioavailable testosterone (T) and estradiol (E2) have been positively associated, and SHBG has been negatively associated, with incident type 2 diabetes (T2DM). Previous studies have not explored possible factors explaining these relationships.

Objective: Our objective was to examine the association of endogenous sex hormones with incident T2DM in postmenopausal women and possible explanatory factors.

Design, Setting, and Participants: The Multi-Ethnic Study of Atherosclerosis (MESA) is a prospective study that included 1612 postmenopausal women aged 45–84 yr, followed between the years 2000–2006, who were not taking hormone replacement therapy, had no prevalent cardiovascular disease or diabetes, and had complete ascertainment of sex hormones.

Main Outcome Measures: T2DM was defined based on fasting glucose and/or treatment for diabetes.

Results: There were 116 incident cases of diabetes during follow-up. Across higher quartiles of bioavailable T and E2 and lower quartiles of SHBG, we found significantly greater hazards of developing incident T2DM (all *P* for trend ≤ 0.001). After adjustment for body mass index and insulin resistance estimated by homeostasis model assessment of insulin resistance, bioavailable T was no longer associated with incident T2DM. The associations of E2 and SHBG with incident T2DM were partially explained by body mass index and insulin resistance but persisted in fully adjusted models (both *P* for trend < 0.02). Dehydroepiandrosterone had no relationship with incident T2DM.

Conclusions: Adiposity and insulin resistance explained most of the association of bioavailable T but only partially explained the associations of E2 and SHBG with incident T2DM among postmenopausal women. (*J Clin Endocrinol Metab* 94: 4127–4135, 2009)

We have previously shown that higher serum bioavailable testosterone (T) and estradiol (E2) and lower SHBG are cross-sectionally associated with impaired fasting glucose (IFG) and type 2 diabetes mellitus in

postmenopausal women not taking hormone therapy (1). Few studies have prospectively examined endogenous sex hormones as predictors of type 2 diabetes in postmenopausal women (2–4). Both higher free or bioavailable T (3,

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Abbreviations: BMI, Body mass index; CI, confidence interval; CRP, C-reactive protein; DHEA, dehydroepiandrosterone; E2, 17 β -estradiol; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment of insulin resistance; IFG, impaired fasting glucose; LDL, low-density lipoprotein; MESA, Multi-Ethnic Study of Atherosclerosis; T, testosterone.

4) and lower SHBG (2) predict development of type 2 diabetes in women; however, results examining E2 are mixed. Although one study showed that total and bioavailable E2 did not predict type 2 diabetes (3), a more recent study showed total and free E2 to be strong predictors of type 2 diabetes (4). Dehydroepiandrosterone (DHEA) has been cross-sectionally associated with impaired fasting glucose but not type 2 diabetes in postmenopausal women (1). In one study, sulfated DHEA was not associated with incident diabetes (4). However, nonsulfated DHEA has not been examined as a predictor of type 2 diabetes.

In postmenopausal women without diabetes, endogenous free T and E2 are positively correlated with markers of insulin resistance, fasting glucose (3, 5–7), and measures of adiposity (8, 9). However, not all studies report a positive association of E2 with fasting glucose (3), and other studies in humans and rodents report that E2 treatment confers a protective effect on glucose metabolism (10). SHBG is inversely associated with both markers of insulin resistance, fasting glucose (1, 2, 11–14), and measures of adiposity (8, 9, 15) in postmenopausal women. DHEA is positively associated with fasting glucose, markers of insulin resistance (1, 11), and central adiposity (16) in postmenopausal women, although not all studies have found a positive association between DHEA and insulin resistance (17). Thus, sex hormones may exert their effects on diabetes risk through their association with glucose metabolism and/or adiposity. In addition, inflammatory factors have been associated with both sex hormones and diabetes (18, 19) and may contribute to this relationship. Possible confounders of the relationship between sex hormones and diabetes include behavioral factors such as physical activity, metabolic factors such as dyslipidemia, and past reproductive history.

We examined the association between endogenous total and bioavailable T, total E2, SHBG, and DHEA with incident type 2 diabetes mellitus among postmenopausal women in the Multi-Ethnic Study of Atherosclerosis (MESA), a cohort with complete assessment of sex hormones. The current analyses are one of the few studies to focus on several possible explanatory factors contributing to the relationship of sex hormones and incident type 2 diabetes, including adiposity, insulin resistance, and inflammatory, behavioral, metabolic, and reproductive factors.

Subjects and Methods

Study population

MESA is a multicenter, longitudinal cohort study of the prevalence and correlates of subclinical cardiovascular disease and

the factors that influence its progression (20). Individuals were excluded from MESA if they had clinical cardiovascular disease (20). Between July 2000 and August 2002, 6814 men and women who identified themselves as White, Black, Hispanic, or Chinese, and were 45–84 yr of age were recruited from six U.S. communities: Baltimore City and Baltimore County, MD; Chicago, IL; Forsyth County, NC; Los Angeles County, CA; Northern Manhattan and the Bronx, NY; and St. Paul, MN. The second examination (visit 2) occurred between 2002 and 2004, the third examination (visit 3) occurred between 2004 and 2005, and the fourth examination (visit 4) occurred between 2005 and 2006. Details on the sampling frames and the cohort examination procedures have been published elsewhere (20). Informed consent was obtained from each participant, and the study was approved by the Institutional Review Boards of each institution.

At baseline, there were 2169 women without prevalent cardiovascular disease who were postmenopausal and not using hormone replacement therapy. A woman was considered postmenopausal if she self-reported being postmenopausal, had undergone a bilateral oophorectomy, and/or was more than 55 yr of age. Women were excluded if they had prevalent diabetes ($n = 301$) or had missing data on diabetes status at any of the study visits ($n = 146$). Of the remaining women, 110 did not have complete measures of sex hormones. Thus, the current analyses were based on a total of 1612 women who fulfilled all the inclusion criteria.

Assessment of exposures: endogenous sex hormones

Participants fasted for 12 h and avoided smoking and heavy physical activity for 2 h before each examination. Fasting blood samples were drawn between 0730 and 1030 h. Serum samples, extracted by centrifugation at $2000 \times g$ for 15 min or $3000 \times g$ for 10 min, were immediately stored at -70°C and shipped to the University of Vermont for long-term freezer storage. Since MESA began, these samples have not been thawed.

Serum hormone concentrations were measured from stored samples at the Sex Hormone Laboratory at the University of Massachusetts Medical Center in Worcester, MA. Total T and DHEA were measured directly using RIA kits, and SHBG was measured by chemiluminescent enzyme immunoassay using Immulite kits obtained from Diagnostic Products Corp. (Los Angeles, CA). Total E2 was measured by use of an ultrasensitive RIA kit from Diagnostic Systems Laboratories (Webster, TX). The minimal detectable limit for each hormone was as follows: 0.14 nmol/liter (total T), 0.02 nmol/liter (SHBG), 0.14 nmol/liter (DHEA), and 0.009 nmol/liter (E2). Concentrations of free T, SHBG-bound T, and albumin-bound T were calculated according to the method of Södergård *et al.* (21), allowing for determination of bioavailable T as the sum of SHBG-bound T and albumin-bound T. Bioavailable T, calculated from T and SHBG as determined by immunoassay, has been shown to be comparable to apparent free T concentration obtained by equilibrium dialysis (22). Assay variability was monitored by including a 5% randomly chosen subset of blinded participant samples in each batch that were analyzed in duplicate. Blinded quality control sera included with the kits were also analyzed in duplicate. The overall coefficient of variation for total T,

SHBG, DHEA, and total E2 were 12.3, 9.0, 11.2, and 10.5%, respectively.

Assessment of outcomes

Diabetes was defined as a fasting glucose of at least 126 mg/dl (7.0 mmol/liter) or use of antidiabetic medication and was assessed at visits 2, 3, and 4. Impaired fasting glucose (IFG) was defined as a fasting glucose of 100–125 mg/dl (5.5–6.9 mmol/liter). Serum glucose was measured by rate reflectance spectrophotometry using thin film adaptation of the glucose oxidase method on the Vitros analyzer (Johnson & Johnson Clinical Diagnostics, Inc., Rochester, NY) (laboratory coefficient of variation is 1.1%).

Covariates

Possible confounding and explanatory factors were assessed at baseline using standard protocols that have been previously described (1, 20). Information on demographic characteristics such as sex, age, race/ethnicity, income (low, medium, or high), education (less than high school, high school, or college or greater), and family history of diabetes was collected. Anthropometric measures such as weight and height for calculation of BMI and waist circumference were measured similar to previous studies (23). Fasting plasma glucose, insulin, IL-6, C-reactive protein (CRP), and lipids were measured by methods previously described (23). Blood pressure was assessed using standardized methods (23). Insulin resistance was estimated using the homeostasis model assessment of insulin resistance (HOMA-IR) [glucose (mg/dl) \times insulin (mU/liter)/405] because of its strong association with fasting insulin, the euglycemic and hyperglycemic clamps, and type 2 diabetes as described previously (1).

Physical activity was assessed using the MESA Typical Week Physical Activity Survey and categorized according to previous studies (24). Daily caloric intake during the last year was calculated using a 120-item food frequency questionnaire as detailed previously (25). Smoking was self-reported and categorized (never, former, or current). Prescription medications such as lipid-lowering and blood-pressure-lowering agents were determined by transcription of medicines brought into the clinic. Reproductive variables including number of live births, age at first live birth (<23 yr, \geq 23 yr, or nulliparous), age at menopause, years since menopause, type of menopause (surgical *vs.* natural), ever use of hormone replacement therapy, and past use of birth control pills was self-reported.

Statistical analyses

The distribution of baseline characteristics was compared by diabetes status at the last visit using *t* tests for normally distributed continuous variables and χ^2 tests for categorical variables. In univariate analyses, because sex hormones, triglycerides, IL-6, CRP, low-density lipoprotein (LDL)-cholesterol, fasting insulin, and intentional exercise were not normally distributed, median levels of each of these variables were compared using a Kruskal-Wallis test for women who maintained normal glucose levels with those who developed IFG or type 2 diabetes over follow-up.

For subsequent analyses, sex hormones were divided into quartiles. Hazard ratios and 95% confidence intervals (CI) were calculated using Cox proportional hazards models to determine the relative hazard of developing type 2 diabetes for each quartile of sex hormone, using quartile 1 as the reference. Multivariable analyses were performed for total T, bioavailable T, total E2, SHBG, and DHEA.

The base model (model 1) included terms for age, race/ethnicity, education, income, family history of diabetes, and examination site. Model 2 included terms for model 1, body mass index (BMI), and HOMA-IR. Waist circumference and BMI were highly correlated (Pearson's correlation coefficient = 0.85; $P < 0.001$), so only BMI was used as a measure of adiposity, consistent with previous studies (3, 4). Model 3 was a fully adjusted model including all covariates in model 2 and metabolic factors [LDL, high-density lipoprotein (HDL), triglycerides, use of lipid-lowering medication, systolic blood pressure, and use of anti-hypertensive medication], behavioral factors (total daily caloric intake, physical activity, and smoking), inflammatory factors (IL-6 and CRP) and reproductive factors (age at menopause, years because menopause, type of menopause, age at first live birth, five or more live births, and past use of hormone replacement therapy or oral contraceptive pill).

We tested for effect modification by creating interaction terms between hormone levels and race/ethnicity, BMI, and IFG status in addition to performing stratified analyses. We also performed sensitivity analyses adjusting for SHBG in bioavailable T and E2 models and substituting waist circumference for BMI in multivariable models.

A two-sided *P* value (<0.05) was used to determine statistical significance. Statistical analyses were performed using SAS version 9.1.

Results

Baseline characteristics

Table 1 summarizes the baseline characteristics of the participants who did not have prevalent diabetes at baseline by diabetes status at their last visit. Of the 1612 postmenopausal women without prevalent diabetes at baseline, 116 developed diabetes and 312 developed IFG over a median follow-up of 4.7 yr.

Compared with postmenopausal women who did not develop diabetes, women who developed diabetes were significantly younger and less educated, exercised less, and were more likely to be of non-White ethnicity and have positive family history of diabetes. These women also had significantly higher BMI, waist circumference, fasting glucose, fasting insulin, HOMA-IR, IL-6, and CRP at baseline. The women who developed diabetes were more likely to be taking anti-hypertensive and lipid-lowering medications and had significantly higher systolic blood pressure and triglycerides and lower HDL-cholesterol at baseline. In addition, women who developed diabetes had their first birth at a younger age and had a greater number of live births.

Univariate analyses

In univariate analyses, women who developed diabetes had significantly higher baseline levels of bioavailable T and total E2 and lower SHBG compared with those who maintained normal glucose levels (Table 1). There was a

TABLE 1. Characteristics of 1612 postmenopausal women without prevalent diabetes at baseline, summarized according to diabetes status at their last visit

	Normal glucose	IFG	Diabetes
n	1184	312	116
Demographics and family history			
Age at baseline (yr) ^b	65.31 (9.48)	65.48 (8.83)	63.11 (8.62)
Ethnicity (%) ^c			
Caucasian	36	28	20
Chinese-American	14	9	17
African-American	28	33	34
Hispanic	22	30	29
Education (%) ^b			
Less than high school	20	29	29
High school	52	49	48
College or greater	28	22	23
Annual income (%)			
Low	33	38	40
Medium	38	37	36
High	29	25	24
Family history of diabetes (%) ^c	33	42	54
Behavioral factors			
Cigarette smoking status (%)			
Never	62	63	63
Former	27	25	28
Current	11	12	9
Daily caloric intake (calories/d)	1434 (660)	1514 (812)	1500 (797)
Intentional exercise (met-min/wk) ^{a,b}	735 (1733)	630 (1576)	375 (1155)
Adiposity and metabolic factors			
BMI (kg/m ²) ^c	27.8 (5.6)	30.1 (5.8)	32.1 (6.81)
Waist circumference (cm) ^c	95.3 (14.6)	101.1 (14.17)	106.9 (15.74)
Fasting glucose (mg/dl) ^c	86.71 (7.63)	97.49 (9.32)	103.63 (12.21)
Fasting insulin (mU/liter) ^{a,c}	4.9 (3.9)	7.15 (5.7)	8.8 (6.7)
HOMA-IR ^{a,c}	1.05 (0.88)	1.74 (1.40)	2.40 (1.85)
Systolic blood pressure (mm Hg) ^c	127.46 (23.41)	133.88 (22.86)	132.62 (22.31)
LDL-cholesterol (mg/dl) ^a	121 (39)	126 (46)	122 (47)
HDL-cholesterol (mg/dl) ^c	56.59 (14.52)	52.66 (15.01)	49.84 (11.59)
Triglycerides (mg/dl) ^{a,c}	102 (70)	120.5 (83.5)	133.5 (87.5)
One or more anti-hypertensives (%) ^c	29	47	49
One or more lipid-lowering agents (%) ^b	15	22	19
Inflammatory factors			
IL-6 (pg/ml) ^{a,c}	1.25 (1.03)	1.43 (1.22)	1.74 (1.50)
CRP (mg/liter) ^{a,c}	1.93(3.30)	2.90 (4.02)	3.03 (4.85)
Reproductive factors			
Age at menopause (yr)	48.06 (6.19)	47.76 (6.60)	47.60 (6.42)
Age at first live birth (yr) ^b	23.52 (5.17)	22.84 (4.98)	22.40 (5.17)
Number of live births ^b	2.99 (2.13)	3.28 (2.15)	3.45 (2.16)
Nulliparous (%)	17	13	15
First birth <23 yr (%) ^b	40	46	50
First birth ≥23 yr (%)	43	41	35
Surgical menopause (%)	19	21	20
Live births ≥5 (%) ^b	8.70	11.86	15.52
Time since menopause (yr)	17.29 (11.22)	17.66 (11.11)	15.51 (10.43)
Ever use of hormone replacement therapy (%)	30%	29%	30%
Ever use of oral contraceptives (%)	39%	41%	47%
Sex hormones (nmol/liter)			
Bioavailable T ^{a,c}	0.24 (0.21)	0.31 (0.31)	0.35 (0.35)
Total E2 ^{a,c}	0.055 (0.040)	0.062 (0.048)	0.079 (0.051)
SHBG ^{a,c}	54.45 (32.85)	43.05 (29.1)	35.75 (26.05)
DHEA ^a	10.76 (7.88)	11.45 (6.78)	11.66 (6.61)

Results are presented as mean (sd) unless indicated otherwise.

^a Median (interquartile range).

^b $P < 0.05$.

^c $P < 0.001$.

nonsignificant trend toward higher DHEA levels in women who developed diabetes.

Multivariable analyses

Among women without prevalent diabetes at baseline (n = 1612), increasing quartiles of bioavailable T were positively associated with incident diabetes (P for trend <0.0001). Women in the highest quartile of bioavailable T had a 2.45-fold risk of developing diabetes compared with those in the lowest quartile (95% CI = 1.48–4.03; Table 2, model 1). After adjustment for adiposity (BMI) and insulin resistance (HOMA-IR), the association weakened further such that it was no longer statistically significant (Table 2, model 2).

There was a strong, graded positive relation between quartiles of E2 and incident diabetes (P for trend <0.0001). Women in the highest quartile had a 3.03-fold greater risk of developing diabetes compared with those in the lowest quartile (95% CI = 1.88–4.84; Table 2, model 1). This association was attenuated after adjustment for adiposity and insulin resistance but remained statistically significant (Table 2, model 2; P = 0.003). Additional ad-

justment for behavioral, metabolic, inflammatory, and reproductive factors did not significantly attenuate this association further (P = 0.013).

There was a strong, graded, inverse relation between SHBG quartile and incident diabetes (P < 0.0001). Women in the highest quartile had a 76% lower risk of developing diabetes compared with women in the lowest quartile (relative hazard = 0.24; 95% CI = 0.14–0.42; Table 2, model 1). This association was weaker but remained statistically significant after adjustment for adiposity and insulin resistance (Table 2, model 2; P = 0.0004). In the fully adjusted model, this association was partially attenuated but remained statistically significant (Table 2, model 3; P = 0.017).

No significant association was found between DHEA and incident type 2 diabetes in any models (Table 2, models 1–3). We also found no significant association of total T and incident type 2 diabetes.

In stratified analysis, no significant interactions were found for sex hormones and BMI, race/ethnicity, or fasting glucose status.

TABLE 2. Relative hazards of developing diabetes by quartiles of baseline sex hormone status among 1612 participants without diabetes at baseline

Quartile of sex hormone	Model 1 (base) ^a	Model 2 (base + BMI + HOMA-IR) ^b	Model 3 (fully adjusted model) ^c
Bioavailable T			
Q1 (0–0.138 nmol/liter)	1.0	1.0	1.0
Q2 (0.139–0.24 nmol/liter)	1.29 (0.74–2.24)	1.13 (0.65–1.97)	0.97 (0.54–1.74)
Q3 (0.25–0.38 nmol/liter)	1.67 (0.98–2.84)	1.26 (0.73–2.15)	0.95 (0.53–1.71)
Q4 (0.39–6.49 nmol/liter)	2.45 (1.48–4.03)	1.35 (0.79–2.31)	1.23 (0.70–2.14)
P for trend	<0.0001	0.26	0.47
E2			
Q1 (0–0.043 nmol/liter)	1.0	1.0	1.0
Q2 (0.044–0.059 nmol/liter)	1.21 (0.70–2.10)	1.03 (0.59–1.80)	1.13 (0.61–2.09)
Q3 (0.060–0.081 nmol/liter)	1.38 (0.80–2.40)	1.08 (0.62–1.89)	1.18 (0.64–2.17)
Q4 (0.084–1.42 nmol/liter)	3.03 (1.88–4.84)	1.96 (1.19–3.23)	1.92 (1.10–3.35)
P for trend	<0.0001	0.003	0.013
SHBG			
Q1 (8.9–37.8 nmol/liter)	1.0	1.0	1.0
Q2 (38.0–51.4 nmol/liter)	0.39 (0.25–0.61)	0.49 (0.31–0.76)	0.41 (0.24–0.69)
Q3 (51.5–71.5 nmol/liter)	0.29 (0.18–0.47)	0.44 (0.26–0.74)	0.53 (0.30–0.92)
Q4 (71.8–255.5 nmol/liter)	0.24 (0.14–0.42)	0.43 (0.24–0.76)	0.52 (0.27–0.98)
P for trend	<0.0001	0.0004	0.017
DHEA			
Q1 (0.52–7.67 nmol/liter)	1.0	1.0	1.0
Q2 (7.70–10.96 nmol/liter)	0.89 (0.54–1.48)	0.93 (0.56–1.56)	0.99 (0.57–1.75)
Q3 (11–15.29 nmol/liter)	1.21 (0.76–1.95)	1.19 (0.74–1.92)	1.11 (0.66–1.86)
Q4 (15.30–52.72 nmol/liter)	0.98 (0.59–1.62)	0.98 (0.59–1.63)	0.97 (0.56–1.69)
P for trend	0.74	0.79	0.99

95% CI are shown in parentheses.

^a Adjusted for age, race/ethnicity, education, income, family history of diabetes, and examination site.

^b Adjusted using model 1 criteria, BMI, and HOMA-IR.

^c Adjusted using model 2 criteria, metabolic factors (LDL, HDL, triglycerides, use of lipid-lowering medication, systolic blood pressure, and use of anti-hypertensive medication), behavioral factors (total daily caloric intake, physical activity, and smoking), inflammatory factors (IL-6 and CRP), and reproductive factors (age at menopause, years since menopause, type of menopause, age at first live birth, five or more live births, and past use of hormone replacement therapy or oral contraceptive pill).

In sensitivity analysis, additional adjustment for SHBG in model 3 did not significantly change the association of bioavailable T (P for trend = 0.74) and estradiol (P for trend = 0.095) with incident diabetes. Furthermore, substituting waist circumference for BMI as a measure of adiposity in our analyses did not alter our observed associations.

Discussion

We found that among postmenopausal women without prevalent disease at baseline and not taking hormone replacement therapy, higher levels of bioavailable T and E2 and lower levels of SHBG were associated with incident type 2 diabetes. Adiposity and insulin resistance mostly explained the association of bioavailable T with incident type 2 diabetes but only partially explained the associations of E2 and SHBG with incident type 2 diabetes. DHEA was not associated with diabetes risk.

T

Higher bioavailable T was associated with incident diabetes, but this association did not persist after adjustment for adiposity and insulin resistance, similar to findings from our cross-sectional study (1). Two previous longitudinal studies have shown that free and bioavailable T predict development of diabetes, independent of BMI; however, neither study adjusted for insulin resistance, which may explain the differences with our results (3, 4). Although the association of insulin resistance with free or bioavailable T has been reported previously (3, 11, 13, 26), our results further suggest that adiposity and insulin resistance largely mediate the association of bioavailable T with diabetes risk.

Androgen excess in women likely impairs insulin action both in skeletal muscle and adipose tissue, resulting in reduced whole-body insulin sensitivity. Although not found in all studies (27), T-treated healthy women or hyperandrogenic women have impairment of insulin-stimulated glucose uptake that is reversed by androgen receptor antagonists (28, 29). Measures of adiposity in postmenopausal women are also positively associated with T levels (9, 30). Adipose tissue induces insulin resistance through various mechanisms including adipokine production (31). T administration increases intraabdominal fat in obese postmenopausal women (27) whereas anti-androgen therapy administration results in greater loss of visceral adipose tissue compared with untreated obese women with polycystic ovarian syndrome (32). Together, these studies suggest a role for adiposity in the association between T and insulin resistance and are consistent with the results of our present study.

E2

We found that E2 was positively associated with incident diabetes in multivariable analysis and that this association was only partially explained by adiposity and insulin resistance. Our results are similar to those of Ding *et al.* (4) who reported a significantly increased risk of developing diabetes in postmenopausal women who were in the highest quintiles of total and free E2 compared with the lowest quintile, which persisted in their multivariable models. In contrast, Oh *et al.* (3) did not find E2 to predict diabetes after adjustment for BMI.

E2 may be associated with diabetes risk through its relation to insulin resistance, adiposity, and/or inflammatory markers. Although Oh *et al.* (3) did not find a significant correlation of E2 with age-adjusted fasting glucose levels and other measures of insulin resistance, many other studies have suggested a strong association of E2 with measures of insulin resistance, independent of adiposity (1, 26). In our present study, the association of E2 with diabetes risk was partially explained by insulin resistance. High endogenous E2 in physiological states, such as puberty, the luteal phase of the menstrual cycle, and late pregnancy, are associated with insulin resistance and may involve reduced GLUT4 muscle expression (17, 33). Although studies of low-dose exogenous E2 in the form of hormone replacement therapy have been associated with a lower risk of diabetes in postmenopausal women, other studies have found exogenous oral E2 administration at higher doses to be associated with greater insulin resistance (10).

Endogenous E2 is also associated with development of adiposity (8, 9, 30, 34), although endogenous E2, itself, results from aromatization of T in adipocytes; thus, the relationship between E2 and adiposity is likely bidirectional. One animal study suggests that E2 may have direct effects on adipocyte enlargement and weight gain (35), although other studies show the opposite effect (36, 37). In our study, the association of E2 with diabetes was partially explained by adjustment for BMI.

E2 may also have effects on diabetes incidence through its association with inflammatory markers. Elevated inflammatory factors such as CRP have been found in women on oral estrogen treatment (38), and inflammatory markers have also been positively associated with endogenous estrone levels (18). Because inflammatory markers have been linked to development of diabetes (19), this might provide another mechanism by which E2 could lead to diabetes. E2 is also associated with lipid abnormalities (39) and lower physical activity (40). Finally, reproductive history, in addition to past use of hormone replacement therapy and oral contraceptive use, contributes to lifetime estrogen exposure and may be expected to impact levels of

endogenous E2 and diabetes risk. However, we found that the association of E2 and incident diabetes persisted in fully adjusted models, suggesting that E2 may have direct effects on glucose transport or metabolism.

SHBG

Our study corroborates previous findings (2) that lower SHBG is associated with a higher risk of incident diabetes. SHBG is an indirect measure of androgenicity with its concentrations mainly (although not exclusively) related to the level of free estrogen and androgens (41). In postmenopausal women, sex hormones are principally derived from the adrenally produced hormone androstenedione and not as greatly regulated by the gonadotropic axis as in premenopausal women. Thus, free levels of androgens and estrogen may be less strongly related to SHBG levels in postmenopausal women. Our results are consistent with previous reports that SHBG is inversely associated with adiposity (8, 9, 13, 15, 30) and measures of insulin resistance (13, 42), although we found BMI and insulin resistance to only partially explain the association of SHBG and incident diabetes.

The association between SHBG and diabetes risk is potentially confounded by behavioral and metabolic factors that affect endogenous SHBG levels as well as diabetes risk. Higher levels of usual physical activity are associated with significantly higher SHBG levels in postmenopausal women (40), although not all studies have reported this association (12, 43). SHBG is positively associated with HDL and inversely associated with triglycerides (39, 42). SHBG is also associated with inflammatory factors (18) and likely associated with past reproductive history. However, we found that the inverse association of SHBG and incident diabetes persisted in fully adjusted models, suggesting that SHBG may also have direct effects on glucose transport or metabolism.

DHEA

Our study showed no association of DHEA with incident diabetes similar to our cross-sectional study (1) and a previous longitudinal study of DHEAS (4). Endogenous DHEA has been positively associated with both insulin resistance and central adiposity in postmenopausal women (16, 44). However, other studies have suggested that DHEA is inversely associated with diabetes and insulin resistance (17). Human investigations of exogenous DHEA and glucose metabolism have yielded mixed results (45, 46). Thus, the variable effects of DHEA on glucose metabolism may account for the lack of association between DHEA and incident diabetes found in our study.

Sex hormones and race/ethnicity

Despite previous reports (47), we found no significant differences in the associations of sex hormones on diabetes risk by race/ethnicity, which confirmed the results of our cross-sectional study (1).

Strengths and limitations

Our study has several strengths. First, we had a relatively large sample of postmenopausal, ethnically diverse women to examine the relationship of sex hormones and incident diabetes. Second, because MESA included only women without prevalent cardiovascular disease, the women were healthier and the results may be more reflective of postmenopausal women in the general population as opposed to a clinic-based population. Third, we had complete ascertainment of sex hormone data on all postmenopausal women as opposed to a preselected subset. Fourth, we had information on DHEA levels that allowed us to examine the relationship between DHEA and incident diabetes, which has not been consistently reported in the literature. Finally, because MESA collected comprehensive data on diabetes risk factors, we were able to investigate adiposity, insulin resistance, and inflammatory, behavioral, metabolic, and reproductive factors as potential mechanisms and/or confounders explaining the relations between sex hormones and diabetes.

However, our study also has several limitations. We used baseline values of sex hormones to characterize each woman's hormonal status and subsequent risk of developing diabetes during follow-up. However, it has been previously reported that a single measurement for most of the plasma sex hormones can reliably categorize average levels over at least a 3-yr period in postmenopausal women, with intraclass correlation coefficients not varying by age or time since menopause (48). We were also unable to account for changes in glucose values during the intervals between follow-up visits, which may have resulted in misclassification of women with diet-controlled diabetes as normal glucose status and an underestimate of diabetes risk. Incident diabetes was defined using only fasting glucose criteria and not an oral glucose tolerance test, which may have misclassified some women as having normal or IFG status when they would have had diabetes using the oral glucose tolerance test. We would expect this definition of diabetes to have biased our associations to the null; however, we still had a large number of incident cases of diabetes and observed significant associations. Our study also had a relatively shorter follow-up time compared with other longitudinal studies that followed women over 8–10 yr (2–4). Although we found significant relationships within the follow-up time of our study, it is possible that the magnitude and precision of our observed

risk between sex hormones and incident diabetes may change with longer duration of follow-up. In addition, we did not have information regarding free E2 or estrone, the principal estrogen found in postmenopausal women (49). However, most previous studies in postmenopausal women have measured E2 and found total or free E2 to predict diabetes risk similarly (3, 4). As with any observational study, residual confounding remains a possibility. Alcohol consumption is positively associated with estrone levels in postmenopausal women (34), lower SHBG levels (43), and reduced risk of type 2 diabetes (50). However, we were unable to include alcohol consumption as a covariate in our behavioral model due to incomplete ascertainment of this information.

Implications

Our study found that endogenous sex hormones were associated with incident diabetes in postmenopausal women and that these associations were explained to varying degrees by adiposity and insulin resistance. Direct measures of glucose transport and metabolism could provide additional insight into the mechanism of sex hormone action on the development of diabetes. Further studies are needed to establish hormone thresholds at which diabetes risk is increased, because this may aid in identifying high-risk postmenopausal women in the clinical setting. Also, it is not known how medications inhibiting the production of adrenally produced sex hormones or aromatase activity may affect development of type 2 diabetes. In the future, studies investigating the effects of such medications on glucose metabolism and diabetes risk may facilitate the development of novel glucose-lowering therapies and diabetes prevention measures.

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