Circulating Estrone Levels Are Associated Prospectively With Diabetes Risk in Men of the Framingham Heart Study

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OBJECTIVE—In postmenopausal women and preclinical murine models, estrogen administration reduces diabetes risk; however, the relationship of estradiol and estrone to diabetes in men is poorly understood. We determined the relationship between circulating estradiol and estrone levels and diabetes risk in community-dwelling men of the Framingham Heart Study (FHS).

RESEARCH DESIGN AND METHODS—Cross-sectional relationships of estradiol and estrone levels with diabetes were assessed at examination 7 (1998–2001) in FHS generation 2 men (n = 1,458); prospective associations between hormone levels at examination 7 and incident diabetes were assessed 6.8 years later at examination 8. Type 2 diabetes mellitus was defined as fasting glucose >125 mg/dL, medication use, or both. Estradiol, estrone, and testosterone levels were measured with liquid chromatography–tandem mass spectrometry, and free estradiol and estrone were calculated.

RESULTS—In cross-sectional models, men with elevated estrone and estradiol had 40% and 62% increased likelihoods of existing diabetes per cross-sectional doubling of estrone and estradiol levels, respectively. Free estrone (cross-sectional odds ratio 1.28 [95% CI 1.02–1.62], P = 0.04) was associated with impaired fasting glucose at examination 7. There was an increase in risk of existing diabetes with increasing quartiles of total and free estrone and estradiol and an increase in risk of incident diabetes with increasing quartiles of estrone levels. In multivariate longitudinal analyses, a twofold increase in total or free estrone levels at examination 7 was associated with 77 and 93% increases, respectively, in odds of incident diabetes at examination 8.

CONCLUSIONS—Although both estradiol and estrone exhibit cross-sectional associations with diabetes in men, in longitudinal analyses estrone is a more sensitive marker of diabetes risk than is estradiol.

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Aging is associated with a decline in glucose tolerance, resulting in higher prevalence of type 2 diabetes mellitus (T2DM) and impaired fasting glucose (IFG) in older adults (1). Previous studies have suggested a role of endogenous sex hormones in the development of T2DM. Age-related decline in testosterone levels has been associated with an increased risk of T2DM in older men (2–5); however, the effects of low or high estrone and estradiol levels on T2DM risk in men are not clear.

Epidemiologic studies (6,7) and randomized trials (8–10) in women have suggested that hormone therapy reduces the risk of T2DM in postmenopausal women. Furthermore, genetic disruption of estrogen receptor α (ERα) in mice is associated with adiposity and insulin resistance (11). Only a few cross-sectional studies in older men have addressed the relationships between estradiol and T2DM, and the data are conflicting; some studies have shown a positive correlation of estradiol levels with T2DM (12,13), whereas others have found no significant association (5,14). The relationship between estrone and T2DM has not been studied in men. Most studies used immunoassays for the measurement of estradiol levels, for which accuracy in the low range has been questioned (15–17).

By using data from the Framingham Offspring Study, we determined whether circulating estrone and estradiol levels are associated with T2DM or IFG in community-dwelling older men. In longitudinal analyses restricted to nondiabetic men, we evaluated whether these hormones were predictive of incident T2DM during a follow-up period of approximately 7 years. This analysis is among the first population-based assessments of the association between estradiol and estrone—here measured with liquid chromatography–tandem mass spectrometry (LC-MS/MS), widely considered the reference method with the highest specificity and sensitivity—with T2DM risk in men (18).

RESEARCH DESIGN AND METHODS

Study sample

The Framingham Heart Study (FHS) design and methods have been described (19). Briefly, the original cohort was recruited from Framingham, Massachusetts, in 1948 to identify risk factors for cardiovascular disease. In 1971, the study enrolled a second-generation cohort (Gen 2):5,124 of the original participants' adult children and their spouses. The men of

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this Framingham Offspring Study cohort who attended examination 7 (1998–2001) were eligible for the current study (n = 1,625). Men with missing estrone and estradiol measurements (n = 159), those with prostate cancer undergoing androgen deprivation therapy (n = 5), and those with missing diabetes data at examination 7 (n = 3) were excluded, resulting in a sample size of 1,458 for the cross-sectional analyses.

For analyses of incident T2DM, the subset of men who attended examination 8 (2005–2008) was examined. The median time between examination 7 and 8 assessments was 6.8 years. For this analysis, we excluded men who had existing T2DM at examination 7 (n = 226), those who did not attend examination 8 (because of death or loss to follow-up), and those who lacked a T2DM assessment at examination 8 (n = 201). The longitudinal analysis was therefore restricted to 1,031 men.

Ascertainment of outcomes in FHS

Subjects were considered to have T2DM if their fasting glucose levels exceeded 125 mg/dL or they reported use of medication to control T2DM. Subjects were considered to have normal glucose levels if they had fasting blood glucose <100 mg/dL without medication; they were considered to have IFG if fasting blood glucose was between 100 and 125 mg/dL in the absence of T2DM treatment. A subject was deemed to have cardiovascular disease if he had coronary artery disease (angina pectoris, myocardial infarction, or sudden or nonsudden death attributable to coronary artery disease), congestive heart failure, cerebrovascular disease (stroke or transient ischemic attack), or intermittent claudication. Cancer was ascertained by self-report of physician diagnosis, supported by medical records when available. The men who reported smoking at least one cigarette per day during the previous year were categorized as current smokers. Alcohol consumption was measured and expressed in terms of ounces consumed per month; as in previous analyses, subjects were categorized into those who consumed no alcohol, those who consumed between 1 and 14 oz/month, and those who consumed >14 oz/month.

Hormone assays

The FHS samples were obtained between 7:30 and 9:30 A.M. after an overnight fast, aliquoted, frozen immediately, and stored at −80°C until the time of assay. At offspring examination 5 in 1991–1995, the stability of these FHS samples in storage was evaluated by measuring the concentrations of cholesterol, HDL cholesterol, and triglycerides before freezing and storage at −80°C and then repeating the measurement in 2007 (20).

Serum estradiol and estrone were measured with a highly sensitive LC-MS/MS assay. Derivatization of estrone and estradiol was performed with dansyl chloride. Estrone-d4 and estradiol-d5 (20 μL each) were added to 200 μL serum samples, extracted with methyl *t*-butyl ether (21,22), derivatized with dansyl chloride (3.7 mmol/L) in sodium carbonate (10 mmol/L, pH10.5) at 60°C for 10 min, and diluted in acetonitrile and water, and the samples were analyzed on API 4000 triple-quadrupole mass spectrometer (Applied Biosystems/MDS Sciex) with turbo ion spray HPLC pumps series 1200 and autosampler HTC PAL (LEAP). The mobile phase and mass spectrometry method used has been described previously (22). The limit of quantitation for both hormones was 2 pg/mL. Interassay coefficients of variation (CVs) for estrone were 4.5, 7.7, and 6.9% at estrone concentrations of 8, 77, and 209 pg/mL, respectively; interassay CVs for estradiol were 6.9, 7.0, and 4.8% at estradiol concentrations of 8, 77, and 206 pg/mL, respectively.

Free estradiol and estrone concentrations were calculated from a previously published law of mass action solution (22,23). We measured total testosterone with a validated LC-MS/MS assay (24). The limit of quantitation was 2 ng/dL. Interassay CVs were 15.8, 7.7, and 4.4% at 12.0, 241, and 532 ng/dL, respectively. Sex hormone–binding globulin (SHBG) levels were measured with an immunofluorometric assay (DELFIA-Wallac, Inc., Turku, Finland) (24). Interassay CVs were 8.3, 7.9, and 10.9%, and intra-assay CVs were 7.3, 7.1, and 8.7% in the low, medium, and high pools, respectively. The analytical sensitivity of the assays was 0.5 nmol/L.

Statistical analyses

Descriptive statistics were generated for outcomes, sex hormones, and covariate factors. Because of the moderate right skew evident in serum estrone, estradiol, total testosterone, and SHBG, these measures were log transformed. Exploratory assessments of associations were obtained by inspecting the relationship between quartiles of hormones and T2DM status.

Unadjusted estimates of the relative risk quantifying the cross-sectional relationship between hormones, divided into quartiles, and outcomes were generated with the modified Poisson regression approach, which uses the robust variance estimator to avoid bias in interval estimation and corresponding significance tests (25). Covariate-adjusted cross-sectional associations were analyzed with separate polytymous logistic regression models for total and free estrone and estradiol. These models simultaneously assessed the odds of IFG and T2DM in comparison with normal glucose levels.

The longitudinal associations between baseline hormone levels at examination 7 with the cumulative incidence of T2DM at examination 8 were assessed with separate regression models for each of the total and free hormones. In this analysis, the men who had T2DM at examination 7 were excluded. Again, the modified Poisson regression approach was used to determine unadjusted associations between hormone quartiles and T2DM status. Multivariate models used multiple logistic regression.

Both cross-sectional and longitudinal models considered the roles of age, BMI, smoking, total testosterone, and SHBG (for total hormone levels).

To enhance clarity, results on log-transformed values were back transformed and thus may be interpreted in terms of relative rather than absolute differences in hormone values. Estimates are scaled such that odds ratios (ORs) reported here may be interpreted in terms of the apparent effect of a between-person doubling of estrone, estradiol, or testosterone; that is, ORs reported here compare a hypothetical man with any estrone or estradiol level versus a man of similar age and covariates but with half that estrone or estradiol level.

All analyses were performed with SAS version 9.3 (SAS Institute, Cary, NC). Graphical data displays given here were constructed with R version 2.15.0 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Sample characteristics

The baseline characteristics of men in our cross-sectional and prospective study population are shown in Table 1. As expected from their lack of T2DM at examination 7, men eligible for analyses of T2DM incidence were slightly younger

Table 1—*Characteristics of the analytic samples for the cross-sectional and longitudinal analyses at initial observation (examination 7)*

	Cross-sectional (*N* = 1,458)	Prospective (*N* = 1,031)
Age, years	61 (10)	59 (9)
Current smoking	187 (13)	121 (12)
Alcohol consumption, oz/month		
0	395 (27)	238 (23)
1–14	543 (37)	414 (40)
≥15	515 (35)	376 (37)
BMI, kg/m^2		
Mean	28.8 (4.5)	28.4 (4.2)
<25	276 (19)	199 (19)
25–29	711 (49)	530 (52)
30–34	336 (23)	229 (22)
35	132 (9)	72 (7)
Cardiovascular disease	258 (18)	123 (12)
Diabetes	226 (16)	—
Cancer	141 (10)	80 (8)
SHBG, nmol/L	53 (40–73)	53 (39–71)
Total testosterone, ng/dL	561 (418–713)	572 (428–728)
Estrone, pg/mL		
Total	50 (39–61)	49 (38–60)
Free	1.8 (1.4–2.2)	1.8 (1.4–2.2)
Estradiol, pg/mL		
Total	25 (20–32)	25 (20–32)
Free	0.50 (0.39–0.63)	0.50 (0.39–0.62)

Data are mean (SD), *n* (%), or median (interquartile range).

than the overall cross-sectional sample. Aside from T2DM, however, the morbidity profiles of the cross-sectional and prospective samples were similar. The mean total and free estrone and estradiol were similar in men included in the cross-sectional and prospective analyses.

Analyses of existing cases of T2DM

Unadjusted associations between sex hormone quartiles and T2DM status at examination 7 are presented in Table 2. There was a general pattern of increase in prevalence of T2DM with both estrone and estradiol levels. These results were confirmed in analyses of continuous hormone levels and were robust to control for covariates (Table 3). After statistical controls for age, BMI, smoking status, SHBG, and total testosterone, both estradiol and estrone levels were significantly related to T2DM status at examination 7. With other factors held equal, men with elevated estrone and estradiol had an increased likelihood of existing T2DM: estimated increases in odds of 40% (cross-sectional OR 1.40 [95% CI 1.01–1.95]) and 62% (1.62 [1.13–2.32]) per cross-sectional doubling of estrone or estradiol, respectively. The free fractions of estrone and estradiol likewise showed multivariate-adjusted associations with T2DM. In similar models, total testosterone demonstrated an association with T2DM even after controlling for estradiol levels. This finding is in agreement with previously reported results that did not consider estradiol (24).

Neither total nor free estradiol demonstrated a cross-sectional association with IFG after adjustment for covariates. There was an estimated 28% increase in existing IFG per cross-sectional doubling of free estrone (cross-sectional OR 1.28 [95% CI 1.02–1.62]); the corresponding OR for total estrone, although similar in magnitude to that for the free fraction, was not statistically significant (1.24 [0.98–1.56], $P = 0.07$).

The influence of covariate factors on the cross-sectional associations between estrone and estradiol and T2DM is described in Fig. 1. Adjusted only for age, total and free estrone were positively associated with existing T2DM; these associations were preserved in a model controlling for BMI, smoking, SHBG, and total testosterone. The trend was similar for total estradiol; the estimated OR was of lesser magnitude, however, and the association was statistically nonsignificant. In contrast, only free estrone retained significant association with IFG after controlling for age and BMI alone (specific submodel not shown), and there was no significant association between either total or free estradiol and IFG after controlling for these and other covariates.

Analyses of incident cases of T2DM

Exploratory analyses indicated an association between estrone concentrations and incident T2DM. Approximately 11% of subjects with normal or IFG and total estrone measurements in the highest quartile had T2DM at examination 8, compared with 4.3% percent of subjects in the lowest total estrone quartile. A linear trend toward increase in incident T2DM was observed with increasing quartiles of total estrone levels (Table 2).

Analyses of the continuum of hormone concentrations confirmed these results. Associations between estrone and estradiol measured at examination 7 and T2DM status at examination 8, obtained from data on men who were not diabetic at examination 7, are presented in Table 3 and Fig. 1. In models controlling only for age effects, both total and free estrone as well as total estradiol were significantly associated with incident T2DM at examination 8. Models considering estrone that adjusted for all covariates were robust, indicating increased risk of incident T2DM among men with elevated total or free estrone levels at examination 7. This model indicates that, compared with a man of similar age and morbidity profile but with half his circulating total or free estrone at examination 7, a man would have estimated 77% (longitudinal OR 1.77 [95% CI 1.08–2.90]) and 93% (1.93 [1.17–3.19]) increases in odds of incident T2DM during approximately 7 years. Total estradiol levels were not significantly associated with incident T2DM, and results for free estradiol were equivocal (1.59 [0.99–2.57], $P = 0.06$).

CONCLUSIONS—The roles of estrone and estradiol in men's health remain poorly understood. In our analyses, elevated total and free estradiol as well as estrone levels were associated with existing T2DM. These associations were preserved in fully adjusted models that incorporated a control for total testosterone. These models therefore provide some evidence of a testosterone-independent association between circulating estrogens and T2DM in men. In contrast, in

Table 2—*Unadjusted associations between hormone quartiles and diabetes status*

	Cross-sectional (N = 1,458)		Prospective (N = 1,031)	
	RR[a] (95% CI)	P value	RR[a] (95% CI)	P value
Total estrone, pg/mL				
12.2–38.3	Referent		Referent	
38.4–48.9	1.31 (0.90–1.90)	0.15	1.63 (0.79–3.38)	0.20
49.9–59.6	1.28 (0.88–1.85)	0.19	2.34 (1.18–4.64)	0.02
59.7–139.0	1.65 (1.17–2.35)	0.005	2.54 (1.29–5.00)	0.008
Free estrone, pg/mL				
0.48–1.38	Referent		Referent	
1.39–1.76	1.01 (0.69–1.47)	0.96	2.18 (1.05–4.52)	0.03
1.77–2.16	1.16 (0.81–1.66)	0.42	1.89 (0.90–3.99)	0.09
2.17–5.34	1.55 (1.11–2.17)	0.01	3.07 (1.54–6.14)	0.001
Total estradiol, pg/mL				
5.03–19.5	Referent		Referent	
19.6–24.7	1.31 (0.89–1.94)	0.17	1.05 (0.58–1.88)	0.88
24.8–31.4	1.94 (1.35–2.77)	<0.01	0.99 (0.55–1.81)	0.99
31.5–118.0	1.51 (1.03–2.20)	0.03	1.04 (0.58–1.87)	0.90
Free estradiol, pg/mL				
0.12–0.39	Referent		Referent	
0.40–0.50	0.98 (0.67–1.44)	0.92	0.83 (0.42–1.60)	0.57
0.51–0.61	1.19 (0.83–1.71)	0.34	1.42 (0.79–2.55)	0.24
0.62–2.42	1.66 (1.19–2.31)	0.003	1.37 (0.77–2.45)	0.28

[a]Relative risk (RR) represents comparison of the upper three quartiles with the reference (lowest) quartile, with estimates obtained by Poisson regression with robust variance estimator.

longitudinal analyses, only the estrone levels were predictive of incident T2DM. Estrone thus may more sensitively capture T2DM risk, expressed either as concurrent prediabetic illness (i.e., IFG) or as incident T2DM. The lack of significant association between estradiol and incident T2DM is consistent with findings reported in the Rancho Bernardo Study (5,14).

The relationship between estrogens and T2DM has been recognized in women but not in men. Epidemiologic studies in postmenopausal women have found lower fasting glucose levels and a lower incidence of T2DM in women taking hormone therapy than in those not taking hormone therapy (26–28). Randomized trials, such as The Heart and Estrogen/Progestin Replacement Study (HERS), Postmenopausal Estrogen/Progestin Interventions (PEPI), and the Women's Health Initiative (WHI) (8–10), have reported a lower incidence of T2DM and lower fasting glucose levels in postmenopausal women assigned to hormone therapy than in those assigned to placebo. Genetic disruption of ERα but not estrogen receptor β (ERβ) in mice is associated with the development of adiposity, insulin resistance, and T2DM (11). These observations have led to speculation that ERα signaling regulates insulin sensitivity through a number of direct and indirect mechanisms, including alterations in insulin secretion and signaling, body composition and adipose biology, neuronal activity within specific hypothalamic nuclei (29), and additional effects on growth hormone and catecholamine secretion.

In the context of these observations in female mice and women, it is interesting that in community-dwelling men in this study estrone, but not estradiol, levels were prospectively associated with incident T2DM. Physiologically, the significant association between estrone but not estradiol and the risk of T2DM could be potentially explained by the differential actions of estrone on ERα and ERβ (30,31). Estrone and 17β-estradiol each have been shown to bind both ERα and ERβ, although 17β-estradiol has greater affinity and activity than estrone in many in vitro assays. The ligand specificity of various estrogens is reflected in the diverse pharmacologic effects of estrogen receptor modulators. For instance, in randomized trials women treated with tamoxifen had an increased risk of T2DM relative to those treated with placebo. In contrast, raloxifen administration has not been associated with an increased risk of T2DM (32). Although the exact mechanistic basis of the diverse effects of estrogen receptor modulators remains unknown, the estrogen receptor subtype specificity of various estrogen receptor ligands may contribute to their differential pharmacologic effects.

The biologic role of estrone in men has remained unappreciated. Although estrone is a weaker estrogen than estradiol in some bioassays, circulating estrone levels in men are higher than those of estradiol. Estrone can also be converted in the body to estradiol. We speculate that the association of estrone but not estradiol with T2DM may be related to the differential activity of these two ligands in estrogen receptor subtypes. Whether estrone exerts additional nongenomic effects on insulin secretion or sensitivity is not known. The mechanisms by which estrone might contribute to T2DM risk should be investigated.

Table 3—*Multiply adjusted cross-sectional and prospective associations between circulating estrogens and IFG or diabetes*

	Cross-sectional (N = 1,458)				Prospective (N = 1,031)	
	IFG		T2DM		T2DM	
	OR[a] (95% CI)	P value	OR[a] (95% CI)	P value	OR[b] (95% CI)	P value
Total estrone[c]	1.24 (0.98–1.56)	0.07	1.40 (1.01–1.95)	0.05	1.77 (1.08–2.90)	0.02
Free estrone[d]	1.28 (1.02–1.62)	0.04	1.40 (1.01–1.95)	0.04	1.93 (1.17–3.19)	0.01
Total estradiol[c]	1.02 (0.80–1.31)	0.86	1.62 (1.13–2.32)	0.008	1.42 (0.85–2.38)	0.18
Free estradiol[d]	1.12 (0.90–1.40)	0.32	1.55 (1.12–2.14)	0.008	1.59 (0.99–2.57)	0.06
Testosterone[e]	0.85 (0.64–1.12)	0.25	0.54 (0.37–0.79)	0.001	0.75 (0.43–1.30)	0.30

[a]Cross-sectional OR represents multiplicative increase in odds of concurrent IFG or T2DM per cross-sectional doubling of estrone, estradiol, or testosterone. [b]Longitudinal OR represents estimated multiplicative increase in odds of incident diabetes at examination 8 per between-person doubling of estrone, estradiol, or testosterone at examination 7. [c]Regression models adjust for age, smoking, BMI, SHBG, and total testosterone. [d]Regression models adjust for age, smoking, BMI, and total testosterone. [e]Regression model adjusts for age, smoking, BMI, SHBG, and estradiol.

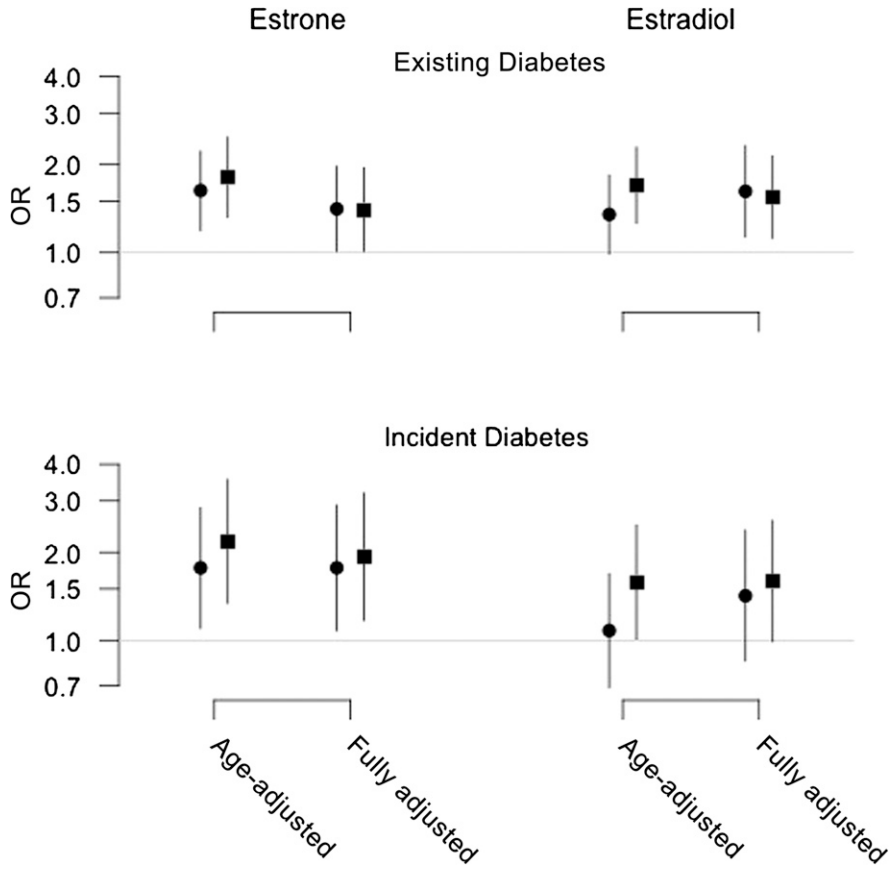

Figure 1—*Estimated ORs (point estimates and 95% CIs are shown) quantify associations between doubling of total (●) and free (■) estrone or estradiol and increases in the prevalence and incidence of T2DM in cross-sectional analyses including all subjects* (top) *and prospective analyses restricted to those without diabetes at baseline* (bottom). *Fully adjusted models control for age, smoking, BMI, and testosterone; models dealing with total (but not free) estrone and estradiol also control for SHBG.*

Our study has several strengths. We measured estrone and estradiol levels with LC-MS/MS, widely considered the reference method with the highest specificity and sensitivity (18). The prospective design of the analyses strengthens the inferences that can be drawn from these analyses. The cohort included community-dwelling men across a wide age range, from 19 to 89 years, and a follow-up of approximately 7 years.

Our study also has some limitations. The FHS population is predominantly white, and these findings may not be generalizable to other populations. Estrone and estradiol levels were measured in single morning samples and thus may not reflect hormone levels over a longer period. With single hormone measurements, we were able to estimate the apparent association of between-person differences with differential downstream risk of T2DM but could not directly capture the association of within-person changes with changes in T2DM status. Survival bias could contribute to the divergence in the apparent cross-sectional association of hormone levels with T2DM but not with IFG. We recognize that fasting glucose alone may fail to diagnose some cases of T2DM and that a 2-h oral glucose tolerance test is a more sensitive indicator of T2DM (33,34). It is therefore possible that some cases of T2DM may have been missed because an oral glucose tolerance test was not performed.

In conclusion, estrone but not estradiol levels were associated with increased risk of incident T2DM in a cohort of community-dwelling men. Future studies should test and confirm this relationship between estrone and T2DM in older men in other populations and investigate the mediating mechanisms.

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G.K.J. and S.B. wrote the manuscript. G.K.J. performed the analyses, and M.D. validated the analyses. T.G.T. wrote portions of the manuscript, guided the analyses, and reviewed and edited the manuscript. A.J.R., A.Z., M.M.K., A.L.R., W.M., A.D.C., R.D., and R.S.V. reviewed the manuscript. A.Z. and M.M.K. performed the estradiol, estrone, and testosterone assays. R.S.V. and S.B. generated the funding for the project. G.K.J. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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