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Relationship Between Higher Estradiol Levels and 9-Year Mortality in Older Women: The Invecchiare in Chianti Study

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Abstract

OBJECTIVES—To investigate the relationship between total estradiol (E2) levels and 9-year mortality in older postmenopausal women not taking hormone replacement therapy (HRT).

DESIGN—Population-based study of persons living in the Chianti geographic area (Tuscany, Italy).

SETTING—Community.

PARTICIPANTS—A representative sample of 509 women aged 65 and older with measures of total E2.

MEASUREMENTS—Serum total E2 was measured at the University of Parma using ultrasensitive radioimmunoassay (RIA).

RESULTS—Women who died (n = 135) during 9 years of follow up were older; had higher total E2 levels; and were more likely to have evidence of stroke, hypertension, diabetes mellitus, and congestive heart failure at baseline than survivors. Higher E2 levels were associated with a greater likelihood of death (hazard ratio (HR) = 1.03, 95% confidence interval (CI) = 1.01–1.06), and the relationship was independent of age, waist:hip ratio, C-reactive protein, education, cognitive function, physical activity, caloric intake, smoking, and chronic disease (HR = 1.08 pg/mL, 95% CI

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= 1.03–1.13, $P = .003$). The excessive risk of death associated with higher total E2 was not attenuated after adjustment for total testosterone (HR = 1.12, 95% CI = 1.02–1.18, $P<.001$) and after further adjustment for insulin resistance evaluated using the homeostasis model assessment (HR = 1.07, 95% CI = 1.03–1.17, $P<.001$).

Total E2 was highly predictive of death after more than 5 years (HR = 1.42: CI 1.01–1.91, $P = .04$) and not predictive of death for less than 5 years ($P = .78$).

CONCLUSION—Higher total E2 concentration predicts mortality in older women not taking HRT.

Keywords

estradiol; older postmenopausal women; mortality

Serum estradiol (E2) levels abruptly decrease at menopause in women. It has been postulated that the greater risk of cardiovascular events in women after menopause is due to sudden withdrawal of the protective effect of E2.[1] Accordingly, several large interventional trials have been conducted to test the effects of estrogen therapy in postmenopausal women. Surprisingly, the administration of estrogen and progesterone hormone replacement therapy (HRT) in late postmenopause was associated with greater risk of cardiovascular disease, stroke, and cancer.[2–4] The prevalence of adverse outcomes was higher in older age groups, suggesting that the timing of HRT initiation after menopause is an important factor that modulates the potential harm induced by estrogen administration.[5]

Because new ultrasensitive methods allow for accurate measurement of very low concentrations of endogenous estrogens, more studies on the effect of estrogens on health status have been published.[6] There is emerging evidence that postmenopausal women with higher E2 levels have a greater risk of developing insulin resistance, type 2 diabetes mellitus, and breast cancer.[7–13] In addition, higher E2 levels predict short-term mortality in older women with severe infections.[14] Despite this initial evidence of detrimental effects of E2, the overall relationship between endogenous E2 levels, health, and mortality in postmenopausal women is still uncertain.[15–18]

AIM OF THE STUDY

The relationship between total E2 levels in postmenopausal women and mortality was investigated over a 9-year period. The effect of free E2 estimated using a formula widely acknowledged in the literature was also analyzed.[19] The reason to conduct separate analyses on both measurements is that, although total E2 is directly measured, free E2 (the unbound hormone fraction) is the more biologically active E2 fraction.

METHODS

Study Population

The study population included 556 women randomly selected from all women aged 65 and older who were resident in the Chianti, Italy, area (the Invecchiare in Chianti (InCHIANTI) Study). Of these, 47 participants were excluded, because they were using HRT, leaving 509 women (aged 65–102) with complete data. Of 509 participants, 469 had natural menopause, and 40 had surgical menopause. All were using oral HRT. None of the participants were taking androgens. The Italian National Institute of Research and Care of Aging institutional review board ratified the study protocol, and all participants received a full description of the study and consented to participate.[20]

Biological Samples

Blood samples were obtained from participants between 7:00 a.m. and 8:00 a.m after a 12-hour fast after a 15-minute rest. Aliquots of serum were stored at −80°C until analyzed.[19]

Laboratory Measurement and Assay Characteristics

E2 was measured in the Laboratory of the University of Parma using ultrasensitive radioimmunoassay (RIA) using a DSL-4800 Kit with a minimum detectable concentration (MDC) of 2.2 pg/mL and intra- and interassay coefficients of variation (CVs) of 8% and 10%, respectively. Total testosterone was assayed using commercial RIA kits (Diagnostic Systems Laboratories, Webster, TX). For total testosterone, MDC was 0.086 ng/mL, and intra-assay and interassay CVs were less than 10%.

Sex hormone–binding globulin (SHBG) was measured using an RIA (Diagnostic Products, Los Angeles, CA) with an MDC of 0.04 nmol/L and inter- and intra-assay CVs for three concentrations of less than 6.9% and 3.6%, respectively. Concentrations of free E2 were calculated using mass action equations described previously.[19] Free testosterone was calculated using the Vermeulen formula.[21]

C-reactive protein (CRP) was measured using an high-sensitivity enzyme-linked immunosorbent assay, a competitive immunoassay that uses purified protein and polyclonal anti-CRP antibodies. The interassay CV was 5.0%. The MDC was 0.03 mg/L. Plasma fibrinogen level was automatically determined using a commercially available STA Fibrinogen Assay (Diagnostic Stago, Roche Diagnostics, Mannheim, Germany) according to the Clauss method and a Sta Stago Boerhinger Mannheim Autoanalyzer. Plasma insulin level was determined using a double-antibody, solid-phase RIA (interassay and intra-assay CVs 3.1% and 0.3%, respectively; Sorin Biomedica, Milan, Italy). Cross-reactivity with human proinsulin was 0.3%. Serum glucose level was determined using an enzymatic colorimetric assay (Roche Diagnostics) and a Roche-Hitachi 917 analyzer. Insulin resistance (IR) was calculated according to homeostasis model assessment (HOMA): IR = fasting insulin × fasting glucose/22.5.[22]

Comorbidity and Other Covariates

Experienced clinicians ascertained diseases according to preestablished criteria that combine information from self-reported physician diagnoses, current pharmacological treatment, medical records, clinical examinations, and blood tests. Diabetes mellitus was defined according to World Health Organization criteria.[23]

Hypertension was defined based on self-reported (physician diagnosis of hypertension and current antihypertensive treatment) or objective (systolic blood pressure >140 mmHg or diastolic blood pressure >90 mmHg) criteria.

Diseases included in the current analysis were cardiovascular disease (any of angina pectoris, myocardial infarction, congestive heart failure, stroke, hypertension), diabetes mellitus, cancer, and chronic obstructive pulmonary disease (COPD).

Waist circumference was measured at the midpoint between the lower rib margin and the iliac crest, and hip circumference was measured at the level of the trochanters to the nearest 0.5 cm using an inelastic plastic tape. Body mass index (BMI) was calculated as weight (kg)/height (m^2). Smoking history was determined from self-report and dichotomized in the analysis as current smoking versus ever smoked or never smoked. Education was recorded as years of school. Cognitive function was evaluated using the validated Italian version of the Mini-Mental State Examination (MMSE), with the total score adjusted for education and age.[24,25]

Physical activity in the year before the interview was coded as (1) sedentary (completely inactive or light-intensity activity <1 h/wk), (2) light physical activity (light-intensity activity 2–4 h/wk), or (3) moderate to high physical activity (light activity ≥5 h/wk or moderate activity ≥1 to 2 h/wk). Daily total energy (kcal) and alcohol (g) intake were estimated using the European Prospective Investigation into Cancer and Nutrition food frequency questionnaire.[26]

Statistical Analyses

Variables with symmetric distribution were reported as means ± standard deviations. Variables with asymmetric distribution were summarized as medians and interquartile ranges and were log-transformed in regression analyses and back-transformed for data presentation. The relationship between E2 and age was explored using a scatterplot. Comparisons between participants who died and those who remained alive over the follow-up were performed using age-adjusted linear regression models (analysis of covariance) and survival analysis including Kaplan-Meier and Cox proportional hazards models. Models that tested the association between E2 and mortality were adjusted for age, waist:hip ratio (WHR), log (CRP), education, cognitive function, physical activity, caloric intake, smoking, diabetes mellitus, cardiovascular disease, cancer, and COPD. Further models were also adjusted for total testosterone and HOMA. Analyses were repeated substituting calculated free E2 (the more biologically active component) for total E2.

A binary time indicator variable (dichotomized as mortality occurring before vs after Year 5) was also created, and the relationship between total E2 and mortality before and after Year 5 was separately analyzed. Then the interaction terms "E2 × dead less than 5 years" and "E2 × dead more than 5 years" were also added into the fully adjusted model.

All analyses were performed using the SAS statistical package, version 9.1 (SAS Institute, Inc., Cary, NC).

RESULTS

During 9 years of follow-up, 135 participants died, and 374 remained alive. Women who died were older, had higher serum total and free E2 levels, and were more likely to report a history of sedentary lifestyle and to be affected by stroke, diabetes mellitus, and congestive heart failure than those who survived (Table 1). Baseline means total E2 levels were 6.0 ± 3.0 pg/mL in survivors and 7.5 ± 5.0 pg/mL in those who died ($P = .02$). Mean total E2 levels in participants who had natural menopause and surgical menopause were 6.5 ± 3.9 pg/mL and 6.4 ± 4.5 pg/mL, respectively ($P = .92$). E2 levels were significantly higher ($P = .003$) in older women (Figure 1).

After adjusting for age, higher total E2 levels were associated with higher risk of death (hazard ratio (HR) = 1.03, 95% confidence interval (CI) = 1.01–1.06, $P = .009$). After further adjustment for multiple confounders (Model 2, Table 2), including age, WHR, log (CRP), education, cognitive function, physical activity, caloric intake, smoking, diabetes mellitus, cardiovascular disease, cancer, and COPD, the risk of death associated with higher E2 level was not attenuated (HR = 1.08, 95% CI = 1.03–1.13, $P = .003$). Furthermore, the relationship between total E2 and mortality remained significant after adjustment for total testosterone levels (HR = 1.12, 95% CI = 1.05–1.18, $P<.001$; Model 3, Table 2) and also for HOMA-IR, after excluding participants with diabetes mellitus (HR = 1.07, 95% CI = 1.03–1.17, $P<.001$; Model 4, Table 2).

Higher free E2 levels were significantly associated with higher mortality (HR = 1.79, 95% CI = 1.07–3.01, $P = .03$), and the relationship was much stronger after adjustment for confounders,

including age, WHR, log (CRP), education, cognitive function, physical activity, caloric intake, smoking, cardiovascular disease, diabetes mellitus, cancer, and COPD (HR = 5.72, 95% CI = 1.69–19.38, $P = .005$) and was approximately eight times as great after further adjustment for total testosterone levels (HR = 7.99, 95% CI = 2.50–25.55, $P<.001$) and HOMA-IR after excluding participants with diabetes mellitus (HR = 8.82, 95% CI = 2.50–31.16, $P<.001$).

Total E2 was highly predictive of mortality after more than 5 years (odds ratio (OR) = 1.42, 95% CI = 1.01–1.91, $P = .04$). The interaction term "E2 × death more than 5 years" was significant ($P = .02$), but E2 was not predictive of death for less than 5 years ($P = .79$), and the interaction term "E2 × death less than 5 years" was not significant ($P = .89$).

Figure 2 depicts the survival analysis (Kaplan-Meier) relating total E2 tertiles and 9-year mortality. Survival time was 7.5 ± 0.2 years for women in the lowest total E2 tertile (reference group), 7.4 ± 0.2 for women in the intermediate total E2 tertile (Group 1), and 6.9 ± 0.2 in women in the highest total E2 tertile (Group 2) (P for trend = .04). The respective mortality rates (per 1,000 person-years) were 32 in the reference group, 40 in the middle tertile, and 54 in the highest tertile of total E2.

DISCUSSION

Independent of age and multiple confounders, higher circulating E2 levels in late postmenopausal women were associated with higher mortality over a 9-year follow up. Despite conflicting data in the scientific literature on the beneficial effects of estrogens on health status, the relationship between E2 and mortality found in the Chianti population is not completely surprising. A few years ago, there was consensus that the sudden decline in estrogens with menopause accounted for the greater risk of cardiovascular disease after menopause in women.[6] Results from observational studies strongly supported this view, although some researchers had seriously questioned their validity.[27] More recently, the Women's Health Initiative (WHI) clearly demonstrated that HRT administered in late menopause was associated with greater risk of cardiovascular disease, stroke, and cancer and the risk of developing adverse health outcomes,[2–4] suggesting a timing hypothesis in the relationship between E2 and mortality.[28] Consistent with these findings, recent studies have demonstrated that E2 levels are high after stressor exposure[29] and that higher E2 levels predict short-term mortality in older women with severe infections.[14]

By showing that high levels of endogenous E2 are independently associated with higher mortality, the current study provides evidence to support the hypothesis that higher E2 levels have detrimental effects on health status in postmenopausal women.

At least three possible mechanisms might mediate the relationship between E2 and long-term mortality in postmenopausal women. A rising incidence of estrogen-related cancers, namely breast and uterine cancers, characterizes late postmenopause. These two forms of cancer account for a sizable portion of mortality in women in this age group. Unfortunately, this hypothesis could not be directly tested, because data on specific-cause mortality were not available for the InCHIANTI study population. Another possible explanation is that high E2 levels might reflect high fat mass and that obesity is a risk factor for mortality. Adipose tissue is probably the main site of conversion of testosterone to E2 in late life. Although adjusting for WHR did not reduce the association between E2 levels and mortality, because WHR is only an approximate measure of obesity and body composition, this hypothesis cannot be completely excluded.

The relationship between E2 and mortality did not change when BMI instead of WHR was included in the multivariate analysis (data not shown).

Finally, recent studies have found that, independent of multiple confounders, higher E2 levels in postmenopausal women were associated with altered glucose tolerance and predicted the development of insulin resistance and type 2 diabetes mellitus.[7–12] This is consistent with the notion that E2, by stimulating the E2 beta receptor, negatively influences GLUT4, the principal glucose transporter in the cells, and may subsequently increase insulin resistance.[30] Contrary to this hypothesis, in the current analysis, the inclusion of HOMA did not change the relationship between E2 and mortality.

The procoagulant effects of E2 are well known, and this mechanism may explain the high rate of thrombotic events observed in older postmenopausal women.[31,32] In the current analysis, fibrinogen levels, the only available marker of coagulation in the InCHIANTI study, were not significantly higher in those who died than in those who did not, and adjusting for fibrinogen did not affect the relationship between total E2 and mortality (data not shown). Consistent with observations, a previous study found a strong positive relationship between E2 and testosterone levels and mortality in postmenopausal women and men with severe infections,[14] although the women in that study were younger (aged 57–63) than those evaluated in the In-CHIANTI population.

Because 80% of circulating E2 levels in postmenopausal women are derived from aromatization from testosterone, especially in adipose tissue, it was hypothesized that testosterone might mediate the relationship between E2 and mortality, but total testosterone was not associated with mortality, and after adjusting for baseline testosterone levels, the strength of relationship between total E2 levels and mortality was unchanged. To exclude the possible influence of testosterone on this relationship, whether the testosterone/E2 ratio was a significant predictor of mortality was tested, but no evidence supporting this hypothesis was found (data not shown). Moreover, because the ovaries produce most testosterone, the history of surgical or natural menopause was accounted for, although type of menopause was not associated with different survival, and when type of menopause was included in the survival model as a potential confounder, the relationship between E2 level and mortality was not affected.

E2 levels were only mild predictors of mortality, suggesting that E2 might merely represent a simple surrogate marker of poor health status. Alternatively, E2 levels may be part of an adaptive process aimed at finding a new homeostatic equilibrium. Furthermore, because CIs for the mortality HR associated with higher E2 were considerably wide, these findings should be considered with caution, and their clinical relevance remains uncertain.

This study had some limitations. First, E2 was measured only at one point in time and in a small number of older postmenopausal women. Thus, these results require further investigation, especially in women immediately after menopause, when the role of endogenous E2 needs to be better elucidated. Second, no information was available on tissue E2 levels, which could have given more details on the biological activity of the hormone. Moreover, the existence of a relationship between E2 and mortality in an older population, even after adjusting for multiple confounders, does not necessarily imply a causal pathway between E2 and mortality in older women.

Third, because diet and lifestyle factors are known to influence E2 levels,[33,34] that dietary habits of this population could have affected the results cannot be excluded, although when caloric intake and physical activity were used as covariates in the fully adjusted analysis, the relationship was unchanged. Fourth, information on cause-specific mortality (cardiovascular and cancer) was also not available. Hence, the disease-specific mechanism underlying the relationship between E2 and mortality could not be directly addressed. Finally, participants

were not evaluated in the perimenopausal transition, and therefore, the findings are valid only for women in late menopause.

The study also had several strengths. This is the first population-based prospective study evaluating the relationship between serum E2 assessed using an ultrasensitive method and mortality in older women.

Participants were carefully selected according to HRT status, even though the sample of participants used in the analysis was comparable with those receiving HRT (data not shown). However, E2 levels in non-HRT users do not necessarily reflect the direct biological effects of higher estrogen exposure. This lesson has been learned from a long history of comparative research of the influence of plasma E2 and oral estrogen on risk of various diseases[8,16,35–38] showing contrasting results between the role of endogenous E2 levels, which largely demonstrate detrimental effects, and HRT in diabetes mellitus, cardiovascular disease, and cancer. In addition, expert clinicians ascertained disease status based on prestandardized criteria that used multiple sources of information.

In conclusion, higher total and free E2 are predictors of mortality in older women, independent of many confounders. Future studies should clarify the link between higher serum E2 levels and cause-specific mortality in older women.

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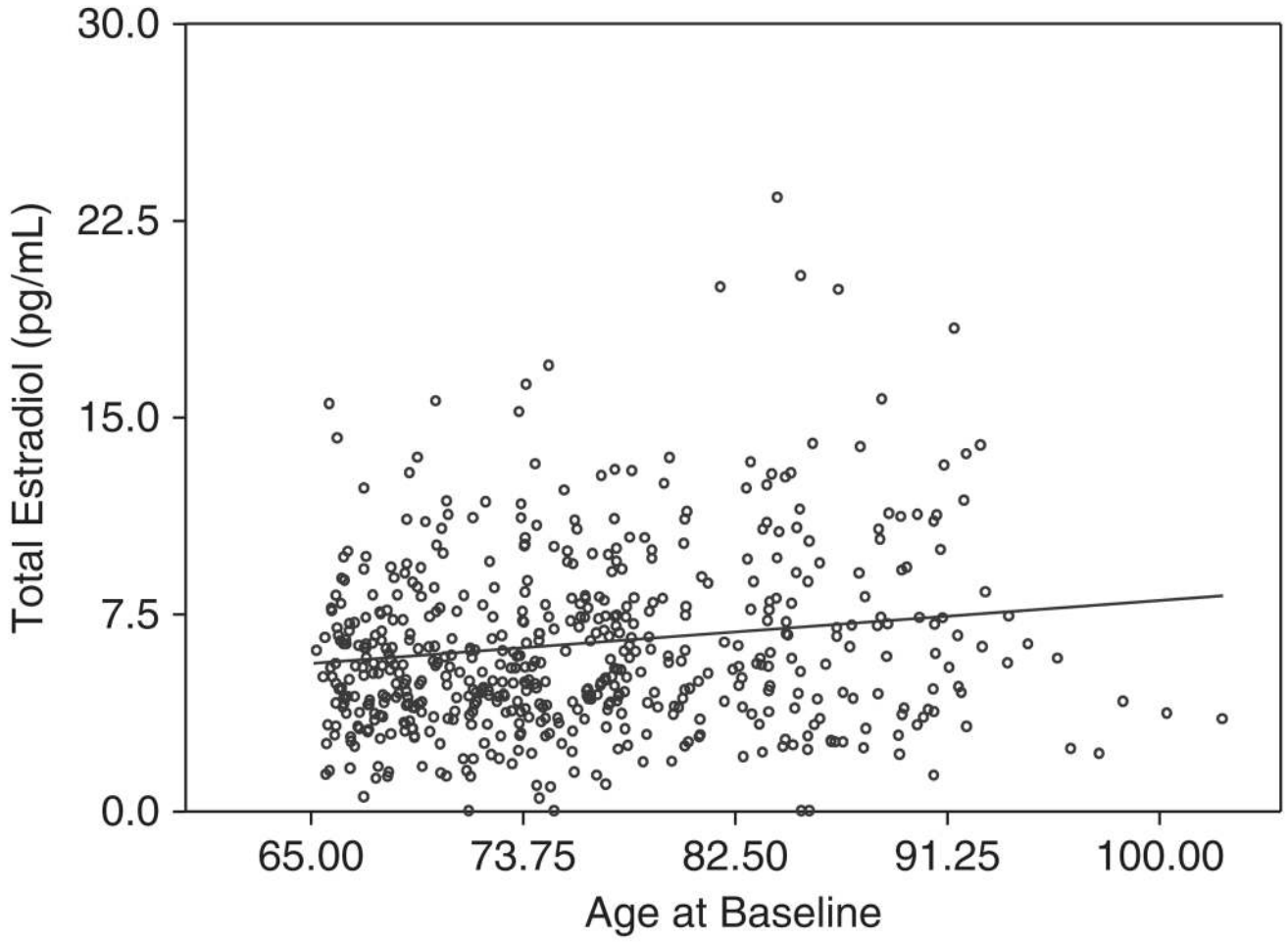

Figure 1.
Scatterplot of the relationship between serum total estradiol levels and age in older women. The relationship is statistically significant ($P = .003$).

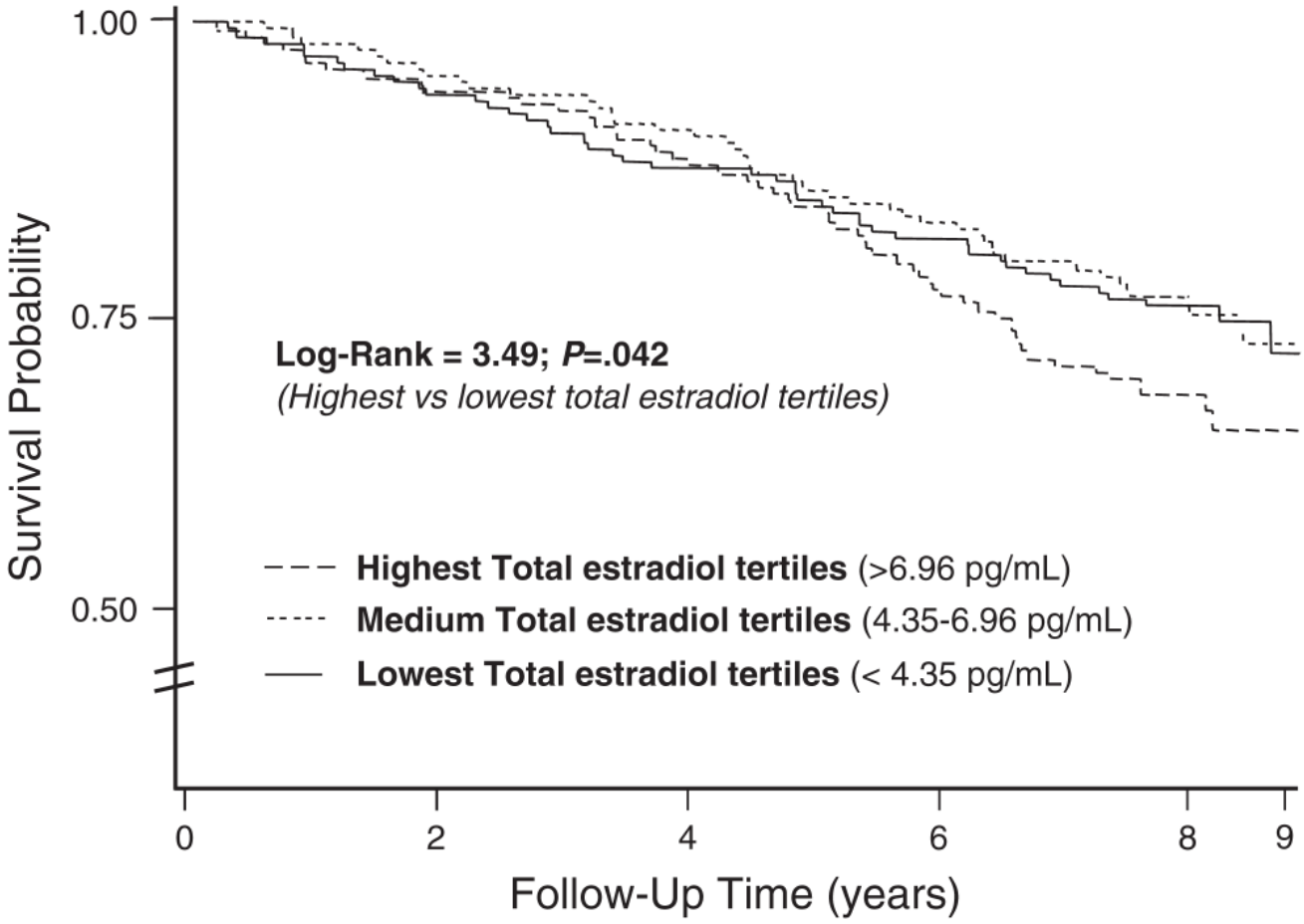

Figure 2.

Survival distribution function according to total estradiol (E2) tertiles shown for the 9 years of follow-up. The solid black line indicates the survival rates for participants in the lowest E2 tertiles (<4.35 pg/mL; reference group). Dashed and dotted lines are the survival rates for participants in the intermediate (4.35–6.96 pg/mL; group 1) and highest (>6.96 pg/mL; group 2) total E2 tertiles. Log-rank = 3.49; $P = .04$ (highest vs lowest E2 tertiles).

Table 1

Participant Charactristics at Enrollment

Characteristic	Survived (n = 374)	Died (n = 135)	*P*-Value*
Age, mean ± SD	73.7 ± 5.9	83.1 ± 7.9	<.001
Total estradiol, pg/mL, mean ± SD	6.0 ± 3.0	7.5 ± 5.0	.02
Free estradiol, pmol/L, mean ± SD	0.22 ± 0.16	0.27 ± 0.14	.04
Total testosterone, ng/mL, mean ± SD	0.62 ± 0.30	0.65 ± 0.33	.25
Free testosterone, ng/dL, mean ± SD	0.53 ± 0.44	0.49 ± 0.40	.76
Sex hormone–binding globulin, nmol/L, mean ± SD	132.7 ± 78.9	152.1 ± 77.5	.80
C-reactive protein, mg/mL, mean ± SD	4.3 ± 5.5	6.5 ± 9.6	.12
Education, years, mean ± SD	4.9 ± 2.6	3.7 ± 2.6	.28
Waist-to-hip ratio, mean ± SD	0.95 ± 0.05	0.96 ± 0.04	.42
Current smoking versus never and ever smokers, n (%)	26 (7.0)	9 (6.7)	.91
Total energy intake, kcal/d, mean ± SD	1,730.5 ± 475.7	1,624.3 ± 456.1	.63
Homeostasis model assessment index, median (interquartile range)	2.3 (1.64–3.26)	2.1 (1.39–3.39)	.003
Congestive heart failure, n (%)	60 (16.0)	38 (28.2)	<.001
Cancer, n (%)	27 (7.2)	12 (8.9)	.53
Stroke, n (%)	9 (2.4)	13 (9.6)	<.001
Diabetes mellitus, n (%)	34 (9.1)	23 (17.0)	.04
Hypertension, n (%)	176 (47.1)	80 (59.3)	.02
Chronic obstructive pulmonary disease, n (%)	16 (4.3)	11 (8.0)	.48
Mini-Mental State Examination score, mean ± SD	25.3 ± 3.2	20.8 ± 6.2	<.001
Physical activity, n (%)			<.001
Sedentary	60 (16.0)	76 (56.3)	
Light	303 (81.0)	57 (42.2)	
Moderate to high	11 (3.0)	2 (1.5)	
Menopause, n (%)			.80
Natural	341 (72.8)	128 (27.3)	
Surgical	33 (82.3)	7 (17.7)	

* Differences between survivors and died were analyzed using age-adjusted linear or multinomial logistic regression models, as appropriate.

SD = standard deviation.

Table 2

Relationship Between Total Estradiol and All-Cause Mortality in Older Women

Characteristic	Hazard Ratio (95% Confidence Interval)	*P*-Value
Model 1		
Total estradiol, pg/mL	1.03 (1.01–1.06)	.009
Age	1.16 (1.14–1.19)	<.001
Model 2		
Total estradiol, pg/mL	1.08 (1.03–1.13)	.003
Age	2.64 (1.52–4.58)	<.001
Education, year	0.99 (0.90–1.11)	.96
Waist:hip ratio	1.06 (0.06–19.4)	.97
Total energy intake, kcal/d	1.01 (0.96–1.06)	.72
Log (C-reactive protein), μg/mL	1.04 (0.98–1.08)	.28
Current smoking, %	1.67 (0.75–3.77)	.21
Cancer, %	2.48 (1.19–5.13)	.01
Diabetes mellitus, %	1.23 (0.58–2.63)	.59
Chronic obstructive pulmonary disease, %	0.91 (0.12–1.64)	.76
Mini-Mental State Examination score	0.89 (0.86–0.94)	<.001
Physical activity	0.65 (0.49–0.87)	.004
Cardiovascular disease, %	1.22 (1.05–1.42)	.009
Model 3 (Model 2+total testosterone)		
Total estradiol, pg/mL	1.12 (1.05–1.18)	<.001
Total testosterone, ng/dL	0.97 (0.30–3.06)	.95
Model 4 (Model 2+HOMA)*		
Total estradiol, pg/mL	1.07 (1.03–1.17)	<.001
HOMA, ng/dL	0.76 (0.33–1.47)	.18

Note: The relationship between total estradiol and mortality was analyzed using the Cox proportional hazard model.

*Removing from the analysis diabetes mellitus (n = 23).

HOMA = homeostasis model assessment.