Circulating Estradiol and Mortality in Men With Systolic Chronic Heart Failure

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Context Androgen deficiency is common in men with chronic heart failure (HF) and is associated with increased morbidity and mortality. Estrogens are formed by the aromatization of androgens; therefore, abnormal estrogen metabolism would be anticipated in HF.

Objective To examine the relationship between serum concentration of estradiol and mortality in men with chronic HF and reduced left ventricular ejection fraction (LVEF).

Design, Setting, and Participants A prospective observational study at 2 tertiary cardiology centers (Wroclaw and Zabrze, Poland) of 501 men (mean [SD] age, 58 [12] years) with chronic HF, LVEF of 28% (SD, 8%), and New York Heart Association [NYHA] classes 1, 2, 3, and 4 of 52, 231, 181, and 37, respectively, who were recruited between January 1, 2002, and May 31, 2006. Cohort was divided into quintiles of serum estradiol (quintile 1, <12.90 pg/mL; quintile 2, 12.90-21.79 pg/mL; quintile 3, 21.80-30.11 pg/mL; quintile 4, 30.12-37.39 pg/mL; and quintile 5, ≥37.40 pg/mL). Quintile 3 was considered prospectively as the reference group.

Main Outcome Measures Serum concentrations of estradiol and androgens (total testosterone and dehydroepiandrosterone sulfate [DHEA-S]) were measured using immunoassays.

Results Among 501 men with chronic HF, 171 deaths (34%) occurred during the 3-year follow-up. Compared with quintile 3, men in the lowest and highest estradiol quintiles had increased mortality (adjusted hazard ratio [HR], 4.17; 95% confidence interval [CI], 2.33-7.45 and HR, 2.33; 95% CI, 1.30-4.18; respectively; $P<.001$). These 2 quintiles had different clinical characteristics (quintile 1: increased serum total testosterone, decreased serum DHEA-S, advanced NYHA class, impaired renal function, and decreased total fat tissue mass; and quintile 5: increased serum bilirubin and liver enzymes, and decreased serum sodium; all $P<.05$ vs quintile 3). For increasing estradiol quintiles, 3-year survival rates adjusted for clinical variables and androgens were 44.6% (95% CI, 24.4%-63.0%), 65.8% (95% CI, 47.3%-79.2%), 82.4% (95% CI, 69.4%-90.2%), 79.0% (95% CI, 65.5%-87.6%), and 63.6% (95% CI, 46.6%-76.5%); respectively ($P<.001$).

Conclusion Among men with chronic HF and reduced LVEF, high and low concentrations of estradiol compared with the middle quintile of estradiol are related to an increased mortality.

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ESTROGENS HAVE NUMEROUS BIOlogical effects in men[1-3] and have a complex effect on the normal cardiovascular system.[4-6] Several studies have identified a role for estrogens in the pathophysiology of cardiovascular disease in men.[4-6]

Estrogens have beneficial effects on the myocardium and vasculature in men, such as attenuation of cardiovascular remodelling[7,8] and reduction in cardiomyocyte apoptosis and necrosis.[9-11] These cardioprotective and vasoprotective properties of estrogens may explain the link between low estradiol concentrations and an increased risk of cardiovascular events in a general male population.[12] In elderly men, hyperestrogenemia is related to an increased risk of stroke[13]; whereas, in men with severe infection, an excess of circulating estrogens has been linked to an increased risk of death.[14]

See also Patient Page.

Recent experimental studies have postulated that the altered metabolism of estrogens and the deranged expression of their receptors may be involved in the pathophysiology of chronic heart failure (HF).[7-11,15] The

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source of estrogens in men is from the aromatization of androgens.[1,2] We have previously demonstrated that deranged metabolism of both gonadal (testosterone) and adrenal androgens (dehydroepiandrosterone sulfate [DHEA-S]) is prevalent in men with chronic HF and has unfavorable clinical and prognostic consequences.[16-18] It has been presumed that along with the progressive derangement of androgen metabolism observed in the course of chronic HF, estrogen synthesis, degradation, or both may be disturbed.

We examined the relationship between serum estradiol concentration and mortality in men with chronic HF and reduced left ventricular ejection fraction (LVEF).

METHODS

Study Sample

The recruitment phase of the study was conducted between January 1, 2002, and May 31, 2006, among men with chronic HF and reduced LVEF who either attended the outpatient clinic or underwent a planned hospitalization (for diagnostic purposes) in 2 tertiary reference cardiology centers (Wroclaw and Zabrze, Poland). The criteria for study inclusion were (1) a documented history of chronic HF for at least 6 months; (2) LVEF of 45% or less as assessed by echocardiography (performed at the time of screening using Simpson planimetric method); and (3) clinical stability and unchanged medications for at least 1 month preceding the study. Exclusion criteria included (1) acute coronary syndrome or coronary revascularization within the 6 months preceding the study; (2) acute or chronic illness that might influence hormonal metabolism (ie, acute or chronic infections, chronic autoimmune diseases, previously established primary endocrine disorders); (3) any treatment with hormones (thyroid hormones, anabolic steroids, corticosteroids) or drugs markedly inhibiting hormone production either at the time of the study or in the past; and (4) unplanned hospitalization due to HF deterioration or other urgent cardiovascular pathology within the month preceding the study.

The study protocol was approved by the local ethics committees and all patients gave written informed consent. The study was conducted in accordance with the Helsinki Declaration.

Serum Concentrations of Hormones and Other Laboratory Measurements

In all men, venous blood samples were taken in the morning following an overnight fast and after a supine rest of at least 15 minutes. After centrifugation, serum blood was collected and frozen at –70°C until it was analyzed.

Serum concentration of estradiol was measured by using electrochemiluminescence on the Elecsys 1010/2010 System (Roche Diagnostics GmbH, Mannheim, Germany) and expressed in pg/mL (to convert to picomoles per liter, multiply by 3.671). The interassay and intra-assay variability coefficients for estradiol were 2.0% and 3.3%, respectively. The cohort was divided into quintiles of serum estradiol where quintile 1 included less than 12.90 pg/mL; quintile 2, 12.90 to 21.79 pg/mL; quintile 3, 21.80 to 30.11 pg/mL; quintile 4, 30.12 to 37.39 pg/mL; and quintile 5, 37.40 pg/mL or more. Quintile 3 was considered prospectively as the reference group.

Serum concentrations of total testosterone and DHEA-S were measured by using immunoassays (Diagnostic Products Corp, San Francisco, California) and expressed in ng/mL (to convert total testosterone to nanomoles per liter, multiply by 3.47, and DHEA-S to micromoles per liter, multiply by 0.00271). The interassay and intra-assay variability coefficients were 12.0% and 6.8% for DHEA-S, and 9.8% and 7.4% for total testosterone, respectively.

Plasma concentration of N-terminal pro-type B natriuretic peptide (NT-proBNP, expressed in pg/mL; to convert to nanograms per liter, multiply by 1.0) was measured by using immunoassay based on electrochemiluminescence on the Elecsys 1010/2010 System (Roche Diagnostics GmbH).

Renal function was assessed by using the estimated glomerular filtration rate (GFR, expressed in mL/min/1.73 m^2), which was calculated from the Modification of Diet in Renal Disease equation.[19]

Serum concentration of total bilirubin (mg/dL), sodium (mEq/L), uric acid (mg/dL), plasma concentration of total cholesterol (mg/dL), and activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) (both expressed in U/L) were assessed using standard methods. To convert total bilirubin to micromoles per liter, multiply by 17.104; sodium to millimoles per liter, multiply by 1.0; uric acid to micromoles per liter, multiply by 59.485; total cholesterol to millimoles per liter, multiply by 0.0259; and AST and ALT to microkatals per liter, multiply by 0.0167.

Fat Tissue Densitometry Measurements

Serum estrogens in men originate predominantly from androgens by the process of peripheral aromatization, in which fat tissue (containing aromatase, a key enzyme in estrogen synthesis) plays a principal role.[1,2] Therefore, in 264 men with chronic HF, we assessed total fat tissue (expressed in kilograms) by using dual-energy x-ray absorptiometry with a scan mode for total body composition assessment (LUNAR DPXIQ 7339 scanner, Madison, Wisconsin). Dual-energy x-ray absorptiometry is a widely applied, noninvasive method of bone densitometry and body composition, which involves only a minimal exposure to ionizing radiation.

Clinical Follow-up

Patients were observed regularly by the study investigators in the outpatient chronic HF clinic for at least 18 months in all survivors. Information regarding survival was obtained directly from patients or their relatives, from the HF clinic database, or from the hospital system. No patient was lost to follow-up. The primary end point for all the survival analyses was all-cause mortality during a 3-year follow-up.

Power Analysis

We set up a priori the hypothesis that in the course of chronic HF estrogen

metabolism may be disturbed and associated with relevant clinical consequences. We had also expected a nonlinear relationship between serum estradiol concentration and patients' outcome (already well-established for several hormonal systems in the endocrinology literature). To evaluate the risk relationship for both low vs normal and high vs normal values of serum estradiol in men with chronic HF, we based the power calculation on 2 separate comparisons. A Cox regression of the log hazard ratio (HR) on a covariate with a standard deviation of 0.5 (ie, 2 groups of equal size) based on a sample of 187 observations achieved 80% power at .05 significance level to detect an HR of 2. The sample size was adjusted for an anticipated event rate of 35%. As 3 groups might not allow for an adequate analysis of a nonlinear effect, we decided that 5 groups would be the required minimum; therefore, our goal was to recruit at least 470 patients.

Statistical Analyses

Continuous variables with a normal distribution (age, LVEF, hemoglobin concentration, estimated GFR, body mass index [BMI, calculated as weight in kilograms divided by height in meters squared], serum sodium, serum uric acid, plasma total cholesterol, systolic blood pressure, and total fat tissue mass) were expressed as mean (standard deviation). The intergroup differences were tested using the *t* test, the χ^2 test, or the analysis of variance where appropriate. The remaining continuous variables had a skewed distribution (plasma NT-proBNP, serum bilirubin, serum activity of ALT and AST, and serum concentrations of estradiol, total testosterone, and DHEA-S) and were expressed as median (interquartile range). The intergroup differences were tested using the Mann-Whitney U test, the χ^2 test, or the Kruskal-Wallis analysis of variance where appropriate. The associations between analyzed variables and survival were established by using Cox proportional hazard regression analyses (both univariable and multivariable models). In the univariable analyses, we included clinical parameters (age, New York Heart Association [NYHA] class [class 1, 2, 3, or 4], LVEF, plasma NT-proBNP, HF etiology, renal function assessed using estimated GFR, BMI, systolic blood pressure, hemoglobin concentration, serum uric acid, serum sodium, plasma total cholesterol, presence of diabetes mellitus, history of hypertension, smoking [never, current, or past], therapy with loop diuretics, and therapy with spironolactone), serum androgen concentrations (total testosterone and DHEA-S), and serum estradiol concentration. The NYHA classes 1, 2, 3, and 4 were defined as class 1, no limitation of physical activity, ordinary physical activity does not result in dyspnoea and/or fatigue; class 2, slight limitation of physical activity, ordinary physical activity results in dyspnoea and/or fatigue, no symptoms at rest; class 3, marked limitation of physical activity, less than ordinary physical activity results in dyspnoea and/or fatigue, no symptoms at rest; and class 4, dysponoea and/or fatigue at rest, any physical activity increases symptoms. For continuous variables, HRs with 95% confidence intervals (CIs) were calculated per interquartile range increment. The assumptions of proportional hazard were tested for all the covariates.

As already mentioned, we expected a nonlinear relationship between estradiol concentration and outcome in men with chronic HF. To assess the shape of the association between serum estradiol and survival in men with chronic HF, univariable and multivariable models were constructed, where serum estradiol was included as (1) a linear variable, (2) a log-transformed variable; (3) a transformation using restricted cubic splines with 3 to 5 knots[20]; and (4) 5 quintiles. To indicate the fitness of these models, χ^2 tests (with *P* values) were reported. Serum estradiol was modeled as a continuous variable with appropriate transformation using restricted cubic splines to account for nonlinear relationships.[20] Cubic splines are well suited because they allow nonlinear relationships, which were expected to exist in our study population. Five knots at quantiles of the predictors were used in our analysis. For the illustration of U-shaped relationship, serum estradiol was categorized into quintiles. In univariable and multivariable Cox proportional hazard regression models, the third quintile of serum estradiol (quintile 3) was chosen as the reference group. The following hierarchical model building approach was used: (1) unadjusted, (2) adjusted for age, (3) adjusted for all clinical and laboratory prognosticators tested in the univariable models (age, BMI, NYHA class, HF etiology, LVEF, plasma NT-proBNP, serum sodium, hemoglobin, estimated GFR, plasma total cholesterol, serum uric acid, therapy with loop diuretic, therapy with spironolactone, systolic blood pressure, history of hypertension, diabetes mellitus, and smoking); (4) adjusted for above mentioned prognosticators as well as androgens; and (5) adjusted for these clinical and laboratory prognosticators and androgens being significant predictors of death in univariable models.

To illustrate the effect of circulating estradiol on 36-month mortality rates, Kaplan-Meier curves for cumulative survival were constructed for men with chronic HF varying of serum estradiol and divided into 5 groups reflecting subsequent quintiles of serum estradiol. Differences in survival rates were tested using the Cox-Mantel log-rank test.

All statistical analyses were performed by using R version 2.5.1 (http://www.r-project.org), Graph Pad Prism 4.0 (GraphPad Software Inc, San Diego, California), MedCalc (MedCalc Software, Mariakerke, Belgium), and SPSS version 16.0 (SPSS Inc, Chicago, Illinois). $P < .05$ was considered statistically significant.

RESULTS

Serum Estradiol and Clinical Indices

TABLE 1 displays the baseline clinical characteristics of the entire cohort and of the cohort divided into quintiles

based on the serum estradiol concentration. All men were treated according to current recommendations. Those men with low serum estradiol (quintile 1) compared with patients with serum estradiol within the middle quintile (quintile 3) were characterized by increased serum total testosterone, decreased serum DHEA-S, advanced

Table 1. Baseline Characteristics of Men With Chronic HF and Reduced LVEF[a]

	All Men With HF (N = 501)	Serum Estradiol Quintile				
		1 (n = 100)	2 (n = 100)	3 (n = 100)	4 (n = 100)	5 (n = 101)
Clinical variables						
Age, y	58 (12)	60 (10)	59 (11)	58 (13)	56 (11)	57 (12)
BMI	26.8 (4.5)	26.3 (4.0)	27.2 (4.8)	27.1 (4.6)	25.8 (4.5)	27.4 (4.6)
LVEF, %	28 (8)	30 (8)	30 (8)	28 (8)	27 (8)	27 (8)
NYHA class, No. (%)						
1-2	283 (56)	47 (47)[b]	62 (62)	66 (66)	54 (54)	54 (53)
3-4	218 (44)	53 (53)	38 (38)	34 (34)	46 (46)	47 (47)
Ischemic HF etiology, No. (%)	353 (71)	77 (77)[c]	74 (74)	63 (63)	72 (72)	67 (66)
Systolic blood pressure, mm Hg	117 (17)	120 (16)	122 (16)	118 (17)	112 (16)[c]	114 (18)
History of hypertension, No. (%)	278 (56)	50 (50)	63 (63)	59 (59)	50 (50)	56 (55)
Diabetes mellitus, No. (%)	139 (28)	21 (21)	27 (27)	29 (29)	29 (29)	33 (33)
Total fat tissue mass, kg	21.9 (7.8)	18.9 (5.4)[b]	22.8 (9.1)	23.7 (8.3)	20.3 (7.1)[c]	23.0 (7.6)
Smoking, No. (%)						
Current	71 (14)	14 (14)	4 (4)[b]	18 (18)	21 (21)	14 (14)
Never	155 (31)	32 (32)	31 (31)	32 (32)	26 (26)	34 (34)
Past	275 (55)	54 (54)	65 (65)	50 (50)	53 (53)	53 (52)
Laboratory variables						
Plasma NT-proBNP, median (IQR), pg/mL	1817 (691-3899)	1794 (725-4104)	1652 (693-3291)	1576 (585-3038)	2138 (843-4562)[c]	1818 (655-3926)
Serum sodium, mEq/L	139 (5)	141 (5)[c]	141 (4)	140 (4)	137 (5)[d]	138 (5)[b]
Hemoglobin, g/dL	14.2 (1.7)	13.7 (1.5)[d]	14.2 (1.8)	14.6 (1.5)	14.0 (1.7)[b]	14.3 (1.7)
Estimated GFR, mL/min/1.73 m^2	75.0 (24.6)	69.1 (21.2)[c]	71.8 (22.9)	77.4 (26.5)	81.9 (26.1)	74.6 (24.3)
Plasma total cholesterol, mg/dL	184 (51)	189 (52)	186 (53)	187 (46)	178 (53)	183 (52)
Serum uric acid, mg/dL	7.1 (2.3)	6.9 (2.3)	6.8 (2.0)	7.2 (2.3)	7.1 (2.5)	7.4 (2.3)
Serum bilirubin, median (IQR), mg/dL	0.91 (0.68-1.31)	0.86 (0.60-1.33)	0.75 (0.63-1.01)	0.87 (0.69-0.99)	0.99 (0.69-1.40)[c]	1.11 (0.79-1.93)[b]
AST, median (IQR), U/L	25 (18-34)	24 (20-32)	22 (17-26)	25 (17-32)	25 (17-37)	29 (22-41)[b]
ALT, median (IQR), U/L	25 (18-35)	23 (20-34)	22 (17-30)	25 (16-32)	25 (17-38)	29 (22-42)[b]
Treatment, No. (%)						
ACE inhibitor and/or ARB	472 (94)	95 (95)	97 (97)	92 (92)	92 (92)	96 (95)
β-Blocker	466 (93)	93 (93)	93 (93)	92 (92)	91 (91)	97 (96)
Spironolactone	287 (57)	34 (34)[d]	38 (38)[d]	62 (62)	77 (77)[c]	76 (75)[c]
Digoxin	180 (36)	23 (23)[c]	29 (29)	38 (38)	45 (45)	45 (45)
Loop diuretic	346 (69)	59 (59)[c]	49 (49)[d]	73 (73)	81 (81)	84 (83)
Thiazide	82 (16)	28 (28)[b]	22 (22)	13 (13)	12 (12)	7 (7)
Statin	348 (70)	76 (76)	76 (76)	72 (72)	61 (61)	63 (62)
Acetylsalicylic acid	273 (55)	63 (63)	60 (60)	50 (50)	53 (53)	47 (47)
Serum hormones, median (IQR)						
Estradiol, pg/mL	26.9 (15.3-35.1)	8.6 (5.0-10.7)[d]	18.1 (15.3-20.0)[d]	26.8 (24.1-28.6)	33.4 (31.8-35.0)[d]	44.5 (41.0-54.5)[d]
Total testosterone, ng/mL	3.54 (2.52-4.70)	4.10 (3.00-5.23)[d]	3.86 (2.83-4.88)[c]	3.02 (2.29-4.24)	3.18 (2.24-4.08)	3.61 (2.53-4.63)
DHEA-S, ng/mL	779 (361-1361)	561 (295-980)[c]	827 (397-1293)	782 (439-1349)	975 (553-1466)	932 (332-1632)

Abbreviations: ACE, angiotensin converting enzyme; ALT, alanine aminotransferase; ARB, angiotensin receptor blocker; AST, aspartate aminotransferase; BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); DHEA-S, dehydroepiandrosterone sulfate; GFR, glomerular filtration rate; HF, heart failure; IQR, interquartile range; LVEF, left ventricular ejection fraction; NT-proBNP, N-terminal pro-type B natriuretic peptide; NYHA, New York Heart Association.

SI conversions: To convert ALT and AST to μkat/L, multiply by 0.0167; bilirubin to μmol/L, multiply by 17.104; DHEA-S to μmol/L, multiply by 0.00271; estradiol to pmol/L, multiply by 3.671; hemoglobin to g/L, multiply by 10.0; plasma NT-proBNP to ng/L, multiply by 1.0; sodium to mmol/L, multiply by 1.0; total cholesterol to mmol/L, multiply by 0.0259; total testosterone to nmol/L, multiply by 3.47; and uric acid to μmol/L, multiply by 59.485.

[a] Values are presented as mean (SD), unless otherwise noted. Men were grouped according to quintiles of serum estradiol: quintile 1 (<12.90 pg/mL), quintile 2 (12.90-21.79 pg/mL), quintile 3 (21.80-30.11 pg/mL; reference group), quintile 4 (30.12-37.39 pg/mL), and quintile 5 (≥37.40 pg/mL). Some men were missing for baseline characteristics; therefore, total numbers of men with HF were 264 for total fat tissue mass, 478 for total cholesterol, 486 for uric acid, 212 for bilirubin, and 219 each for AST and ALT. See the "Methods" section for explanation of variables. All *P* value comparisons were the other quintiles vs quintile 3 (reference group).

[b] $P < .01$.

[c] $P < .05$.

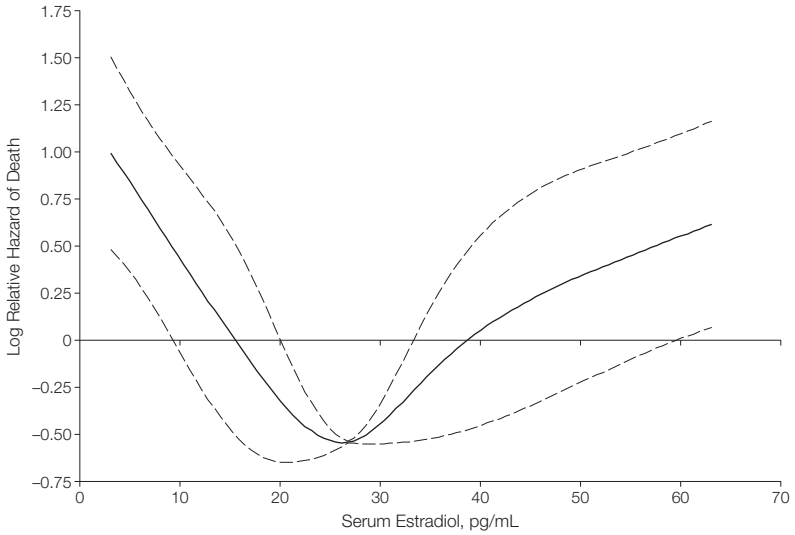
[d] $P < .001$.

Table 2. Effect of Different Transformation of Serum Estradiol on Fitness of Cox Proportional Hazard Regression Models Including Serum Estradiol as a Death Predictor During a 3-Year Follow-up in Men With Chronic HF and Reduced LVEF

Serum Estradiol Transformation	Univariable Models		Multivariable Model[a]		Contribution of Serum Estradiol in a Multivariable Model	
	χ^2 Test	*P* Value	χ^2 Test	*P* Value	χ^2 Test	*P* Value
Linear	0.2	.66	154.5	<.001	1.17	.28
Log-transformed	9.8	.002	162.5	<.001	9.83	.002
Cubic splines						
With 3 knots	36.9	<.001	181.4	<.001	30.7	<.001
With 4 knots	39.6	<.001	180.0	<.001	29.5	<.001
With 5 knots	40.8	<.001	186.6	<.001	32.6	<.001
5 Quintiles	26.7	<.001	184.0	<.001	29.9	<.001

Abbreviations: HF, heart failure; LVEF, left ventricular ejection fraction.
[a] Adjusted for age, body mass index, New York Heart Association class, HF etiology, LVEF, plasma N-terminal pro-type B natriuretic peptide, serum sodium, hemoglobin, estimated glomerular filtration rate, plasma total cholesterol, serum uric acid, loop diuretic, spironolactone, systolic blood pressure, history of hypertension, diabetes mellitus, smoking, serum total testosterone, and serum dehydroepiandrosterone sulfate.

Figure 1. Serum Estradiol by Log Relative Hazard of Death Using Cubic Splines With 5 Knots During 3-Year Follow-up in Men With Chronic Heart Failure and Reduced Left Ventricular Ejection Fraction



Serum estradiol by log relative hazard of death was calculated by using restricted cubic splines with 5 knots with 95% confidence intervals (dashed curves). To convert serum estradiol to pmol/L, multiply by 3.671.

NYHA class, more prevalent ischemic etiology of HF, decreased hemoglobin concentration, decreased estimated GFR, and decreased total fat tissue mass. These patients compared with quintile 3 were also less frequently treated with spironolactone, loop diuretic, and digoxin, and more often received thiazides.

Those men with high serum estradiol (quintile 5) compared with quintile 3 were characterized by increased serum bilirubin, increased serum activity of both AST and ALT, and decreased serum sodium. These patients compared with quintile 3 were also more frequently treated with spironolactone (Table 1).

Serum Estradiol and Prognosis

Among 501 men with chronic HF, 171 deaths (34%) occurred during the 3-year follow-up. The mean (SD) follow-up duration in all patients was 785 (331) days; follow-up in survivors, 955 (176) days; and time to death, 458 (313) days. In the whole group, the 3-year survival rate was 62.3% (95% CI, 57.5%-66.7%).

TABLE 2 presents univariable and multivariable Cox proportional hazard regression models in men with chronic HF, where serum estradiol as a prognosticator of 3-year survival was differently transformed and the fitness of these models had been tested. The Cox proportional hazard regression model of the spline-transformed serum estradiol with 5 knots was statistically significant. The estimated shape of the predictor is shown in FIGURE 1. The Cox proportional hazard regression model with serum estradiol quintiles was also significant and performed only slightly worse than the spline-transformed model (Table 2). In further survival analyses, serum estradiol was expressed as 5 quintiles.

The proportionality assumption and the assumption of a log-linear relationship between the prognosticators and the hazard function were fulfilled for all tested variables.

Univariable Analyses

In the univariable model, the most favorable outcome was in patients with circulating estradiol within the middle quintile, whereas the highest 3-year mortality rates were observed in those men with low (quintile 1) and high (quintile 5) circulating estradiol (TABLE 3 and TABLE 4). This was also shown in Kaplan-Meier survival curves in FIGURE 2.

In univariable Cox proportional hazard regression models, the following variables were shown to predict in-

creased 3-year mortality in 501 men with chronic HF: older age, higher NYHA class, low LVEF, high plasma NT-proBNP, decreased estimated GFR, decreased hemoglobin, decreased serum sodium, decreased plasma total cholesterol, high serum uric acid, low BMI, low systolic blood pressure, therapy with loop diuretic, and therapy with spironolactone (Table 4). However, HF etiology, presence of diabetes mellitus, history of hypertension, and smoking did not show statistical significance (all $P > .05$). Decreased serum concentrations of total testosterone and DHEA-S predicted poor outcomes in 501 men with chronic HF.

Multivariable Analyses

Serum estradiol expressed as 5 quintiles remained a significant predictor of death during a 3-year follow-up in men with HF also in multivariable models, when adjusted for clinical variables and androgen levels (Table 4 and TABLE 5). In all constructed multivariable models, the lowest and the highest estradiol quintiles were related to increased 3-year mortality compared with the middle estradiol quintile in men with congestive HF (Table 4 and Table 5). For increasing estradiol quintiles, 3-year survival rates adjusted for clinical variables and androgens were 44.6% (95% CI, 24.4%-63.0%), 65.8% (95% CI, 47.3%-79.2%), 82.4% (95% CI, 69.4%-90.2%), 79.0% (95% CI, 65.5%-87.6%), and 63.6% (95% CI, 46.6%-76.5%), respectively ($P < .001$) (Table 3).

The U-shape relationship between serum estradiol and 3-year survival in men with HF was also preserved when adjusted for clinical variables and androgen levels (Table 2).

COMMENT

There are 2 major findings arising from our study. First, we have shown that in men with stable chronic HF and reduced LVEF, there is a U-shaped outcome in relationship to serum estradiol concentrations. Both low and high concentrations of circulating estradiol are significant predictors of a poor prognosis, independently of gonadal and adrenal androgen deficiencies and conventional clinical prognostic indicators. Second, we have observed that men with either decreased or increased concentrations of serum estradiol have different clinical characteristics, suggesting that the underlying pathophysiological mechanisms are not the same.

Many recent studies indicate that estrogens are as important as androgens in male physiology and pathophysiology, including their cardiovascular protective effects.[4-6,21] Estrogens in men originate predominantly from peripheral aromatization of androgens and male adipocytes demonstrate a particularly high activity of aromatase, a key enzyme in estrogen synthesis.[1,2] Specific estrogen receptors are localized in the myocardium and vessel walls.[4-6,21] Data regarding the role of estrogens in maintaining health in men comes from clinical and epidemiological studies in humans, animal experimental studies,[4-6,21] and genetic diseases associated with estrogen deficiency in men (congenital aromatase deficiency and estrogen resistance due to specific receptor mutations).[2,3,22,23] There is evidence that estrogen deficiency may lead to impaired skeletal maturation, decreased lean mass, disturbed glucose and lipid metabolism, and premature atherosclerosis in men.[1,5,6,22]

The U-shaped relationship between serum estradiol concentrations and mortality in men with chronic HF and reduced LVEF is not easily explained. The major problem in attempting to discern a mechanism is the issue of whether the estradiol concentrations are related to critical body changes in the progression of HF or merely markers of that progression without a mechanistic role. There were differences in the characteristics of those men with low and high serum estradiol concentrations. In previous studies in normal

Table 3. Three-Year Survival Rates in Men With Chronic Heart Failure and Reduced LVEF

Survival Data	Serum Estradiol Quintile[a] 1 (n = 100)	2 (n = 100)	3 (n = 100)	4 (n = 100)	5 (n = 101)
No. of deaths/No. of patients at risk	56/100	29/100	20/100	27/100	39/101
Death rate, %	56	29	20	27	39
Follow-up, mean (SD), d					
For all patients	749 (372)	841 (345)	861 (275)	731 (297)	746 (340)
For survivors	1064 (77)	1015 (134)	954 (174)	848 (191)	937 (180)
For nonsurvivors	502 (321)	414 (332)	487 (292)	413 (302)	441 (313)
Death rate per 100 patients, y	26.9	12.4	8.4	13.3	18.6
3-Year survival rate, % (95% CI)					
Unadjusted	43.5 (33.6-53)	69.7 (59.4-78)	77.7 (67.2-85.2)	66.9 (54.2-76.7)	56.8 (45.4-66.7)
Adjusted for clinical variables[b] and androgens[c]	44.6 (24.4-63.0)	65.8 (47.3-79.2)	82.4 (69.4-90.2)	79.0 (65.5-87.6)	63.6 (46.6-76.5)

Abbreviations: CI, confidence interval; LVEF, left ventricular ejection fraction.
[a] Men were grouped according to quintiles of serum estradiol: quintile 1 (<12.90 pg/mL), quintile 2 (12.90-21.79 pg/mL), quintile 3 (21.80-30.11 pg/mL; reference group), quintile 4 (30.12-37.39 pg/mL), and quintile 5 (≥37.40 pg/mL).
[b] Included age, body mass index, New York Heart Association class, heart failure etiology, LVEF, plasma N-terminal pro-type B natriuretic peptide, serum sodium, hemoglobin, estimated glomerular filtration rate, plasma total cholesterol, serum uric acid, loop diuretic, spironolactone, systolic blood pressure, history of hypertension, diabetes mellitus, and smoking.
[c] Included serum total testosterone and serum dehydroepiandrosterone sulfate.

male populations, mortality was highest in those men with estradiol concentrations toward the low end of the normal range.[12] In our study, these men had a loss of renal function and decreased hemoglobin concentration, which may have contributed to the low estradiol and mortality. On other hand, the high estradiol group demonstrated a deterioration of liver function. Because estradiol is largely synthesized in fatty tissue and broken down in the liver, the relationship may relate to the severity of HF. Men with chronic HF and decreased serum estradiol were characterized by reduced total fat tissue mass. We may hypothesize that low circulating estradiol is due to a decreased activity of adipose aromatase. This concept is supported by the observation that low serum estradiol was accompanied by increased serum total testosterone due to an inhibition of the synthesis of estradiol from testosterone in fatty tissue. Observed associations between low serum estradiol and reduced fat tissue amount in men with chronic HF may explain some of the features of cardiac cachexia (particularly in those with marked fat tissue deficiency), such as endothelial dysfunction, increased systemic vascular resistance, impaired muscle capacity, and very high mortality.[24,25]

We have also found that men with chronic HF and high estradiol concentrations had increased serum bilirubin, and serum activities of AST and ALT were increased, whereas sodium level was decreased. Analogous to changes in steroid metabolism occurring in men with liver cirrhosis, high serum estra-

Table 4. Univariable and Multivariable Cox Proportional Hazard Regression Models of Mortality During a 3-Year Follow-up in Men With Chronic HF and Reduced LVEF

Variables[a]	Median (IQR) or Grouping Variable	Univariable Models			Multivariable Model[b]		
		χ^2 Test	HR (95% CI)	*P* Value	χ^2 Test	HR (95% CI)	*P* Value
Age, y	57 (52-66)	9.8	1.36 (1.12-1.64)	.002	3.2	1.29 (0.98-1.70)	.07
BMI	26.3 (23.7-29.4)	7.5	0.76 (0.62-0.93)	.006	0.1	0.96 (0.76-1.22)	.75
LVEF, %	28 (22-35)	38.4	0.42 (0.32-0.56)	<.001	9.2	0.58 (0.41-0.82)	.002
NYHA class		102.5		<.001	13.1		.004
2 vs 1			1.98 (0.85-4.62)			1.20 (0.49-2.92)	
3 vs 1			5.15 (2.25-11.80)			1.58 (0.65-3.89)	
4 vs 1			17.68 (7.33-42.67)			3.58 (1.30-9.85)	
HF etiology	Nonischemic vs ischemic	0.9	0.85 (0.60-1.20)	.34	0.04	0.96 (0.63-1.45)	.84
Plasma NT-proBNP, pg/mL	1817 (691-3899)	44.2	2.21 (1.75-2.79)	<.001	2.8	1.31 (0.95-1.80)	.10
Serum sodium, mEq/L	140 (137-143)	19.5	0.66 (0.55-0.80)	<.001	0.5	0.92 (0.72-1.17)	.49
Hemoglobin, g/dL	14.3 (13.2-15.2)	21.1	0.67 (0.57-0.80)	<.001	1.9	0.87 (0.71-1.06)	.17
Estimated GFR, mL/min/1.73 m^2	73.9 (59.2-89.1)	13.5	0.69 (0.57-0.84)	<.001	0.5	1.09 (0.86-1.38)	.49
Plasma total cholesterol, mg/dL[c]	179 (147-215)	4.6	0.79 (0.64-0.98)	.03	0.04	1.02 (0.82-1.29)	.84
Serum uric acid, mg/dL[d]	6.7 (5.5-8.0)	8.9	1.27 (1.08-1.48)	.003	1.9	1.14 (0.95-1.36)	.16
Loop diuretics	Yes vs no	22.2	2.53 (1.72-3.71)	<.001	0.7	1.24 (0.75-2.06)	.41
Spironolactone	Yes vs no	12.6	1.78 (1.29-2.44)	<.001	2.0	1.38 (0.88-2.16)	.16
Systolic blood pressure, mm Hg	120 (105-130)	14.5	0.64 (0.51-0.80)	<.001	0.2	0.93 (0.68-1.27)	.63
History of hypertension	Yes vs no	2.4	0.79 (0.58-1.06)	.12	0.7	0.85 (0.59-1.23)	.40
Diabetes mellitus	Yes vs no	2.9	1.32 (0.96-1.82)	.09	0.01	0.98 (0.67-1.43)	.91
Smoking		5.1		.08	3.0		.22
Never vs current			1.92 (1.07-3.44)			1.68 (0.85-3.36)	
Past vs current			1.83 (1.05-3.21)			1.78 (0.93-3.42)	
Serum total testosterone, ng/mL	3.54 (2.52-4.70)	42.0	0.70 (0.62-0.78)	<.001	9.9	0.79 (0.68-0.91)	.002
Serum DHEA-S, ng/mL	779 (361-1361)	55.1	0.44 (0.35-0.54)	<.001	10.0	0.63 (0.47-0.84)	.002
Serum estradiol, pg/mL		26.1		<.001	29.9		<.001
Q1 vs Q3			3.19 (1.91-5.32)			4.17 (2.33-7.45)	
Q2 vs Q3			1.48 (0.84-2.62)			2.15 (1.16-3.99)	
Q4 vs Q3			1.61 (0.90-2.87)			1.22 (0.64-2.31)	
Q5 vs Q3			2.23 (1.30-3.83)			2.33 (1.30-4.18)	
Total					173.3		<.001

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); CI, confidence interval; DHEA-S, dehydroepiandrosterone sulfate; GFR, glomerular filtration rate; HF, heart failure; HR, hazard ratio; IQR, interquartile range; LVEF, left ventricular ejection fraction; NT-proBNP, N-terminal pro-type B natriuretic peptide; NYHA, New York Heart Association; Q, quintile.

SI conversions: To convert DHEA-S to µmol/L, multiply by 0.00271; hemoglobin to g/L, multiply by 10.0; plasma NT-proBNP to ng/L, multiply by 1.0; sodium to mmol/L, multiply by 1.0; total cholesterol to mmol/L, multiply by 0.0259; total testosterone to nmol/L, multiply by 3.47; and uric acid to µmol/L, multiply by 59.485.

[a] For continuous variables, HRs with 95% CIs were calculated per IQR increment.

[b] n=463.

[c] n=478.

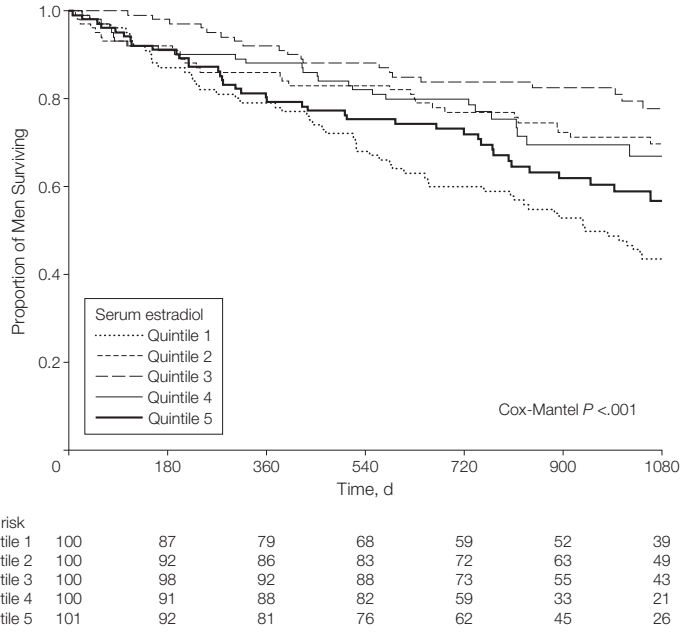
[d] n=486.

diol observed in the course of chronic HF could at least partially be due to deranged liver metabolism and secondary hepatocyte failure.[26-28] High estradiol concentrations observed in men during major illness may be due to increased peripheral aromatization.[29] The origin of high-circulating estradiol observed in men with stable chronic HF and reduced LVEF remains unknown.

In the literature, there is no consensus regarding the associations between circulating estrogens and cardiovascular risk in men.[12,13,30] There are links between certain polymorphisms of estrogen receptors (modifying tissue responsiveness to estrogen stimuli) in men and the severity of coronary artery disease,[31] a risk of fatal and nonfatal myocardial infarction,[32,33] and a risk of stroke.[34] High-circulating estradiol has been shown to predict an increased risk of death in men with severe infection[14] and an increased risk of stroke in elderly men.[13] Links between serum estradiol and cardiovascular disease have not been confirmed by other studies.[30] According to Arnlov et al,[12] low estradiol concentrations have been related to increased risk for cardiovascular disease in white men from a community-based study in a Framingham cohort.

Data from experimental models of HF in male animals indicate potential mechanisms by which estradiol could reveal cardioprotective effects.[7-11,15,35-37] Nakamura et al[38] have shown that cardiomyocytes are actively involved in the steroidogenesis and this process is markedly disturbed in failing human hearts. In the course of chronic HF, a reduced synthesis of DHEA in cardiomyocytes is observed, which subsequently promotes pathological hypertrophy and myocardial remodelling.[38] Estrogen receptors alpha are redistributed in human failing heart compared with controls.[15] Estrogen supplementation ameliorates myocardial remodelling and cardiac dysfunction after myocardial infarction in male mice and dogs.[10,35]

Figure 2. Kaplan-Meier Curves Reflecting 3-Year Cumulative Survival Rates in Men With Chronic Heart Failure and Reduced Left Ventricular Ejection Fraction According to Subsequent Quintiles of Serum Estradiol



No. at risk							
Quintile 1	100	87	79	68	59	52	39
Quintile 2	100	92	86	83	72	63	49
Quintile 3	100	98	92	88	73	55	43
Quintile 4	100	91	88	82	59	33	21
Quintile 5	101	92	81	76	62	45	26

Quintiles of serum estradiol were divided into quintile 1 (<12.90 pg/mL), quintile 2 (12.90-21.79 pg/mL), quintile 3 (21.80-30.11 pg/mL), quintile 4 (30.12-37.39 pg/mL), and quintile 5 (≥37.40 pg/mL). Quintile 3 was considered prospectively as the reference group. To convert serum estradiol to pmol/L, multiply by 3.671.

Table 5. Risk of Death During 3-Year Follow-up in Men With Chronic HF and Reduced LVEF According to Subsequent Quintiles of Serum Estradiol in Unadjusted and Adjusted Models

	Serum Estradiol Quintile Hazard Ratio (95% Confidence Interval)[a]						
Models	1	2	3	4	5	χ^2 Test	*P* Value
Unadjusted	3.19 (1.91-5.32)	1.48 (0.84-2.62)	1 [reference]	1.61 (0.90-2.87)	2.23 (1.30-3.83)	26.1	<.001
Adjusted for age	3.12 (1.87-5.20)	1.45 (0.82-2.57)	1 [reference]	1.70 (0.95-3.04)	2.27 (1.32-3.90)	24.9	<.001
Adjusted for all clinical covariates[b]	4.07 (2.28-7.25)	2.03 (1.11-3.71)	1 [reference]	1.06 (0.56-2.00)	2.12 (1.19-3.80)	31.7	<.001
Adjusted for all clinical covariates[b] and androgens[c]	4.17 (2.33-7.45)	2.15 (1.16-3.99)	1 [reference]	1.22 (0.64-2.31)	2.33 (1.30-4.18)	29.9	<.001
Adjusted for clinical covariates[d] and androgens[c] being significant predictors in univariate models	4.20 (2.37-7.44)	2.26 (1.22-4.16)	1 [reference]	1.24 (0.66-2.31)	2.40 (1.35-4.28)	30.7	<.001

Abbreviations: HF, heart failure; LVEF, left ventricular ejection fraction.
[a] Men were grouped according to quintiles of serum estradiol: quintile 1 (<12.90 pg/mL), quintile 2 (12.90-21.79 pg/mL), quintile 3 (21.80-30.11 pg/mL; reference group), quintile 4 (30.12-37.39 pg/mL), and quintile 5 (≥37.40 pg/mL).
[b] Included age, body mass index, New York Heart Association class, HF etiology, LVEF, plasma N-terminal pro-type B natriuretic peptide, serum sodium, hemoglobin, estimated glomerular filtration rate, plasma total cholesterol, serum uric acid, loop diuretic, spironolactone, systolic blood pressure, history of hypertension, diabetes mellitus, and smoking.
[c] Included serum total testosterone and serum dehydroepiandrosterone sulfate.
[d] Included age, body mass index, New York Heart Association class, LVEF, serum sodium, hemoglobin, estimated glomerular filtration rate, serum uric acid, loop diuretic, spironolactone, and systolic blood pressure.

Knockout mice lacking estrogen receptor beta develop severe cardiomyopathy with a disarray of cardiomyocytes and unfavorable molecular changes (eg, within gap junctions and nuclear structure).[37] In in vitro models, estradiol down-regulates angiotensin type 1 receptor on cardiac fibroblasts and inhibits angiotensin II–stimulated proliferation and collagen production by these cells.[36] In stretched isolated male rat heart, estradiol prevents heart dysfunction and decreases myocardial concentrations of tumor necrosis factor and nuclear factor kappa B activity.[8] In an experimental cardiomyopathy due to cardiac tumor necrosis factor overexpression, estradiol inhibits remodelling and left ventricular dilatation in male mice.[11] Estradiol interacts with proteins modulating cardiac excitation-contraction coupling and can lessen hypertrophic myocardial response to intracellular calcium derangements.[7] Estradiol exerts antioxidative properties[9,10] and makes male cardiomyocytes resistant to angiotensin II–induced apoptosis.[9] Moreover, estrogen signaling can activate bone marrow–derived endothelial progenitor cells, stimulate their mobilization and incorporation into neovascularization areas of ischemic myocardium; however, these effects have been demonstrated so far only in female mice.[39,40]

In contrast, there is no evidence whether and in what manner excessive amounts of estradiol in circulation might be detrimental in the context of survival for men with cardiovascular disorders.

Study Limitations

The observational character of our study is acknowledged. The study was not designed to elucidate the underlying detrimental mechanisms of low and high concentrations of serum estradiol in men with chronic HF and reduced LVEF. No simple explanation is evident to explain the findings. We propose that deranged liver metabolism and secondary hepatocyte failure may be a possible mechanism underlying increased serum estradiol level, whereas decreased serum estradiol may be due to a decreased amount or function of adipose tissue. Unfortunately, data on liver function tests and fat tissue mass are available only in about half of the entire study population, which limits any conclusion on the operating mechanisms. Measurement of additional cytokines or corticosteroid hormones might prove revealing.

The limitation of our analyses is a lack of the reference data on circulating estradiol measured in age-matched healthy men. Such information would allow us to determine the magnitude of impaired estradiol metabolism in men with chronic HF and reduced LVEF.

The studies on novel prognostic factors may comprise inherent methodological limitations. The selection of traditional prognosticators vs which new parameter is being tested will always be arbitrary. Our goal was to include a broad spectrum of prognosticators as comparators for estradiol describing clinical, laboratory, and hormonal aspects of congestive HF in our study, and the results appear very stable. Nevertheless, the possibility of an effect of unmeasured confounders cannot be entirely excluded.

Clinical Perspective

Adamopoulos et al[41] demonstrated that estrogens improved cardiac systolic performance and decreased pulmonary and systemic vascular resistance in patients with advanced chronic HF. In 2 other studies, low doses of estrogens have been shown to improve endothelial dysfunction in men with hypogonads[42] and increase myocardial perfusion in healthy men.[43] Based on the presented data (strong associations between low-circulating and high-circulating estradiol and mortality), the response to estradiol therapy in men with HF may depend on the baseline concentration. At present, there is insufficient evidence to consider estradiol supplementation as a treatment for HF, and our results suggest the response may be complex. Another much more controversial question would be the potential role of aromatase inhibition, estrogen receptor blockade, or both in those men with high-circulating estradiol.

CONCLUSIONS

We have demonstrated a U-shaped curve relating mortality in men with chronic HF and reduced LVEF to serum estradiol concentrations. Further studies are needed to explain the origin of these hormonal derangements.

Author Contributions: Dr Jankowska had full access to all of 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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