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Serum Estradiol Associates With Blood Hemoglobin in Elderly Men: The MrOS Sweden Study

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Context: Blood hemoglobin (Hb) declines with age in healthy elderly men, in whom decreasing T has been regarded as part of normal aging. However, the association between Hb and serum estradiol is incompletely known.

Objective: To determine whether estradiol is associated with anemia/Hb and established determinants of Hb in elderly men without prostate cancer.

Design, Setting, and Participants: The MrOS (Osteoporotic Fractures in Men) is a population-based study (n = 918; median age, 75.3 y; range, 70–81 y).

Main Outcome Measures: We evaluated total estradiol in relation to Hb and adjusted for potential confounders (ie, age, body mass index [BMI], erythropoietin [EPO], total T, cystatin C, and iron and B-vitamin status).

Results: Estradiol correlated negatively with age ($r = -0.14$; $P < .001$). Hb correlated (age adjusted) positively with estradiol ($r = 0.21$; $P < .001$) and T ($r = 0.10$; $P < .01$). Independent predictors for Hb in multivariate analyses were estradiol, EPO, BMI, transferrin saturation, cystatin C, and free T_4, but not T. After exclusion of subjects with Hb <130 g/L and/or T < 8 nmol/L (n = 99), the correlation between Hb and T was no longer significant, whereas the associations between Hb and estradiol remained. After adjusting for age, BMI, and EPO, men with lower estradiol levels were more likely to have Hb in the lowest quartile of values (odds ratio per SD decrease in estradiol = 1.61 [95% confidence interval, 1.34–1.93]). Anemic subjects (Hb < 130 g/L) had lower mean estradiol than nonanemic subjects (67.4 vs 79.4 pmol/L; $P < .001$).

Conclusions: Estradiol correlated positively and independently with Hb. Decreased estradiol might partly explain the age-related Hb decline observed in healthy elderly men. **(*J Clin Endocrinol Metab* 99: 2549–2556, 2014)**

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Abbreviations: BMI, body mass index; CI, confidence interval; CRP, C-reactive protein; CV, coefficient of variation; EPO, erythropoietin; EVF, erythrocyte volume fraction; Hb, hemoglobin; holoTC, holotranscobalamin; OR, odds ratio; WBC, white blood count.

The prevalence of anemia according to the World Health Organization (WHO) (blood hemoglobin [Hb] <130 g/L in men and <120 g/L in women) varies in the elderly and is seen in up to 40–50% of individuals, depending on the population studied (1, 2). Anemia in the elderly can be a significant health problem that confers increased risk of falls and fractures, decreased physical ability and quality of life, depression, cognitive impairment, and congestive heart failure and is associated with excess mortality (3). The most common causes of anemia in the elderly are chronic inflammatory disease and iron deficiency. However, the cause of anemia in the elderly is unknown in up to 36% (4, 5). Blood Hb declines with age in apparently healthy elderly subjects (6, 7) and is more pronounced in men (6, 7). In a longitudinal Swedish study, the decline in Hb between the ages of 70 and 88 years was 0.53 g/L/y for men and 0.05 g/L/y for women (7). The reason for this decline is incompletely known, but elderly men have been shown to have a lower number of bone marrow erythroid and myeloid precursors than women (8). The main proposed hormonal modulators of erythropoiesis are T, erythropoietin (EPO), and thyroid hormone (9, 10). In addition, GH and/or serum IGF-1 have been reported to be determinants of Hb in elderly subjects (11). In healthy men, there is a gradual but progressive age-dependent decline in T levels (12, 13); lower T levels are associated with lower Hb levels in middle-aged and older men and women (14), and androgens increase erythroid mass (15). Testosterone supplementation in hypogonadal men resulted in elevated hematocrit (16, 17), and T has been used for treating aplastic anemia in the past (18). The influence of age on levels of estradiol, the major biologically active estrogen, is complex. In males, estradiol is a metabolite (via aromatase) of T and androstenedione produced by the adrenals, and both production rates and plasma concentrations are higher in elderly men as compared to women (19). The age-related decline in T production does, however, not cause a corresponding decline in estradiol due to its increased tissue production by aromatization in fat mass (19). There is less information about estradiol levels in elderly men. In a large cross-sectional study of 1555 men (age range, 25–84 y; mean age, 60 y), total and free estradiol were higher in men over the age of 70 than below 40 years (20); no data on Hb were reported. Total (21, 22) and free estradiol (12) has been shown to decline with age in some studies, but not others (20, 23). Recently, high estradiol has been associated with higher hematocrit (24). We thus hypothesized that estradiol, independently from other proposed erythropoietic modulators, would correlate to Hb in a community-based population of older men.

Subjects and Methods

Study subjects—MrOS

The MrOS (osteoporotic fractures in men) study is an international multicenter prospective epidemiological investigation of elderly men. In this study, men participating in the Gothenburg part of the Swedish study (n = 1010; median age, 75.3 y; range, 69–81 y) were investigated. The general study design has been described previously (25). Participants were identified from national population registries and invited by mail to participate. To be eligible for the study, the participants had to be able to walk without physical aids and provide self-reported data about medical history, current medication, and lifestyle characteristics during the previous 12 months. Written informed consent was obtained from all study participants. The study was approved by the ethics committee at Gothenburg University (M 014-01) and conducted according to the guidelines in The Declaration of Helsinki.

Assessment of covariates

Body height and weight were measured using standard equipment. All measurements were carried out by the same trained staff. Body mass index (BMI) was calculated as weight (in kilograms) divided by height (in meters) squared. Levels of habitual physical activity were quantified using parts of the questions in the Physical Activity Scale for Elderly (26).

Blood sampling and analytical methods

Blood samples were collected at 8 AM after an overnight fast and nonsmoking. Hb, erythrocyte indices, erythrocyte volume fraction (EVF), former hematocrit, and blood cells were analyzed immediately on an automated cell counter (CELL-DYN 4000; Abbott) at Sahlgrenska University Hospital, Gothenburg, Sweden. Serum and plasma samples were obtained from April 2002 to December 2004, frozen within 1 hour, and stored at −80°C until analyzed. Serum samples for sex steroid levels were available for 99% of the subjects and were analyzed in one laboratory. We used a validated gas chromatography-mass spectrometry system (27, 28) to analyze total T (lower limit of quantitation, 0.05 ng/mL; intra-assay coefficient of variation [CV], 2.9%; interassay CV, 3.4%) and total estradiol (lower limit of quantitation, 2.0 pg/mL; intra-assay CV, 1.5%; interassay CV, 2.7%) in baseline serum samples (25). The accuracy was 102.1% (at 0.47 ng/mL) for T and 96.3% (at 19.9 pg/ml) for estradiol.

An HP5973 quadrupole mass spectrometer equipped with a chemical ionization source detected analytes and internal standards. We used immunoradiometric assay (Orion Diagnostics; detection limit, 1.3 nmol/L; intra-assay CV, 3%; interassay CV, 7%) to measure serum SHBG. IL-6 was measured using a quantitative sandwich immunoassay technique (Quantikine HS human IL6; R&D Systems, Inc; intra-assay CV, 7.4%; interassay CV, 6.5%). Measurements of the plasma EPO concentrations were carried out by using an immunoenzymatic assay (Quantikine IVD Human EPO DEP 00; R&D Systems, Inc) with a normal range of 3.1–14.9 IU/L in adults and total CV of 6.2% for a human serum control 11.2 IU/L. In addition, renal function was estimated from both serum creatinine and cystatin C (29). Serum iron, serum transferrin, serum ferritin, serum free T_4, serum TSH, and serum albumin were analyzed on the Roche Modular system (Roche Diagnostics Scandinavia AB). Total iron-binding capacity was calculated from transferrin, and transferrin satu-

Table 1. The MrOS Study Cohort: Demographic Data and Laboratory Values

	Proportion % or Mean (±SD)			
	All	Subjects Without Prostate Cancer	Subjects Without Prostate Cancer and No Deficiency of Iron or B Vitamins	Subjects Without Prostate Cancer and With T > 8 nmol/L and Hb > 130 g/L
n	1010	918	773	810
Age, y	75.3 (3.2)	75.3 (3.2)	75.3 (3.2)	75.2 (3.2)
Smoking, %	8	8	7.4	7.6
BMI, kg/m^2	26.2 (3.5)	26.2 (3.5)	26.1 (3.5)	26.1 (3.4)
Serum/plasma variables				
B-Hb, g/L	146.7 (11.8)	147.2 (11.5)	147.8 (10.8)	148.9 (9.6)
Total estradiol, pmol/L	76.9 (30.5)	78.7 (27.9)	78.5 (27.3)	81.1 (27.5)
Total T, nmol/L	15.4 (6.5)	15.8 (5.9)	15.9 (5.8)	16.5 (5.5)
SHBG, nmol/L	47.4 (24.6)	47.3 (24.6)	47.4 (23.6)	48.4 (24.5)
WBC, $\times 10^9$/L	6.6 (4.0)	6.5 (4.2)	6.5 (4.4)	6.3 (1.8)
Platelet count, $\times 10^9$/L	233.4 (60.9)	233.5 (60.9)	230.8 (55.6)	233.1 (59.4)
Ery-MCV, fL	93.7 (4.1)	93.7 (4.1)	93.9 (3.8)	93.7 (3.8)
B-EVF, %	44.5 (3.5)	44.6 (3.4)	44.8 (3.2)	45.1 (3.0)
S-Cobalamin, pmol/L	398.4 (228.0)	396.1 (226.1)	402.0 (218.1)	388.1 (206.8)
P-HoloTC, pmol/L	81.6 (95.8)	81.6 (118.9)	80.0 (79.1)	73.9 (71.3)
S-Folate, nmol/L	20.6 (11.9)	20.6 (12.0)	21.9 (11.8)	20.3 (11.6)
S-TIBC (μmol/L)	65.2 (9.1)	65.3 (9.1)	64.8 (8.9)	65.1 (8.9)
Transferrin saturation, %	30.5 (0.1)	30.6 (0.1)	31.6 (0.1)	31.2 (0.1)
S-Ferritin, μg/L	182.6 (164)	183.3 (167.2)	191.4 (168.8)	182.2 (146.5)
S-Iron, μmol/L	19.6 (6.2)	19.7 (6.3)	20.2 (6.0)	20.0 (6.1)
P-EPO, IU/L	11.5 (9.0)	11.5 (9.2)	11.2 (9.2)	10.6 (4.4)
S-Cystatin C, mg/L	1.13 (0.23)	1.13 (0.23)	1.13 (0.23)	1.12 (0.21)
IL-6, ng/L	3.36 (6.4)	3.35 (6.4)	2.92 (4.5)	3.02 (5.3)
Free T_4, pmol/L	17.5 (4.0)	17.3 (2.5)	17.4 (2.5)	17.4 (2.4)

Abbreviations: MCV, mean corpuscular volume; S, serum; B, blood; P, plasma; TIBC, total iron-binding capacity.

ration was calculated from serum iron and transferrin. Methods for holotranscobalamin (holoTC), total cobalamin, and serum folate have been described previously (30). C-reactive protein (CRP) was measured by an ultrasensitive particle-enhanced immunoturbidimetric assay (Orion Diagnostica). The analyses were performed on a Konelab 20 autoanalyzer (Thermo Fisher Scientific), with a detection limit of 0.1 mg/L and interassay CV below 5%.

Statistical analyses

Standard methods were used for tests of correlations between variables. The Pearson correlation test was used for determination of univariate correlations, and linear regression models were used for test of correlations adjusted for confounding factors, giving standardized β-values. Differences in means between two groups were tested with the Welch-Satterthwaite *t* test and linear regression models to adjust for different confounders. Because many of the variables showed skewed distributions, skewed continuous variables were analyzed in the log scale. Stepwise multiple regression analyses with demographic and laboratory variables as possible explanatory variables for Hb were performed. To study the nonlinearity of the association between Hb and estradiol/T, a spline regression model was fitted using knots at the 10th, 50th, and 90th percentiles of estradiol/T (31). The splines were second-order functions between the breakpoints and linear functions at the tails, resulting in a smooth curve.

Double-sided tests were used throughout, and a significance level of $P < .05$ was regarded as statistically significant. The software used was SAS for Windows, version 9.1 (SAS Institute, Inc); a database and statistics program package developed at the Department of Community Medicine and Public Health, Gothenburg University, was used.

Results

Characteristics of study population

Demographics and selected laboratory data from the total study group (n = 1010) as well as the different subgroups are presented in Table 1. Nine percent (92 of 1009) of the subjects had a diagnosis of prostate cancer at the start of the study according to the Swedish Cancer Registry; 40% (36 of 91) of those with prostate cancer had T < 8 nmol/L. Subjects with prostate cancer had significantly lower mean Hb compared to subjects without cancer (141.3 vs 147.2 g/L; $P < .001$). To avoid prostate cancer as a potential confounder, all men with prostate cancer were excluded at the outset. The remaining subjects (n = 918) are henceforth described as the total study group. Mean age was 75.3 ± 3.2 years (range, 70–81 y). Mean Hb level was 147.2 ± 11.5 g/L. Hb < 130 g/L was found in 5.6% (51 of 915), 6.1% (55 of 908) had T < 8 nmol/L, and 10.9% (99 of 909) had Hb < 130 g/L or T < 8 nmol/L. The remaining 810 individuals had Hb ≥ 130 g/L and T ≥ 8 nmol/L, and 257 of 734 (35.0%) had EVF ≥ 46%. To exclude subjects in whom deficiency of cobalamin, folate, and iron might have caused a low Hb, those with holoTC < 19.6 pmol/L (30) (2.9%, 26 of 910) and/or serum folate < 10 nmol/L (10.4%, 90 of 863) and/or transferrin saturation < 15% (3.7%, 34 of 912) were excluded. After these exclusions, 779 subjects remained—defined as non-B-vitamin- and non-iron-deficient subjects.

Table 2. Age-Adjusted Partial Correlations Between Hb and Selected Variables

	All (n = 918)		No Deficiency of Iron or B Vitamins (n = 773)		T > 8 nmol/L and Hb > 130 g/L (n = 807)	
	r	*P* Value	r	*P* Value	r	*P* Value
BMI	0.18	<.001	0.17	<.001	0.21	<.001
Total estradiol	0.21	<.001	0.22	<.001	0.19	<.001
Total T	0.10	<.01	0.08	.02	0.04	.25
SHBG	0.03	.32	−0.03	.48	−0.02	.58
P-EPO	−0.38	<.001	−0.31	<.001	−0.17	<.001
Transferrin saturation	0.27	<.001	0.18	<.001	0.19	<.001
B-EVF	0.91	<.001	0.90	<.001	0.88	<.001
S-Cystatin C	−0.15	<.001	−0.10	<.01	−0.03	.39
S-Cobalamin	−0.04	.21	−0.05	.22	−0.02	.50
P-HoloTC	−0.10	<.01	−0.11	<.01	−0.05	.16
S-Folate	−0.06	.06	−0.09	.02	−0.06	.11
IL-6	−0.10	<.01	−0.02	.56	−0.02	.53
TSH	−0.01	.88	0.03	.47	0.04	.22
Free T_4	0.11	<.001	0.09	.01	0.08	.02

Abbreviations: r, Pearson's correlation coefficient; S, serum; B, blood; P, plasma. Skewed variables (cobalamin, holoTC, S-folate, SHBG, transferrin saturation, IL-6, TSH) are log-transformed.

Correlation between Hb/sex steroids and age, white blood count (WBC)/platelet count

In the total study group, age correlated negatively with Hb (r = −0.11; $P < .001$) and estradiol (r = −0.14; $P < .001$), but not with T (r = −0.05; $P = .09$). In subjects with T > 8 nmol/L (n = 853), estradiol correlated positively with T (r = 0.48; $P < .001$). Age was positively associated with SHBG (r = 0.19; $P < .001$). There were no significant age-adjusted partial correlations between Hb and WBC or platelet count (r = 0.06, $P = .07$; and r = 0.02, $P = .64$).

Correlations between Hb and sex steroids

In univariate analyses of the total study group (n = 918), Hb correlated positively with estradiol (r = 0.23; $P < .001$) and T (r = 0.11, $P < .01$), but not with SHBG (r = 0.01; $P = .70$). The corresponding figures when subjects with prostate cancer were included (n = 1010) were: r = 0.26, $P < .001$; r = 0.16, $P < .001$; and r = −0.01, $P = .75$. Partial (age-adjusted) correlations between Hb and selected variables are shown in Table 2. Analysis of non-B-vitamin and non-iron-deficient subjects showed in principle the same results. However, when excluding all subjects with Hb < 130 g/L and/or T < 8 nmol/L (n = 99), the association between Hb and T was no longer significant, whereas the association between Hb and estradiol remained (Table 2). To explore a possible threshold effect, subjects with Hb < 130 g/L and/or estradiol < 48.4 pmol/L (10th percentile) were excluded; however, the age-adjusted correlation between Hb and T was not significant (r = −0.01; $P = .82$). Partial correlations between Hb and estradiol—cumulatively adjusted for age, EPO, BMI, transferrin saturation, cystatin C, smoking, IL-6, alcohol consumption, physical activity, albumin, and T in the total study group as well as when subjects with Hb < 130 g/L and T < 8 nmol/L were excluded—are shown in Table 3. These results did not differ substantially when subjects with either B-vitamin or iron deficiency or ongoing medication with B-vitamin supplements (n = 111) or pharmacological doses of vitamin B12, folic acid, and/or vitamin B6 (n = 103) or combinations thereof (n = 12; a total of 202 [22%]) were excluded (data not shown). The above

Table 3. Partial Correlations Between Hb and Estradiol, Cumulative Adjusted for Age, EPO, BMI, Transferrin Saturation, Cystatin C, Smoking, IL-6, Alcohol Consumption, Physical Activity, Albumin and Testosterone

	All (n = 849)		T > 8 nmol/L and Hb > 130 g/L (n = 756)	
Model	r	*P*	r	*P*
1	0.22	<.001	0.20	<.001
2	0.18	<.001	0.17	<.001
Model 1	Unadjusted			
Model 2	Cumulative adjusted for age, EPO, BMI, transferrin saturation, cystatin C, current smoking, IL-6, alcohol consumption, physical activity last week, albumin, and T			

Abbreviation: r, Pearson's correlation coefficient.

age-adjusted partial correlations between Hb and sex hormones were not altered substantially when subjects with prostate cancer were included (data not shown).

EVF and associations with sex steroids

In univariate analyses of the total study group, EVF correlated positively with estradiol (r = 0.24; $P < .001$) as well as T (r = 0.13; $P < .001$), but not with SHBG (r = 0.03; $P = .50$). We then performed the same analysis with EVF as with Hb and did not find any substantial differences in these results (Supplemental Tables 1–3). In multiple regression analysis, with EVF as dependent variable, estradiol was a significant predictor (standardized β = 0.18; $P < .001$).

Correlations between sex steroids and markers of iron status, WBC/platelet count, and inflammation

In univariate analysis, estradiol correlated positively both with serum iron (r = 0.11; $P < .01$) and transferrin saturation (r = 0.10; $P < .01$), but not significantly with ferritin (r = 0.05; $P = .16$). After further adjustment for age and EPO, the associations between estradiol and serum iron (r = 0.10; $P < .01$) as well as transferrin saturation (r = 0.11; $P < .01$) were still statistically significant. There were no age-adjusted correlations between estradiol and IL-6 (r = −0.05; $P = .13$), albumin (r = 0.03; $P = .30$), WBC (r = −0.01; $P = .80$), platelet count (r = −0.02; $P = .46$), or CRP (r = −0.05; $P = .13$). Testosterone correlated positively with serum iron (r = 0.16; $P < .001$) and transferrin saturation (r = 0.18; $P < .001$), but not significantly with ferritin (r = −0.01; $P = .74$). No correlations between T and WBC or platelet count were seen (data not shown). In age-adjusted partial correlations, T was significantly associated with IL-6 (r = −0.19; $P < .001$), CRP (r = −0.19; $P < .001$), but not with albumin (r = 0.04; $P = .27$).

Distribution of estradiol across quartiles of Hb

Men in the lowest quartile of Hb (84–140 g/L) had lower mean estradiol compared to quartiles 2–4 (70.3 pmol/L and 81.6 pmol/L; $P < .001$). Per SD decrease in estradiol adjusted for age, BMI, and EPO, the odds ratio (OR) for being in the lowest quartile of Hb was 1.61 (95% confidence interval [CI], 1.34–1.93) compared to quartiles 2–4. No substantial differences were seen when subjects with Hb < 130 g/L and T < 8 nmol/L as well as subjects defined as B-vitamin- or iron-deficient were excluded (data not shown). There was a positive linear trend between Hb and quartiles of estradiol ($P < .001$); the same pattern was seen when subjects with Hb < 130 g/L and/or T < 8 nmol/L ($P < .001$) as well as subjects with possible B-vitamin or iron deficiency were excluded ($P < .001$).

Anemia and estradiol

In subjects with anemia (Hb < 130 g/L according to the WHO definition), mean estradiol was significantly lower compared to subjects with Hb > 130 g/L (67.4 vs 79.4 pmol/L; $P < .001$). The results did not change substantially when subjects with ongoing medication with B-vitamin supplements (n = 111) or pharmacological doses of vitamin B12, folic acid, and/or vitamin B6 (n = 103) or combinations thereof (n = 12) at the start of the study (n = 202) were excluded (data not shown).

After adjustment for age, current smoking, physical activity, alcohol consumption, BMI, EPO, transferrin saturation, cystatin C, IL-6, and albumin, the difference in estradiol between subjects with WHO anemia and the remaining subjects was 9.54 pmol/L (95% CI, 0.99–18.1). In age-adjusted regression analysis with anemia as the categorical variable (5.6% of the cohort), the OR of having anemia increased with 71% per SD decrease in estradiol (OR = 1.71; 95% CI, 1.20–2.43).

Multivariate analysis

Multiple regression analysis with Hb as the dependent variable was performed. Significant explanatory variables were estradiol, EPO, BMI, transferrin saturation, cystatin C, holoTC, and smoking (Table 4). Variables originally in the model but excluded by stepwise forward procedure were physical activity, age, folate, T, IL-6, alcohol consumption, and free T_4. The results were not significantly

Table 4. Significant Predictors of Hb Levels in Multiple Stepwise Linear Regression Analysis in Subjects Without Prostate Cancer (n = 798)

Dependent Variable Hb	Explanatory Variable Entered at Each Step	Cumulative R^2	*P*
Step 1	EPO	0.13	<.001
Step 2	Estradiol	0.20	<.001
Step 3	Transferrin saturation	0.25	<.001
Step 4	BMI	0.30	<.001
Step 5	Cystatin C	0.31	<.001
Step 6	HoloTC	0.32	.04
Step 7	Current smoking	0.32	.04

Variables not included after the stepwise selection were physical activity, age, folate, T, IL-6, alcohol consumption, and free T_4.

affected when subjects with prostate cancer were included in the analyses; estradiol was still a significant explanatory variable. When subjects with T < 8 nmol/L and/or Hb < 130 g/L were excluded, significant explanatory variables for Hb were transferrin saturation, BMI, estradiol, EPO, and smoking (data not shown).

Spline models of the association between Hb and estradiol/T

Further evaluation of the association between Hb as an dependent variable and estradiol as an independent variable were performed using regression with spline models adjusted for age, BMI, and EPO (Figure 1A). Breakpoints in the spline curves are at the 10th, 50th, and 90th percentiles, corresponding to estradiol of 48, 77, and 115 pmol/L. A corresponding figure for Hb and T is shown in Figure 1B. Breakpoints in the spline curves are at the 10th, 50th, and 90th percentiles, corresponding to T of 9, 15, and 24 pmol/L.

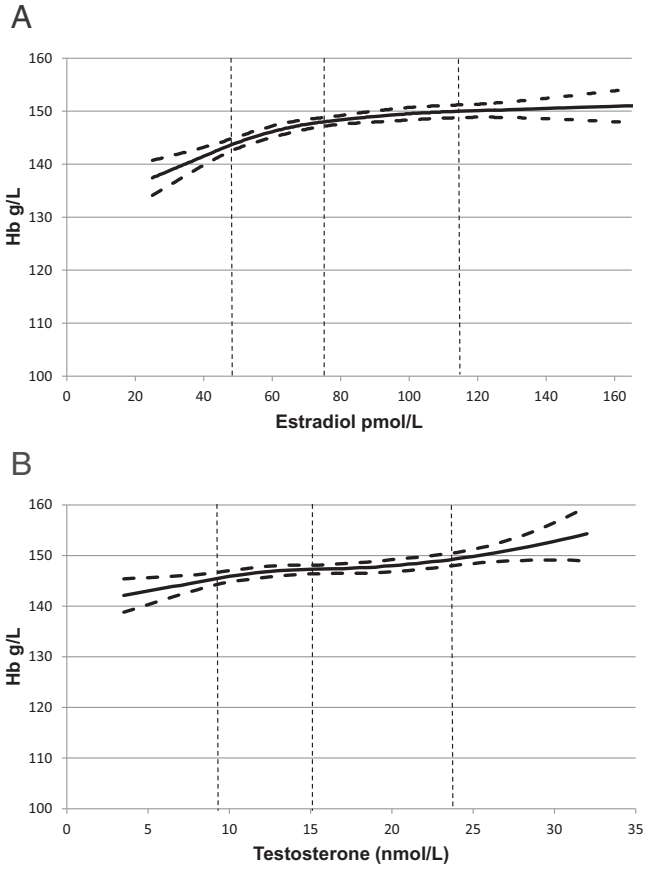

Figure 1. A, The association between Hb and estradiol, described with regression using spline functions and knots at the 10th, 50th, and 90th percentiles of estradiol. The association is adjusted for age, EPO, and BMI, which are set to average value of the cohort. The vertical dashed lines in the figure represent the 10th, 50th, and 90th percentiles. B, The association between Hb and T, described with regression using spline functions and knots at 10th, 50th, and 90th percentiles of T for men without prostate cancer at baseline. The association is adjusted for age, EPO, and BMI, which are set to average value of the cohort. The vertical dashed lines in the figure represent the 10th, 50th, and 90th percentiles.

Discussion

The regulation of erythropoiesis in elderly men is incompletely known. Hb declines significantly from age 70 to 88 in seemingly healthy men, as shown in both cross-sectional and longitudinal studies (6, 32). Women show much smaller age-related changes in Hb. However, conditions causing a drop in Hb to a level above commonly utilized decision limit levels for anemia (ie, individual anemia) are rather common. These include nutritional deficiency states (iron, folate, and vitamin B12), endocrine deficiency (eg, EPO, thyroid hormone, sex hormones), and inflammation. In addition, primary myeloid disorders (eg, myelodysplastic syndromes, myeloproliferative neoplasms) become more prevalent with advancing age. We investigated a large group of ambulatory elderly men, invited to studies regarding bone metabolism. Their mean age was just above 75 years, the prevalence of low Hb ("anemia," as defined by the WHO) was 5.6%, and laboratory investigations showed very low numbers of subjects with nutritional deficiency, impaired renal function, thyroid deficiency, or inflammation. Nine percent suffered from prevalent cancer of the prostate, 40% of whom had low T levels (<8 nmol/L), and to avoid prostate cancer as a potential confounder, all men with prostate cancer were excluded at the outset. We took advantage of this population of elderly men to study a hitherto not fully appreciated potential regulator of Hb in elderly men, estradiol. After exclusion of subjects with deficiency states diagnosed using updated methods, eg, holoTC for cobalamin status (30), a larger "healthy" fraction remained, compared with our earlier studies on population samples (7), facilitating analyses of estradiol as a possible regulator of erythropoiesis in seemingly healthy elderly men. First, univariate analyses of the total study group with a large number of factors documented to influence Hb were performed, and the results formed the basis for further multivariate analyses. Hb correlated negatively with age, in line with other studies (6, 7, 32). Furthermore, there were no correlations between T and Hb after exclusion of subjects with low T and Hb, indicating limited influence of T on the erythropoiesis of healthy elderly men. We found a negative correlation between age and estradiol, in line with some other studies (12, 13, 33).

The main finding in this study was the positive correlation between Hb and estradiol. There was a univariate linear correlation in the total study group, which remained after adjustment for age and exclusion of subjects with

potential deficiency of T, iron, cobalamin, and folate. In multivariate analysis, estradiol, but not T, was an independent predictor of Hb (Table 4). The results were not substantially altered if individuals with prostate cancer were included in the analyses or not. Additional analyses showed that this association remained significant after cumulative adjustment for age, EPO, BMI, transferrin saturation, cystatin C, smoking, IL-6, physical activity, alcohol consumption, serum albumin, and T. Taken together, our data indicate that estradiol might be an independent regulator of Hb in elderly men.

In a cross-sectional study of 1273 men, aged 20+, high total and free estradiol was associated with high hematocrit (24). Adjustments were made for T but not for EPO and iron status, ie, factors that influence Hb, hematocrit, and estradiol. In this study, immunoassays were used to analyze sex steroids; in our present study, we used mass spectrometry which is seen as the "gold standard." Recently, a positive association between estradiol and Hb was demonstrated in community-dwelling men using mass spectrometry; however, no data on EPO or iron status were reported (34). The underlying mechanism by which estradiol might influence erythropoiesis remains, however, unclear. It has become more evident in the last few years that estradiol in men is responsible for a number of effects that earlier were thought to be attributable to T. In the Swedish MrOS study, it was previously shown that low estradiol, but not T, was an independent predictor of fracture risk (25) and low lean mass (35), suggesting that estradiol has an anabolic effect in men. Recently, it was shown that estrogens have a role in the regulation of body fat in men through aromatization of T to estradiol, whereas lean mass, muscle size, and strength were regulated by androgens (36). Whether the well-known side effect of T treatment, elevated Hb/EVF, is due to aromatization of T to estradiol is, however, not clear. In a study of hypogonadal men treated with either transdermal or im injection of T, an association between EVF and estradiol levels was seen in both groups (16). However, the increase in EVF was more pronounced in subjects receiving im injection of T, probably related to higher estradiol levels deriving from im injection of T compared with transdermal administration. In a randomized placebo-controlled trial in men treated with dihydrotestosterone, a nonaromatizable pure androgen, Hb was increased during dihydrotestosterone treatment and returned to baseline after cessation of treatment (37) with no change of Hb in the placebo group, suggesting that the effect of androgens on erythropoiesis is mediated via androgen and not estrogen receptors. Recently, data on the interrelations between estrogen and iron homeostasis were presented, indicating a negative effect on hepcidin by estrogen, resulting in an increased availability of iron for the erythropoiesis (38). A putative mechanism for this effect was described in mice in which estradiol was shown to increase iron absorption and iron release from storage cells by a functional estrogen response element, located in the promoter region of the hepcidin gene. Thus, estradiol reduced hepcidin levels in hepatocytes by inhibiting hepcidin gene expression (39). Furthermore, estradiol has been shown to increase iron uptake in human liver cells by suppressing hepcidin transcription (40). This increased availability of iron by estradiol might thus facilitate maintenance of erythropoiesis, and indeed we found a significant positive correlation between total estradiol and serum iron (including transferrin saturation). To what extent estradiol might compensate for the increased hepcidin synthesis, seen in anemia secondary to inflammation, would require detailed investigations in elderly anemic men. The strengths of our study are the standardized collection of blood samples; data on Hb, EVF, and EPO; detailed information on iron, cobalamin, and folate status; and the use of mass spectrometry to measure estradiol and T. The present study has the limitations of a cross-sectional study, in respect to a possibly causality between estradiol and hematopoiesis. In future studies, free hormone levels of estradiol and T would preferably be measured and related to Hb. The mechanisms for estradiol synthesis in, eg, healthy vs anemic elderly men and its influence on erythropoiesis and hepcidin levels could indeed be the subject of further studies. However, our findings indicate an independent role of estradiol for maintaining Hb in healthy elderly men independently of previously recognized modulators of erythropoiesis, thus adding to the understanding of why seemingly healthy elderly men show a decline in blood Hb.

Acknowledgments

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