The Association of Reproductive Hormone Levels and All-Cause, Cancer, and Cardiovascular Disease Mortality in Men

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Context: Testosterone (T) levels have been associated with mortality, but controversy exists.

Objective: Our objective was to investigate associations between serum levels of total T, SHBG, free T, estradiol, LH and FSH, and subsequent mortality with up to 30 years of follow-up.

Design: This was a prospective cohort study consisting of men participating in four independent population-based surveys (MONICA I–III and Inter99) from 1982 to 2001 and followed until December 2012 with complete registry follow-up.

Setting and Participants: A total of 5350 randomly selected men from the general population aged 30, 40, 50, 60, or 70 years at baseline participated.

Main Outcomes and Measures: All-cause mortality, cardiovascular disease (CVD) mortality, and cancer mortality were the main outcomes.

Results: A total of 1533 men died during the follow-up period; 428 from CVD and 480 from cancer. Cox proportional hazard models revealed that men in highest LH quartile had an increased all-cause mortality compared to lowest quartile (hazard ratio [HR], 1.32; 95% confidence interval [CI], 1.14–1.53). Likewise, increased quartiles of LH/T and estradiol increased the risk of all-cause mortality (HR, 1.23; 95% CI, 1.06–1.43; HR, 1.23; 95% CI 1.06–1.43). No association to T levels was found. Higher LH levels were associated with increased cancer mortality (HR, 1.42; 95% CI, 1.10–1.84) independently of smoking status. Lower CVD mortality was seen for men with T in the highest quartile compared to lowest (HR, 0.72; 95% CI, 0.53–0.98). Furthermore, negative trends were seen for SHBG and free T in relation to CVD mortality, however insignificant.

Conclusion: The observed positive association of LH and LH/T, but not T, with all-cause mortality suggests that a compensated impaired Leydig cell function may be a risk factor for death by all causes in men. Our findings underpin the clinical importance of including LH measurement in the diagnostic work-up of male patients seeking help for possible androgen insufficiency. (*J Clin Endocrinol Metab* **100:** 4472–4480, 2015)

Abbreviations: BMI, body mass index; CI, confidence interval; CVD, cardiovascular disease; HR, hazard ratio; ICD, International Classification of Diseases.

Testosterone (T) is essential for male reproductive function but also exerts effects in various nonreproductive organs. Thus, actions of T include anabolic effects of muscle mass, mineralization of bones, and stimulation of hematopoiesis. It also influences cognitive function (1). Numerous studies suggest that low serum T in men is associated with risk factors for cardiovascular disease (CVD) and type 2 diabetes (2). Furthermore, several studies have investigated the association between T levels and subsequent mortality, although with conflicting results (3).

LH is the primary stimulator of T production, and the ratio between LH and T (LH/T) is a clinical marker of Leydig cell function. The male ability to produce T is associated with a large buffer capacity. Thus, if Leydig cell function is somewhat compromised, a normal or near-normal T level can often be sustained by increased LH levels, as seen in the clinical entity "compensated primary hypogonadism" (4). Whether such a compensated state is associated with morbidity or increased mortality remains unknown. Few inconclusive studies have focused on the association between gonadotropins and mortality (5–7).

In this study, we have investigated the associations of serum LH and T levels and related reproductive hormones, with subsequent mortality in a comprehensive data material of 5350 men aged 30–70 years. All men belonged to the background population and mean length of follow-up was 18 years.

Materials and Methods

Study population

The study comprised serum samples from 5350 randomly selected men participating in four independently population-based surveys at Research Centre for Prevention and Health, Glostrup University Hospital. The surveys included the three Danish Monitoring Cardiovascular Risk Factors Surveys (MONICA I, II, and III) conducted in 1982, 1987, and 1992, with the aim of monitoring trends and determinants of coronary heart disease as part of an international World Health Organization project. The Inter99 survey conducted between 1999 and 2001 was an intervention study aiming to investigate the effect of changes in lifestyle on the incidence of ischemic heart disease. However, in the present study, only data from the baseline examination are included.

All participants were drawn from the background population in 11 municipalities in the southwestern part of the Copenhagen area. Random samples of 30-, 40-, 50-, 60-, and 70-year-old men were drawn from the Danish Civil Registration System in which all Danish inhabitants are registered (8, 9). The participation rates in the four surveys were 81%, 75%, 69%, and 51%, respectively. Prior informed written consent was obtained from all participants.

At baseline, the study participants completed a detailed self-administered questionnaire regarding sociodemographic characteristics, lifestyle, and health status, and went through a clinical examination where anthropometrics and blood pressure were measured according to standardized protocols (8–10). Study participants had a blood sample drawn in the morning after an overnight fast. All of the variables included in the current study were collected in the same way across the four surveys with the exception of waist circumference, which was added from MONICA II and onwards. Serum samples were analyzed for lipids and stored in aliquots at −18°C until further analyses.

Hormone measurements

T and estradiol were measured by time-resolved fluoroimmunoassay (DELFIA; Wallac Oy) and SHBG, LH, and FSH were measured by time-resolved immunofluorometric assay (DELFIA) as described previously (11, 12). All hormone measurements were performed in the same laboratory in 2004. Serum volume was limited and by analyzing all hormones on the same platform (DELFIA), it was possible to obtain data on all five hormones. The T DELFIA assay has subsequently been compared to a turboflow-liquid chromatography tandem mass spectrometry method (established in the laboratory in 2012) and a strong correlation between the two methods was found in the relevant range (Pearson's r, 0.805; $P < .01$; mean bias, 1.2%). Because of varying storage periods (3–22 years) and various numbers of thawings, the hormone levels were initially corrected for evaporation according to median serum Na^+ levels measured in samples from each of the four surveys as previously validated (11). Free T was calculated based on the T and SHBG concentrations according to Vermeulen et al (13).

Follow-up and outcome variables

In Denmark, all residents have a unique identification number allowing linkage of data from registries on an individual level and thereby obtaining almost complete follow-up of all individuals with regard to mortality and diseases. Using the unique identification number, participants were linked to The Central Office of Civil Registration for information on vital status. Four men were excluded because of a loss of follow-up. Follow-up time for the remaining participants was calculated as the time from their baseline examination until the time of death or end of follow-up (December 17, 2012), whichever occurred first. Participants who emigrated during the follow-up period (n = 62) contributed with person time at risk until date of emigration, after which they were censored. Information about the specific causes of death was obtained from The Danish Register of Causes of Death. Because of a delay in the registration of the causes of death, there was a minor proportion of deaths (n = 183) not registered with a specific cause and therefore censored in the analyses of CVD and cancer mortality.

Initially, men who were diagnosed with an impaired testicular function before entry of study, including prostate cancer, testis cancer, and impaired pituitary gland function (International Classification of Diseases [ICD]-8: 185–186, 253.19, 257; ICD-10: C61–C62, E23.0, E29) were excluded (n = 23), leaving 5323 men for the analyses. Furthermore, 191 men who had been diagnosed before participation with either ischemic heart disease, stroke, or atherosclerosis (ICD-8: 410–414, 430–438, 440; ICD-10: G45, I20–I25, I60–I70) were excluded in the analysis of CVD mortality. The information on hospitalization diagnoses were obtained from the National Patient Register in which in-

formation on all admissions to Danish hospitals since 1977 is registered (14).

Statistical analysis

Differences in levels of total T, SHBG, free T, estradiol, LH, and FSH stratified by baseline characteristics were investigated using general linear regression. Potential confounders were identified based on published literature and confirmed in our data or were statistically associated with hormone levels and mortality predictors. To rule out the age-related differences in hormone levels, we calculated age-standardized z-scores for each hormone. To obtain normality, LH and FSH were initially transformed using the natural logarithm. Thus, each of the age-standardized hormone variables were calculated as the difference between a man's individual hormone level (eg, T) and the mean of the hormone level of his age group divided with the SD of the hormone level within the age group.

Cox proportional hazard models were used to analyze the association of age-standardized hormone z-scores in relation to all-cause, CVD, and cancer mortality adjusted for potential confounders. Because of the wide age range at entry, survival depends on age rather than time at risk. Thus, age was used as the underlying time scale in all models. To accommodate potential nonlinear associations, hormone z-scores were analyzed in quartiles and a trend test was performed. In fully adjusted models, individuals with a missing value of at least one of the included covariates were excluded and quartiles recalculated. In the fully adjusted model of all-cause mortality, 164 (3%) individuals were thus excluded because of one or more missing values. Absolute hormone levels stratified according to quartiles of z-scores can be seen in the Supplemental material (published on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org). Models were stratified according to smoking status (yes/no) allowing different baseline hazards for smokers and nonsmokers to fulfill the proportional hazards assumption. For consistency, all final models were adjusted for the same covariates; body mass index [BMI] (<20.0, 20.0–24.99, 25.0–29.99, >30), study (MONICA I, MONICA II, MONICA III, and Inter99), weekly alcohol units (0, 1–14, >14), and exercise (sedentary, modest exercise, moderate exercise, and competitive sports). P values < .05 were considered statistically significant. We used interaction tests to investigate whether the effect of a given hormone level differed according to differences in each covariate. This was done by testing potential differences between a model including main effects and the interaction term and a model restricted to including only main effects using an ANOVA test. Despite insignificant tests for interaction between hormones and smoking in relation to all survival outcomes, the associations were elucidated in separate analyses of smoking and nonsmoking men. In this case, the age-standardized hormone z-scores were divided into quartiles within the group of smokers and nonsmokers to obtain equal distributions within the two groups. Models were adjusted for the same covariates as in the full model, with the exception of smoking. To further elucidate the potential differences in survival across quartiles of hormones, differences in life expectancy up to 90 years of age were estimated according to hormone level and stratified according to smoking status based on Kaplan-Meier estimates of the related survival functions. All models were investigated for the assumption of proportional hazards using cumulative marginal residuals.

Sensitivity analyses were conducted to elucidate the robustness of our findings. Deaths occurring within the first 2 years from baseline were excluded to minimize reverse causation between hormones and potential subclinical illness. In the three most recent studies, information on waist circumference was available. These were used to further explore the potentially reversible relationship between T and CVD (Supplemental Material). The statistical analyses were performed using R (version 3.0.3) and SPSS version 20.0 (SPSS Inc.).

Results

A total of 5323 men with serum samples were included in the study with a mean follow-up of 18.5 years (25th percentile, 12.0; 75th percentile, 25.3). A total of 1533 men (28.9%) died during the follow-up period; 428 deaths were caused by CVD and 480 by cancer. The men contributed with 98 286 person-years at risk corresponding to an all-cause mortality rate of 15.6 per 1000 person-years and mortality rates of 4.4 and 4.9 per 1000 person-years at risk for death caused by CVD and cancer, respectively.

Table 1 shows mean hormone levels stratified by baseline characteristics. As expected, total and free T decreased with age, whereas SHBG, LH, and FSH increased. Men participating in MONICA I had higher hormone levels compared to men participating in the subsequent surveys with the exception of FSH, which was highest for men from MONICA III. Smokers had significantly higher hormone levels compared to nonsmokers. For BMI, a significantly inverse trend was seen with T, SHBG, free T, and LH, whereas FSH was significantly higher for men with a BMI greater than 30 kg/m^2 compared to normal-weight men. Men with a known diagnosis of CVD at baseline had significantly lower total T and free T levels and significantly higher SHBG, LH, and FSH levels.

All-cause mortality

Cox proportional hazard models revealed no significant differences in all-cause mortality across age-standardized quartiles of T or FSH (Figure 1; for unadjusted and adjusted model estimates see Supplemental Material, Table 1). Higher all-cause mortality was seen for men with an age-standardized SHBG level in the second quartile or higher. When adjusted for confounders, no significant differences in survival were seen between first and higher quartiles of age-standardized free T. However, test for trend indicated a linear association toward lower mortality with increasing quartile of free T (P trend = .024). An increased all-cause mortality was seen for men in the highest quartiles of estradiol (hazard ratio [HR], 1.23; 95% confidence interval [CI], 1.06–1.43) and LH (HR, 1.32; 95% CI, 1.14–1.53) compared to men in the lowest quar-

TABLE 1. Mean Hormone Levels Stratified by Baseline Characteristics (n = 5323)

	n[a]	Total T (nmol/L)	SHBG (nmol/L)	Free T (nmol/L)	Estradiol (nmol/L)	LH (IU/L)	LH/T (IU/nmol)	FSH (IU/L)
Age (y)								
30[b]	1001	21.6	26.7	534.1	92.5	3.3	0.18	3.3
40	1474	19.7[c]	28.1[c]	464.8[c]	89.5[c]	3.2	0.19	3.7
50	1542	18.7[c]	32.0[c]	404.1[c]	89.2[c]	3.6[c]	0.21	4.7[c]
60	1108	19.1[c]	39.9[c]	368.3[c]	96.9[c]	4.5[c]	0.39[c]	6.2[c]
70	198	17.9[c]	41.5[c]	329.4[c]	87.3[c]	5.0[c]	0.84[c]	7.7[c]
Study								
MONICA I [b]	1920	20.6	34.5	445.5	96.5	4.0	0.25	4.5
MONICA II	737	19.5[c]	31.0[c]	437.9	92.2[c]	3.5[c]	0.26	4.3
MONICA III	1002	18.9[c]	31.4[c]	420.8[c]	85.8[c]	3.7[c]	0.33	4.9[c]
Inter99	1664	18.9[c]	29.6[c]	430.7[c]	88.6[c]	3.4[c]	0.23	4.5
Education (y)								
0–7	1358	19.6	37.0[c]	397.9[c]	94.2[c]	4.3[c]	0.32	5.5[c]
8–10	2848	19.6	30.6[c]	443.7[c]	90.9	3.5[c]	0.23	4.3
>10[b]	1013	19.7	28.9	460.4	89.2	3.3	0.23	4.1
Currently smoker								
Nonsmoker[b]	2536	18.5	29.3	425.1	89.6	3.4	0.24	4.4
Smoker	2779	20.6[c]	34.3[c]	444.3[c]	93.2[c]	3.9[c]	0.27	4.7[c]
BMI								
<20	150	25.2[c]	45.7[c]	487.0	90.8	4.4[c]	0.20	5.9[c]
20.0–24.9[b]	2209	21.7	35.2	467.6	90.6	3.7	0.24	4.5
25–30	2277	18.5[c]	29.8[c]	422.1[c]	91.4	3.6[c]	0.26	4.5
> 30	683	15.1[c]	25.4[c]	362.4[c]	94.6[c]	3.7	0.35	4.9[c]
Physical exercise								
Sedentary[b]	1207	19.1	30.8	432.4	92.2	3.8	0.27	4.4
Modest exercise	2712	19.5	32.2[c]	429.7	91.3	3.7	0.27	4.7
Moderate exercise	1226	20.0[c]	32.4[c]	442.0	90.6	3.6	0.24	4.5
Competitive sports	108	22.1[c]	30.8	512.0[c]	95.0	3.2*	0.16	3.4[c]
Alcohol consumption (units/week)								
0	404	19.8	34.2[c]	423.1	92.5	4.0 [c]	0.25	5.0
1–14[b]	3220	19.8	31.9	438.7	90.1	3.6	0.27	4.5
15+	1634	19.3[c]	31.5	431.4	93.6[c]	3.7	0.24	4.6
Known CVD at baseline								
No	5132	19.7	31.8	437.8	91.5	3.7	0.26	4.5
Yes	191	18.0[c]	35.3[c]	364.5[c]	90.1	4.0[c]	0.26	5.4[c]

Cardiovascular disease according to International Classification of Diseases-10: G45, I20–I25, I60–I70.

Abbreviations: BMI, body mass index; CVD, cardiovascular disease.

[a] Total numbers may vary slightly because of missing values for some variables.

[b] Reference category.

[c] $P < .05$ general linear regression model for the difference in mean hormone levels compared to the reference category.

tile. Likewise, the LH/T ratio reflected a significantly higher all-cause mortality with increasing quartile (HR, 1.23; 95% CI, 1.06–1.43). Separate analyses of smokers and nonsmokers showed similar associations between reproductive hormone levels and survival.

Cancer-related mortality

Cancer mortality tended to increase with higher age-standardized quartiles of total T and SHBG (Figure 2; for unadjusted and adjusted model estimates see Supplemental Material, Table 2), with the differences being most pronounced for smokers (HR, 1.53; 95% CI, 1.14–2.08 and HR, 1.71; 95% CI, 1.10–2.11, respectively). No significant differences in cancer mortality was seen for free T, but men with estradiol and LH levels in the highest quartile showed higher cancer mortality compared to lower quartiles independently of smoking status. No significant differences in cancer mortality were seen across LH/T quartiles. However, a trend toward higher mortality with higher quartiles was seen among nonsmokers (P trend = .045). Only for nonsmoking men was an association with FSH seen with lowest cancer mortality for men with lower FSH quartile; however, this was only significant for the second quartile.

CVD-related mortality

A negative linear association between age-adjusted T and CVD mortality was found (Figure 1; for unadjusted and adjusted model estimates see the Supplemental Material and Table 1). Thus, men with total T levels in the
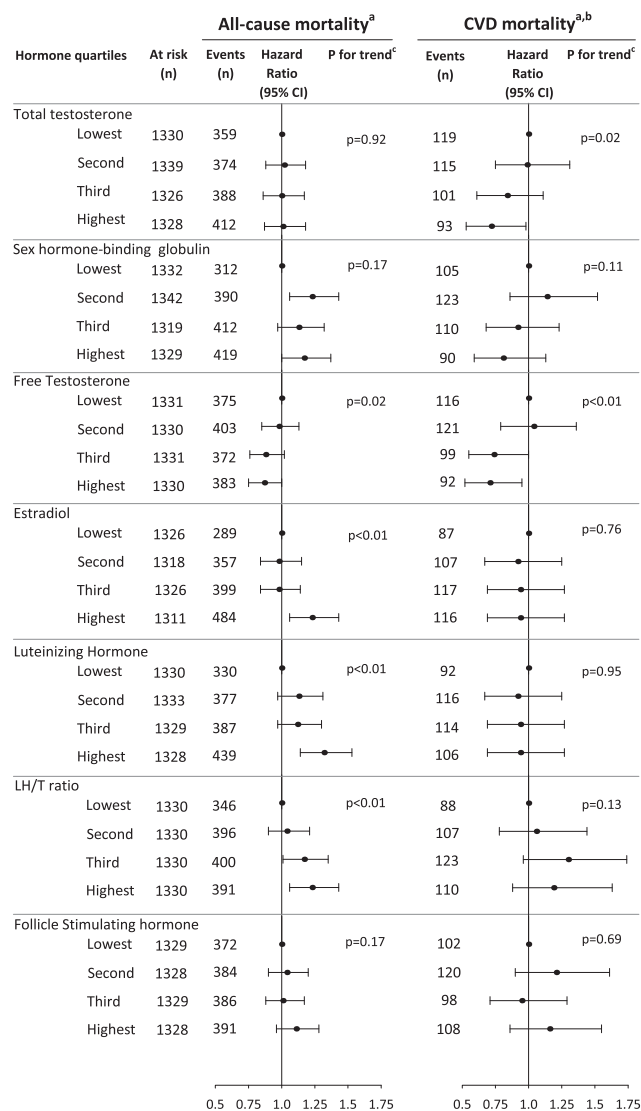
Hormone quartiles	At risk (n)	All-cause mortality[a]			CVD mortality[a,b]		
		Events (n)	Hazard Ratio (95% CI)	P for trend[c]	Events (n)	Hazard Ratio (95% CI)	P for trend[c]
Total testosterone							
Lowest	1330	359		p=0.92	119		p=0.02
Second	1339	374			115		
Third	1326	388			101		
Highest	1328	412			93		
Sex hormone-binding globulin							
Lowest	1332	312		p=0.17	105		p=0.11
Second	1342	390			123		
Third	1319	412			110		
Highest	1329	419			90		
Free Testosterone							
Lowest	1331	375		p=0.02	116		p<0.01
Second	1330	403			121		
Third	1331	372			99		
Highest	1330	383			92		
Estradiol							
Lowest	1326	289		p<0.01	87		p=0.76
Second	1318	357			107		
Third	1326	399			117		
Highest	1311	484			116		
Luteinizing Hormone							
Lowest	1330	330		p<0.01	92		p=0.95
Second	1333	377			116		
Third	1329	387			114		
Highest	1328	439			106		
LH/T ratio							
Lowest	1330	346		p<0.01	88		p=0.13
Second	1330	396			107		
Third	1330	400			123		
Highest	1330	391			110		
Follicle Stimulating hormone							
Lowest	1329	372		p=0.17	102		p=0.69
Second	1328	384			120		
Third	1329	386			98		
Highest	1328	391			108		

0.5 0.75 1.0 1.25 1.5 1.75   0.5 0.75 1.0 1.25 1.5 1.75

[a]Models assuming different baseline hazards for non-smokers and smokers with age as the underlying time scale and adjusted for BMI, study, alcohol consumption and exercise.
[b]Men with known cardiovascular disease at baseline (n=191) excluded.
[c]Age-standardized hormone quartiles inserted in the model as a continuous variable assuming a linear association.

Figure 1. Hazard ratios (95% confidence interval [CI]) for quartiles of age-standardized hormone z-scores associated with all-cause and cardiovascular disease (CVD) mortality (International Classification of Diseases-10: I00–I99).

highest quartile had a reduced risk of CVD mortality compared to men in the lowest quartile (HR, 0.72; 95% CI, 0.53–0.98). Likewise, a trend toward lower CVD mortality was seen for increasing age-standardized SHBG and free T but only significant for free T. No significant differences in CVD mortality were seen across quartiles of age-standardized estradiol, LH, LH/T, and FSH. Similar associations were seen in separate analyses of smokers and nonsmokers. Because of the potentially reversible relationship between T and CVD, we further investigated the impact of different CVD risk factors on the observed associations. Exclusion of BMI from the analyses of reproductive hormones and CVD mortality increased the dif-

ference in estimates slightly (Supplemental Material, Table 1). When including waist circumference instead of BMI in the fully adjusted model, the risk estimates decreased slightly and no longer differed significantly between T quartiles (Supplemental Material, Table 2). Adding number of symptoms of the metabolic syndrome instead of waist circumference as a covariate decreased the risk estimate differences between the T quartiles further and they were all insignificant (Supplemental Material, Table 2).
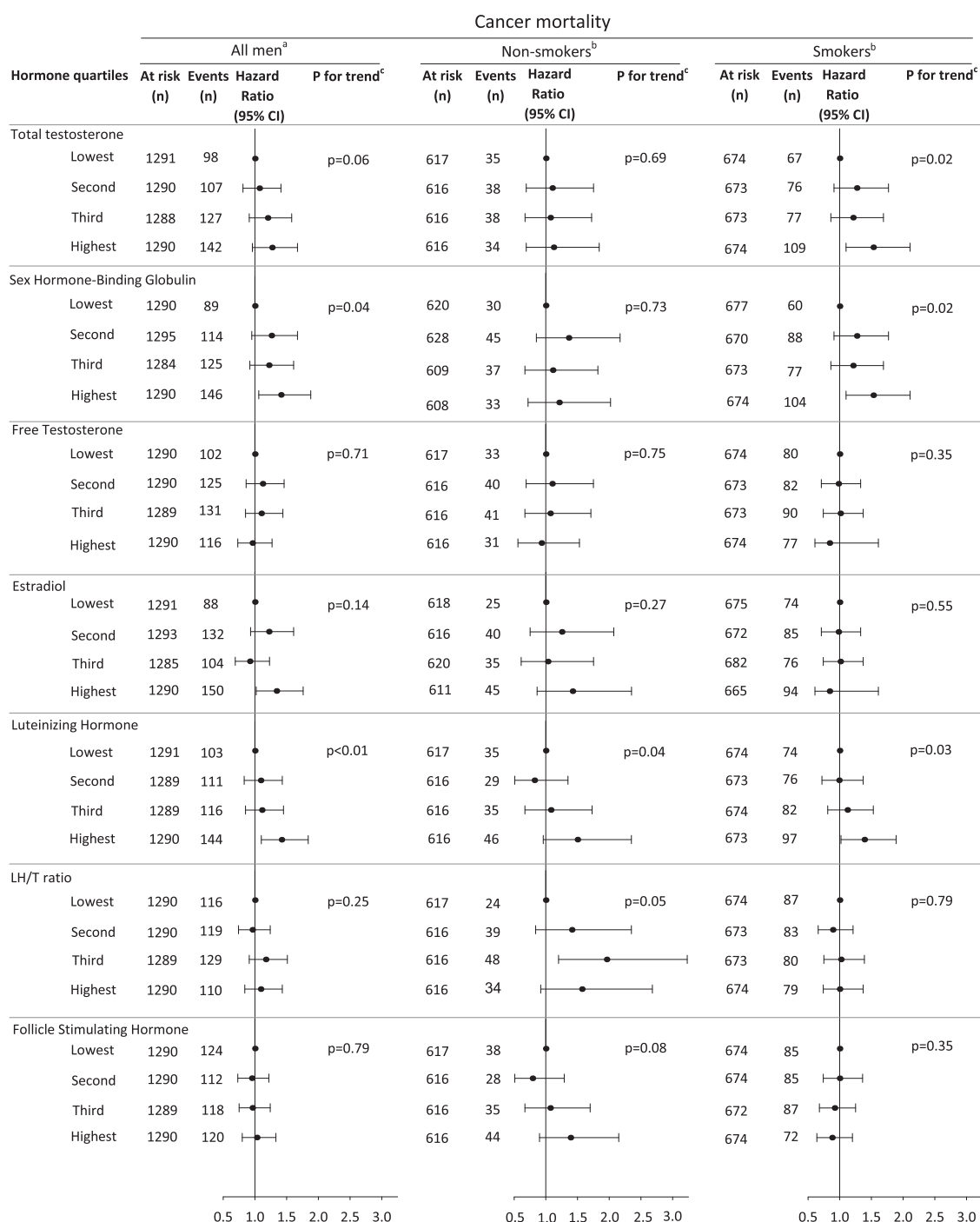
Life expectancy

Life expectancy differed significantly for smokers and nonsmokers (Figure 3). Furthermore, estimations indicated minor differences in survival across hormone quartiles, yet insignificant in most cases. For total T and free T, expected survival for nonsmoking men increased 2.4 years from lowest to highest quartile. Among smoking men, no difference in expected survival was seen for the age-adjusted T quartile, whereas for free T, survival seemed to increase almost 2 years for men with levels of free T in the two highest quartiles compared to the lowest. Life expectancy decreased with increasing age-adjusted LH quartiles. The difference was most pronounced for smokers going from 74.9 to 71.7 years, from lowest to highest LH quartile, compared to 80.9 to 79.2 years for nonsmokers. In accordance, the expected survival decreased with increasing quartiles of LH/T ratio ranging from 80.4 to 78.6 years for nonsmokers and 74.4 to 71.7 years for smokers.

Discussion

In our population-based study of middle-aged Danish men followed for up to 30 years, we observed that increased LH and LH/T were associated with increased all-cause and cancer mortality. Interestingly, the profiles of the hormone associations differed among the three survival outcomes as low T, but not increased LH, was a predictor of CVD mortality. This indicates that different underlying biological pathways are at play.

All-cause mortality. The observed positive association between LH and all-cause mortality presumably does not reflect a direct effect of LH levels on male mortality. A main role of LH in the male is a stimulatory effect on Leydig cell steroidogenesis. A limited number of studies have reported the presence of the LH receptor in extragonadal tissues (eg, skin, prostate); however, the physiological role of LH in these tissues remains unknown (16). We speculate that the higher LH levels associated with increased mortality are more likely a risk marker than a risk factor. Because of the negative feedback regulation be-

Cancer mortality

Hormone quartiles	All men[a]				Non-smokers[b]				Smokers[b]			
	At risk (n)	Events (n)	Hazard Ratio (95% CI)	P for trend[c]	At risk (n)	Events (n)	Hazard Ratio (95% CI)	P for trend[c]	At risk (n)	Events (n)	Hazard Ratio (95% CI)	P for trend[c]
Total testosterone												
Lowest	1291	98		p=0.06	617	35		p=0.69	674	67		p=0.02
Second	1290	107			616	38			673	76		
Third	1288	127			616	38			673	77		
Highest	1290	142			616	34			674	109		
Sex Hormone-Binding Globulin												
Lowest	1290	89		p=0.04	620	30		p=0.73	677	60		p=0.02
Second	1295	114			628	45			670	88		
Third	1284	125			609	37			673	77		
Highest	1290	146			608	33			674	104		
Free Testosterone												
Lowest	1290	102		p=0.71	617	33		p=0.75	674	80		p=0.35
Second	1290	125			616	40			673	82		
Third	1289	131			616	41			673	90		
Highest	1290	116			616	31			674	77		
Estradiol												
Lowest	1291	88		p=0.14	618	25		p=0.27	675	74		p=0.55
Second	1293	132			616	40			672	85		
Third	1285	104			620	35			682	76		
Highest	1290	150			611	45			665	94		
Luteinizing Hormone												
Lowest	1291	103		p<0.01	617	35		p=0.04	674	74		p=0.03
Second	1289	111			616	29			673	76		
Third	1289	116			616	35			674	82		
Highest	1290	144			616	46			673	97		
LH/T ratio												
Lowest	1290	116		p=0.25	617	24		p=0.05	674	87		p=0.79
Second	1290	119			616	39			673	83		
Third	1289	129			616	48			673	80		
Highest	1290	110			616	34			674	79		
Follicle Stimulating Hormone												
Lowest	1290	124		p=0.79	617	38		p=0.08	674	85		p=0.35
Second	1290	112			616	28			674	85		
Third	1289	118			616	35			672	87		
Highest	1290	120			616	44			674	72		

0.5 1.0 1.5 2.0 2.5 3.0   0.5 1.0 1.5 2.0 2.5 3.0   0.5 1.0 1.5 2.0 2.5 3.0

[a]Models assuming different baseline hazards for non-smokers and smokers with age as the underlying time scale, adjusted for BMI, study, alcohol consumption and exercise.

[b]Model adjusted for BMI, study, alcohol consumption and exercise with age as the underlying time scale.

[c]Age-standardized hormone quartiles inserted in the model as a continuous variable assuming a linear association.

Figure 2. Hazard ratios (95% confidence interval [CI]) for quartiles of age-standardized hormone z-scores and cancer mortality (International Classification of Diseases-10: C00–C96) for all men, and stratified according to smoking status.

tween T and LH, higher LH levels reflect lowered androgen action (eg, from impaired Leydig cell steroidogenesis, antiandrogenic effects, genetic variation in androgen sensitivity). Considering the widespread and important role of androgens in the male body, it is more likely that low-ered androgen action could be a risk factor for increase mortality in men. However, we did not observe any association between total T and all-cause mortality. This combination of an association with increased LH but not total T points toward a link between all-cause death and a
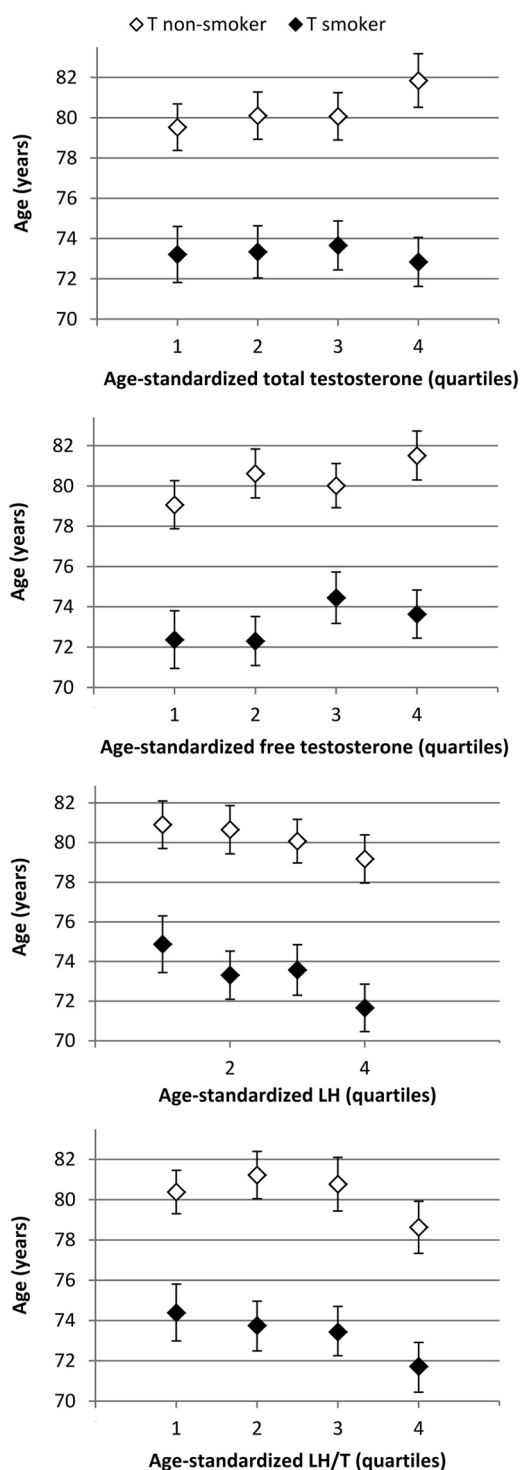
Figure 3. Estimated life expectancy up to 90 years of age according to age-standardized hormone quartile for smokers and nonsmokers.

"compensated primary hypogonadism" rather than a lowered androgen action by antiandrogenic effects or lowered androgen sensitivity because increased LH as well as increased T levels would be expected in the two latter conditions. Compensated primary hypogonadism is a clinical condition characterized by increased LH and normal or low-normal T (4). Interestingly, increased estradiol

levels are also associated with compensated primary hypogonadism and were, in our study, also associated with increased all-cause mortality. Men in a clinical state of compensated primary hypogonadism may be at greater risk of developing an overt primary hypogonadism with aging (4). Furthermore, compensated primary hypogonadism is associated with impaired sperm production (17). In accordance, an inverse association between sperm count and all-cause mortality was shown in a study including more than 43 000 men (18).

A recently published meta-analysis based on 11 community-based studies observed that low serum T was associated with increased all-cause mortality, but also identified considerable heterogeneity between the studies. Thus, the studies in which an association was observed were more likely to include older men, generally reported lower levels of T, had a shorter follow-up period, and were more likely to have blood samples drawn throughout the day (3). In accordance with our findings, a study of 4256 middle-aged men observed a significant increased risk of all-cause mortality for individuals with high LH (6).

Cancer mortality

In the analysis of all men, we observed a tendency toward higher cancer mortality with higher T, SHBG, and estradiol levels, which seemed to be explained by a more pronounced effect for smoking men. It is well-known that smoking men have higher T and SHBG levels compared to nonsmokers (19), and some studies have observed a dose-dependent association between number of cigarettes smoked and T levels (20), indicating that in our study the positive association between T levels and cancer mortality was driven by smoking. Only the positive association between LH and cancer mortality was consistent for both smokers and nonsmokers and was in line with the observed pattern for risk of all-cause mortality.

Our finding of no significant association between T level and cancer mortality is in accordance with some previously published studies (6, 21, 26, 27), but not all (22–25). In general, studies observing no association were characterized by the inclusion of younger men and reported higher smoking frequencies compared to studies observing a positive association.

CVD mortality

Interestingly, the analyses of male reproductive hormones in relation to CVD mortality revealed a very different pattern of associations compared to the trends seen for all-cause mortality. Overall, no associations were observed for LH, FSH, SHBG, and estradiol in relation to CVD mortality, whereas increased levels of total and free T were associated with decreased CVD mortality risk. Our

findings are in accordance with the recent meta-analysis by Araujo et al (3), reporting an association between low T levels and increased CVD mortality. Entities of the metabolic syndrome such as obesity and insulin resistance are known risk factors for CVD disease (28). At the same time, metabolic syndrome is associated with lower levels of total T, SHBG, and free T independently of age (29). The underlying cause seems to be a high blood glucose–mediated downregulation of SHBG, the major binding protein of T in serum (30). In men with metabolic syndrome, T levels are therefore considered to be lowered secondary to a decreased binding capacity for T in serum (2), although a direct effect on testicular T production may also be at play, especially in severe obesity.

Weight loss normalizes both SHBG and T levels in obese men (31), indicating a reversible relationship between T and known risk factors for CVD. Taking into consideration the known physiological relationship between the different male reproductive hormones, the observed association of increased CVD mortality with low T, but not LH, may indicate that metabolic disorders is the confounder for both decreased T and increased CVD mortality. The observed tendency toward lower CVD mortality with higher SHBG quartile, albeit insignificant after adjustments, points in the same direction. Furthermore, the association between low T and increased CVD mortality was observed when we used BMI as a confounder to adjust for obesity, but became insignificant when instead we used waist circumference, an alternative anthropometric measure of obesity (32). Likewise, no association between T levels and CVD mortality was observed when the model was adjusted for number of symptoms of metabolic syndrome.

Our findings of no association between LH and FSH levels and CVD mortality are in accordance with the study of Phillips et al (6). However, in their study, total T did not associate to CVD mortality. In a study of older men, high LH and SHBG levels were associated with increased risk of CVD (5). In the same study, low free T, but not total T, was associated with CVD mortality.

Strengths and limitations

Our findings are based on a comprehensive data material including serum samples from 5323 randomly selected men in a wide age span participating in four cross-sectional surveys, with long follow-up on mortality from virtually complete national registries and analyzed for several reproductive hormones. All samples were analyzed at the same time in the same laboratory; therefore, the storage time and storage conditions vary from study to study. However, all Cox regression analyses were adjusted for study period to rule out potential differences (including storage period) between the four surveys and we therefore find it highly unlikely that the storage methodology systematically can have affected the observed findings in any direction.

Male reproductive hormones change with aging; eg, in adult men, serum T levels on average decline 1%–2% per year (15). Because of the large age range of our participants we used age-standardized hormone levels to ensure that any observed associations of hormone levels with mortality were independently of the age of each man at baseline. To our knowledge, this is the first study making this age correction; our results may therefore not be directly comparable to other studies.

Inherent in the observational study design is the limited ability to determine the direct causal role of changes in hormone levels and disease status. Participation rates differed across the studies and decreased over time, indicating that especially the Inter99 study is more likely to compose a selected group of men that are more likely to be nonrandomly assigned factors that could have an influence on hormone levels and the outcomes of interest. However, analyses in which men participating in Inter99 were excluded did not change our findings. Thus, we believe that our findings are generalizable to similar Caucasian populations in the same age range despite declining participation rates over time.

Conclusion

Our findings of a positive association between LH and LH/T and all-cause mortality in combination with no association for total T suggest that primary Leydig cell dysfunction, even when compensated by increased LH, is a male risk factor for death by all causes. In contrast, secondary decreased Leydig cell function, possibly following a decrease in SHBG, seems to be a marker of increased risk for CVD mortality.

Our findings emphasize the importance of not focusing solely on T levels in the clinical work-up of male patients seeking help for androgen insufficiency. Inclusion of related hormones, particularly LH, contributes to a better mechanistic understanding of the observed findings and may flag risk of comorbidities that otherwise would be overlooked.

Acknowledgments

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