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Individual testosterone decline and future mortality risk in men

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Abstract

Objective: Male aging is characterized by a decline in testosterone (T) levels with a substantial variability between subjects. However, it is unclear whether differences in age-related changes in T are associated with general health. We investigated associations between mortality and intra-individual changes in serum levels of total T, SHBG, free T, estradiol and LH during a ten year period with up to 18 years of registry follow-up.

Design: 1,167 men aged 30 to 60 years participating in the Danish Monitoring Trends and Determinants of Cardiovascular Disease (MONICA1) study and who had a follow-up examination ten years later (MONICA10) were included. From MONICA10 the men were followed up to 18 years (mean: 15.2 years) in national mortality registries via their unique personal ID number.

Methods: Cox proportional hazard models were used to investigate the association between intra-individual hormone changes and all-cause-, CVD-, and cancer mortality.

Results: A total of 421 men (36.1%) died during the follow-up period. Men with most pronounced decline in total T (<10th percentile) had a higher all-cause mortality risk compared to men within the 10th to 90th percentile (hazard ratio [HR], 1.60; 95% confidence interval [CI], 1.08-2.36). No consistent associations were seen in cause-specific mortality analyses.

Conclusion: Our study showed that higher mortality rates were seen among the men who had the most pronounced age-related decline in T, independent of their baseline T levels.

Introduction

Testosterone (T) is essential for male reproductive function. Besides stimulating Sertoli cell function and spermatogenesis T exerts effects in non-reproductive organs, and stimulate bone mineralization, muscle growth, erythropoiesis and cognitive function (1-3). In men, aging is characterized by a physiological decline in T level with a substantial variability between subjects (4). The decline is paralleled by an age-related increase in sex hormone-binding globulin (SHBG), the primary binding-protein for T; thus a more pronounced decline in the free fraction of T occurs with increasing age (5, 6).

Recently, a number of large prospective studies have shown that lifestyle and health changes are associated with changes in T levels (4, 7, 8). Weight gain is closely associated to an accelerated decline in total T, free T and SHBG, and deterioration in health also contributes to this decline (4). Interestingly, smoking men have higher T levels compared to non-smoking men, and smoking cessation is associated with a decrease in the T level (4, 7). Thus, age-related changes in T seem to be modified by changes in health and lifestyle factors indicating that the physiological decline in T with aging is to some degree preventable.

Previously, two studies in elderly men have investigated whether changes in T levels and related hormone levels were associated to all-cause mortality, however with conflicting results (9, 10). In Australian men a decrease in T levels were associated with all-cause mortality (9) whereas no consistent association was seen in American men (10).

We hypothesized that the degree of age-related changes in testosterone may be of clinical relevance and a possible risk marker of health also in younger men. In the present study, we therefore examined whether intra-individual changes in levels of total and free T, SHBG, ~~estradiol,~~ and LH over a ten year period were associated with all-cause mortality in 1,167 men aged 30 to 60 years at baseline and on whom ~~where~~ we had follow-up information from national mortality registries up to 18 years later. This is to our knowledge the first population-based study to relate intra-individual changes in reproductive hormone levels to mortality in men including men as young as 30 years of age.

Subjects and methods

Study population

The study included 1,930 men who participated in the Danish Monitoring Trends and Determinants of Cardiovascular Disease 1 (MONICA1) study conducted between November 1982 and February 1984 and for whom serum samples were available for hormone analyses. The MONICA1 study was part of an international project initiated by the World Health Organization with the aim of monitoring trends and determinants of cardiovascular disease (11, 12). Men born in 1922, 1932, 1942 or 1952 were randomly selected from 11 municipalities in the southwestern part of the Copenhagen area and the participation rate was 81%. The men were invited to participate in a follow-up study between 1993 and 1994 (MONICA10). Follow-up serum samples were available for hormone analyses from 1,167 of men agreeing to participate (Figure 1). The study protocol for the health examination was the same at baseline and follow-up and included a physical examination, and a self-administered questionnaire with detailed information on lifestyle, sociodemographic characteristics and health status. Furthermore, a blood sample was drawn in the morning after an overnight fast. Serum samples were stored in aliquots at -20°C until the hormone analysis was performed. Informed consent was obtained from all participants.

Hormone measurements

To be able to include as many reproductive hormones as possible all samples were analyzed using the same assay platform due to a limited amount of serum available; T was measured by time-resolved fluorimmunoassay (DELFIA; Wallac Oy, Turku, Finland) and SHBG and LH were measured by time-resolved immunofluorometric assay (DELFIA; Wallac Oy, Turku, Finland). Limits of detection were 0.23 nmol/L for T and SHBG and 0.05 IU/L for LH. Intra- and interassay coefficients of variation were less than 12% for T, less than 8% for SHBG and less than 6% for LH. All samples were analyzed for the three hormones in the same laboratory, within the same period in 2004. Thus, samples from the different surveys had been stored for a varying period (10 to 22 years) before hormone analysis. Furthermore, the samples from the different surveys have to a varying degree been thawed and analyzed for other purposes prior to the hormone analyses whereas others have been kept in the freezer exclusively. Therefore, the hormone levels

were initially corrected for evaporation according to a general correction factor based on median serum Na+ levels measured in a subset of samples from each of the two surveys as previously extensively validated (13). Free T was calculated based on the T and SHBG concentrations according to the method suggested by Vermeulen et al. with the assumption of an average albumin concentration of 43.0 g/L (14).

Follow-up and outcome definition

In Denmark, every citizen has a unique personal identification number (CPR-number) through which information from national registries can be obtained. Based on the CPR-numbers of the participants we obtained information on outcomes from The Central Office of Civil Registration for information on vital status with complete follow-up. Follow-up time was calculated as the time from the follow-up examination MONICA10 until the time of death, censoring or end of follow-up (December 17, 2012), whichever occurred first (Figure 1). Participants who emigrated during the follow-up period (n=8) contributed with person time at risk until date of emigration, after which they were censored. Information about specific causes of death was obtained from The Danish Register of Causes of Death. A minor proportion of deaths (n=56) was not registered with a specific cause of death and was therefore censored in the analyses of CVD and cancer mortality. Finally, information on hospital admissions related to CVD prior to baseline was obtained from the National Patient Register in which all admissions to Danish hospitals since 1977 is registered. Thus, men who had been diagnosed with either ischemic heart disease, stroke, or atherosclerosis (ICD-8: 410–414, 430–438, 440; ICD-10: G45, I20–I25, I60–I70) prior to the baseline examination were excluded in the analysis of CVD mortality (n=66).

Statistical analysis

Initially, the changes (delta) per year in total and free T, SHBG, and LH were calculated as the difference between the hormone levels at the follow-up examination and the baseline examination divided with the time span between the two examinations (e.g. $T_{MONICA10}$ - $T_{MONICA1}$)/time). Thus, negative delta (Δ) values reflect a decline whereas positive numbers reflect an increase over time. The marginal association between the

hormone changes per year and the baseline hormone levels was investigated in plots and the Pearson correlation coefficient was reported. Furthermore, mean differences in baseline and ~~delta~~ Δ hormone values per ten years stratified by lifestyle characteristics were investigated using general linear regression. The baseline level of LH was log-transformed to obtain normality and geometric means were reported.

To investigate the association of Δ hormone levels in relation to all-cause, CVD, and cancer mortality we used Cox proportional hazard models with age as the underlying time scale. To accommodate potential nonlinear associations Δ hormone levels were analyzed in tertiles and in percentiles (<10th, 10th-90th, >90th). Potential confounders were identified based on prior literature and confirmed in our data. All final models were adjusted for the baseline hormone level, baseline age (30, 40, 50 or 60 years), smoking habits in MONICA1 to MONICA10 (nonsmoker-nonsmoker, nonsmoker-smoker, smoker-smoker or smoker-nonsmoker), baseline BMI (<20.0, 20.0-24.9, 25.0-29.9 or ≥30), changes in weight from MONICA1 to MONICA10 (>-5kg, +/- 5kg or >+5kg), exercise level in MONICA1 to MONICA10 (sedentary-sedentary, sedentary-active, active-active, active-sedentary), baseline alcohol intake per week (0, 1-14 or +14), and changes in number of alcohol units per week between MONICA1 and MONICA10. In the fully adjusted models, 29 (2.5%) individuals were excluded because of one or more missing values and the tertiles and percentiles were recalculated. P-values <0.05 were considered statistically significant. All models were tested for the assumption of proportional hazards. Furthermore, we tested for interaction between the Δ hormone values and each of the covariates including the baseline hormone level. In sensitivity analyses, we restricted the population to include only eugonadal men at baseline defined as men with T level ≥ 10.5 nmol/L and LH ≤ 9.4 IU/L (n=1,089) as suggested in the European Male Aging Study (EMAS) (15) and repeated the Cox proportional hazards models and compared the results with the models without restrictions. The statistical analyses were performed using R (version 3.2.3).

Results

A detailed description of baseline hormone levels and changes in hormone levels in relation to lifestyle characteristics is shown in Table 1. Mean time span between the two examinations (MONICA1 and

MONICA10) was 10.9 years (min-max: 9.7-12.0). The estimated mean (SEM) intra-individual percentage change in hormone levels per year were -1.5% (0.16) for total T, 0.9% (0.18) for SHBG, -1.9% (0.39) for free T, and 1.0% (0.27) for LH. In comparison, when estimated cross-sectionally, based on baseline hormone levels, mean percentage change in hormone levels were per year -0.4% (0.06) for total T, 1.2% (0.08) for SHBG, -1.1% for free T (0.05), and 1.1% (0.12) for LH.

Δ hormone levels in relation to baseline hormone levels

Higher baseline T level was associated with a more pronounced absolute decline in T per year ($r=-0.52$, $p<0.01$, Supplementary Figure 1) which seemed to be explained by age as the youngest men were characterized by higher baseline level and experienced the largest decline (Table 1). Similarly, higher level of free T was strongly associated with a more pronounced decline in free T per year ($r=-0.69$, $p<0.01$). A weak correlation was seen for SHBG ($r=-0.12$, $p<0.01$) and LH ($r=-0.20$, $p<0.01$).

Baseline and Δ hormone levels in relation to lifestyle factors

At baseline an inverse association between BMI and levels of total and free T and SHBG was found. Men with higher BMI had a less pronounced decline in total and free T. Men who gained more than 5 kg from baseline to follow-up examination had the most pronounced decline in total and free T compared to men who had a stable weight (+/- 5 kg) and men who lost more than 5 kg. Furthermore, men who gained more than 5 kg in weight showed a decrease in SHBG in contrast to the other two groups who were characterized by an increase in SHBG with aging.

Men who were smokers at both time points were characterized by significantly higher baseline hormone levels compared to the other groups. Smoking cessation was associated with the most pronounced decline in total and free T and was associated with a minor decrease in SHBG (whereas mean SHBG increased from baseline to follow-up among the other groups). The higher the number of alcohol units per week, the lower the baseline SHBG level, and the more pronounced decline in free T was seen. No significant differences in baseline hormone levels were seen according to baseline leisure time physical activity. Finally, baseline level

of free T was lower among men with a cardiovascular disease at baseline and they experienced a larger increase in SHBG within the time period.

Mortality

Mean follow-up from MONICA10 until end of follow-up was 15.2 years and up to 18.6 years. A total of 421 men (36.1%) died during the follow-up period of which 106 cases were cancer-related and 119 CVD-related. Figure 2 shows the Cox Proportional hazard models for intra-individual changes in hormone levels in relation to all-cause mortality where Δ hormone levels were categorized into either tertiles or percentiles (<10th, 10th-90th, >90th). In both models the middle category served as the reference category. A significant increased risk in all-cause mortality was observed for the tertile of men with the most pronounced decline in total T, corresponding to an annual decline of at least -0.6 nmol/L (HR, 1.34, 95% CI, 1.02-1.78, for specific model estimates see Supplementary Table 1). A more pronounced effect was seen for men in the lowest 10th percentile (HR, 1.60, 95% CI, 1.08-2.38) compared to the reference category. No significant difference in all-cause mortality was seen for tertiles of Δ SHBG levels. However, men with Δ SHBG levels below the 10th percentile (< -0.7 nmol/L/yr) or above the 90th percentile (>1.1 nmol/L/yr), had an increased mortality compared to the middle group (HR, 1.51, 95% CI, 1.05-2.17; HR, 1.42, 95% CI 1.08-1.88, respectively). In accordance with the findings for total T, men with the most pronounced decline in free T had an increased risk of all-cause mortality, however only significant in the tertile model (HR, 1.45, 95% CI, 1.09-1.92). No association was seen for differences in Δ LH levels and Δ T/LH levels in relation to all-cause mortality.
In analyses with CVD mortality as the outcome no significant associations were seen (Supplementary Table 2). Similarly, in analyses with cancer as the outcome no significant associations were seen (Supplementary Table 3). Finally, similar results were seen when we restricted the population to men who at baseline were eugonadal (data not shown).

Discussion

In this large population-based follow-up study of men aged 30 or older we observed that higher mortality rates were seen among those who had the most pronounced decline in T levels. This association was independent of age, baseline hormone levels and lifestyle factors.

Few other studies have looked at intra-individual declines in T levels in aging men in relation to mortality. In line with our findings a study of older Australian men observed that progressive declines in testosterone were associated with increased all-cause mortality (9) whereas a study of elderly men participating in the Framingham Heart Study, did not find any consistent association between changes in total T and all-cause mortality (10). However, the findings of the latter study may be explained by the older age range in this population and a reduced statistical power caused by a limited study size (n=254) and number of cases (n=104). Furthermore, no adjustment for weight changes and changes in smoking habits were performed, yet both these two lifestyle factors are known to have effects on circulating levels of T.

The association between serum T, measured at a single time point, and all-cause mortality has been investigated in a number of population-based studies, where some (15-23) but not all (24-34) concluded that low T is associated with an increased all-cause mortality risk. Based on 11 community-based studies a meta-analysis from 2011 found a significant association between a single point measurement of low T and all-cause mortality but also identified significant heterogeneity between studies (36). In a population of 5,350 men from the age of 30 years with hormone measurements at a single time point we did not see an association between low T and all-cause mortality but showed that compensated Leydig cell function, characterized by elevated LH levels and LH/T levels and normal T levels, was associated with increased all-cause mortality (30).

It has been suggested that men with a compensated Leydig cell function may be at greater risk of developing an overt primary hypogonadism (characterized by low T levels and elevated LH levels) with age (15). A link between impaired testicular function and increased morbidity and mortality was recently also indicated in

three studies showing associations between lower semen quality and increased morbidity and mortality risk (37, 38, 39). However, while we in the present study observed an association between the tempo in the age-related T and free T decline and mortality we did not observe any association between age-related changes in LH levels and all-cause mortality as would be expected if the association for T was linked to a primary Leydig cell insufficiency.

In accordance with previous longitudinal studies we observed a close link between weight changes and changes in levels of total T and free T where men experiencing weight loss were characterized by the least pronounced decline in T and weight gaining men were characterized by an accelerated decline in T compared to age-matched men with stable weight. Interestingly, weight gaining men were characterized by a decline in SHBG compared to stable weight men and men who lost weight for whom an age-related increase in SHBG was observed.

In our analyses of all-cause mortality a U-shaped association was seen when categorizing Δ SHBG levels into percentiles. However, no association was seen when categorizing Δ SHBG levels into tertiles in line with the findings by Hsu et al, where no association was seen after adjustment for confounders (9). It is well known that SHBG levels increase with age but also smokers have higher levels, whereas lower levels are seen in case of obesity. However, in fully adjusted models the inclusion of age, BMI, smoking and other lifestyle factors as confounders did not change the estimates significantly. We cannot rule out that men with the most extreme SHBG trajectories; either below the 10th percentile or above the 90th percentile has a higher mortality. Alternatively, the lower power when categorizing according to percentiles could have resulted in chance findings. Thus, more studies are needed to confirm these findings.

Our study has a number of strengths including the comprehensive study population with repeated measurements on 1,167 men. In addition, besides being part of a longitudinal study with a ten year interval the men were further followed via registries in up to 18 years. Finally, the same hormone assays were used to measure the hormone levels from both the baseline and follow-up examination reducing potential interassay variation. One of the major methodological limitations inherent in longitudinal studies is subject attrition

which can lead to selection bias and reduce generalizability. In our study, 1,167 men attended the follow-up examination out of the 1,930 men (60.5%) participating in the baseline examination. It is possible that men who participated in the follow-up examination differ in lifestyle and health characteristics compared to men who did not participate in the follow-up examination. As expected, in subanalyses (Supplementary Table 4) we found that men who did not survive until the follow-up examination (n=208) were older compared to the men who participated in the follow-up examination and also more likely to be smokers. Therefore, the men who participated in our longitudinal study population to some degree represents a slightly younger and possibly healthier part of the original study population which could have biased our findings towards less pronounced changes in T levels compared to the original baseline population. T levels were analyzed using fluorimmunoassays (DELFIA). For sex steroids tandem mass spectrometry has been recommended because of higher specificity than immunoassays (40). We have subsequently compared the testosterone DELFIA assay to a turboflow-liquid chromatography tandem mass spectrometry method (established in our laboratory in 2012) and a strong correlation between the two methods was found in the relevant ranges (data not published). Thus, methods of measurements are highly unlikely to have any influence of the obtained results.

In conclusion, our findings suggest that a more pronounced age-related decline in T is associated with mortality in men independent of baseline hormone level. A possible causal link between an increased tempo in age-related T decline and subsequent health is unknown and remains to be investigated.

Declaration of interest

The authors declare no conflicts of interest that could be perceived as prejudicing the impartiality of the research reported.

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Figure Legends

Figure 1 Lexis diagram reflecting the study design

Figure 2 Hazard ratios and 95% confidence intervals for tertiles and percentiles (<10th, 10th-90th, >90th) of delta hormone levels in relation to all-cause mortality.

All models are adjusted for baseline hormone level, baseline age, changes in smoking status, baseline BMI, weight changes, exercise level and baseline and changes in alcohol consumption with age as the underlying time scale.

Tables

Table 1 Mean baseline levels and absolute changes per 10 years (Δ) in hormones stratified according to lifestyle characteristics (n=1,167)

		Total T (nmol/L)		SHBG (nmol/L)		Free T (pmol/L)		LH (IU/L)	
	n	Baseline	Δ[b] per 10yr	Baseline	Δ[b] per 10yr	Baseline	Δ[b] per 10yr	Baseline [a]	Δ[b] per 10yr
Age (years)									
30[c]	305	22.7	-5.1	28.3	0.6	545.9	-146.3	3.0	0.0
40	329	20.4[d]	-4.1[d]	29.7	1.2	464.7[d]	-116.7[d]	3.0	0.1
50	304	19.3[d]	-2.9[d]	34.0[d]	3.3[d]	401.1[d]	-89.1[d]	3.4[d]	0.2
60	229	20.1[d]	-3.1[d]	40.1[d]	3.3[d]	383.3[d]	-81.6[d]	3.9[d]	0.5[d]
Baseline BMI (kg/m^2)									
<20	42	25.7[d]	-4.1	40.9[d]	1.0	520.5	-109.8	3.3	0.7
20-25[c]	581	22.4	-4.5	34.7	1.1	484.2	-121.6	3.2	0.1
25-30	441	18.9[d]	-3.3[d]	30.2[d]	2.8[d]	424.2[d]	-100.5[d]	3.4	0.2
30+	103	16.1[d]	-2.6[d]	26.4[d]	3.7[d]	377.6[d]	-89.1[d]	3.1	0.0
Absolute changes in weight (kg) from baseline to follow-up examination									
> - 5	82	18.5[d]	-1.8[d]	31.3	9.5[d]	405.4[d]	-97.2	3.6	0.7[d]
+/- 5[c]	663	20.6	-3.2	33.4	3.3	444.9	-99.7	3.3	0.1
> +5	420	21.2	-5.4[d]	31.3[d]	-1.6[d]	476.4[d]	-129.8[d]	3.1	0.1
Changes in smoking habits from baseline to follow-up examination									
ns-ns[c]	497	19.4	-3.4	30.8	1.9	431.7	-98.0	3.2	0.2
ns-s	34	18.4	-1.5[d]	27.6	4.9[d]	427.9	-74.8	3.2	0.5
s-s	483	22.2[d]	-4.0[d]	34.6[d]	2.9	477.0[d]	-117.6[d]	3.4[d]	0.1
s-ns	153	20.6	-5.5[d]	32.6	-1.1[d]	455.3	-135.6[d]	3.1	0.2
Alcohol intake (units/week)									
0	59	18.9[d]	-2.5[d]	34.5	2.4	394.8[d]	-70.5[d]	3.1	0.7
1-14[c]	749	21.0	-4.0	33.2	1.5	455.8	-108.0	3.3	0.2

<15	359	20.3	-3.9	30.8[d]	2.9[d]	458.0	-121.7	3.3	0.0
Leisure time physical activity									
Sedentary	220	20.3	-3.6	31.1	3.5	450.4	-114.0	3.4	0.2
Active[c]	947	20.8	-3.9	32.8	1.6[d]	454.1	-109.5	3.2	0.1
Known CVD at baseline									
No[c]	1101	20.7	-3.9	32.5	1.8	455.9	-110.7	3.7	0.1
Yes	66	19.6	-3.5	33.2	4.7[d]	412.4[d]	-104.1	3.9	0.2

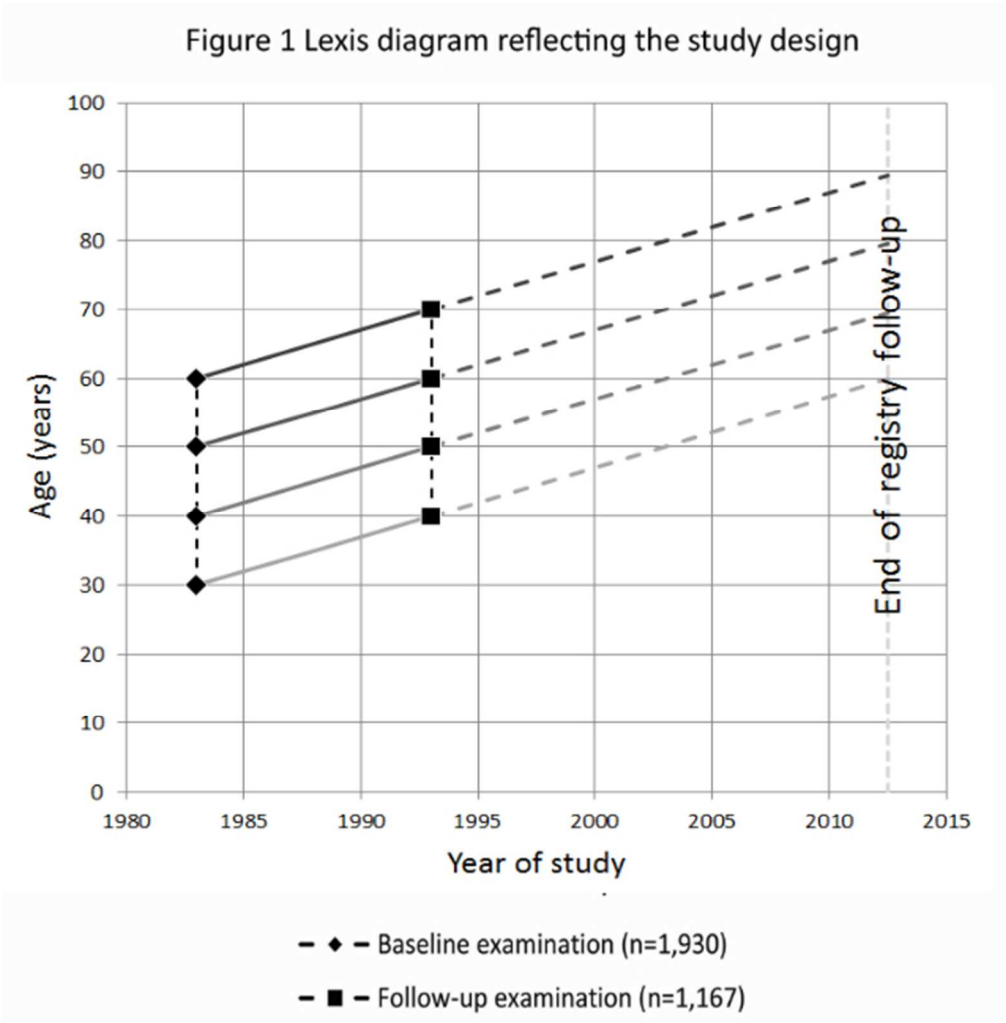

Figure 1 Lexis diagram reflecting the study design

168x170mm (96 x 96 DPI)

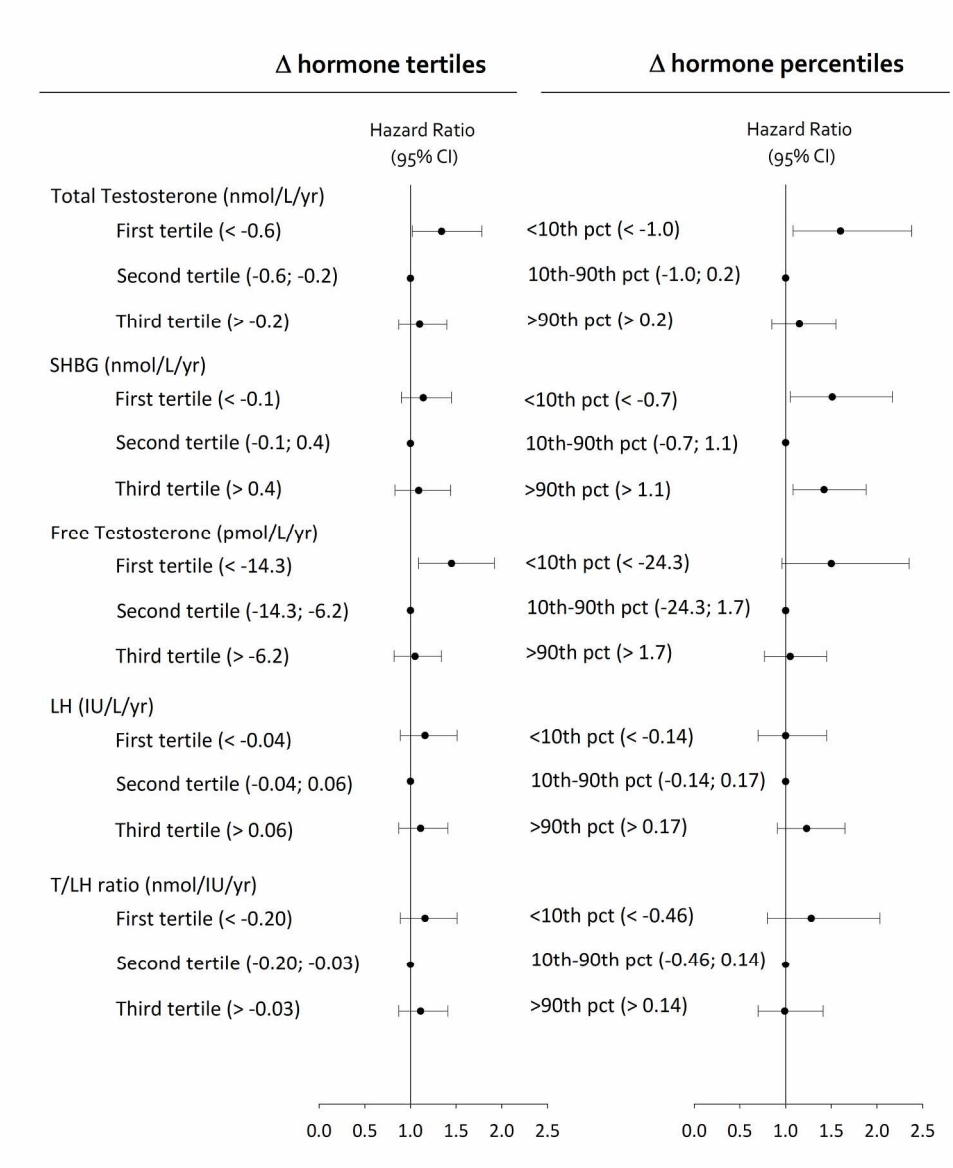

Figure 2 Hazard ratios and 95% confidence intervals for tertiles and percentiles (<10th, 10th-90th, >90th) of delta hormone levels in relation to all-cause mortality

117x138mm (600 x 600 DPI)