Muscles growing older

Schaap, L.A.

2010

Chapter 7

Low testosterone levels and mortality in older men: results from the Longitudinal Aging Study Amsterdam (LASA)

submitted

Abstract

Context: It has been well documented that as men age, their serum testosterone levels fall. The clinical implications are uncertain; especially its effect on mortality.

Objectives: To examine the association of total and free testosterone levels with mortality in older men.

Design, Settings and Participants: The study included 607 men aged ≥65 years (Median age 75.4 years) of the Longitudinal Aging Study Amsterdam (LASA). These persons had a median follow-up of 10.9 ± 3.7 years.

Main Outcome Measures: Association between testosterone and mortality using Cox Proportional Hazards model with adjustment for physical and lifestyle factors.

Results: During follow-up, 373 persons died, of whom 83 died from cardiac causes. There was no significant association between low levels of total or free testosterone and all-cause or cardiovascular mortality after adjustment for age, lifestyle factors, education level, and cognitive impairment. Additional adjustment for chronic diseases did not change these findings.

Conclusions: This study showed no association between low levels of total or free testosterone and increased all-cause or cause-specific mortality in older men.

Introduction

In men, unlike women, there is no abrupt and definite decline in sex hormone synthesis. There is however, a general acceptance that there is an age related decline with progressively more men at the lower end of the spectrum with each passing decade[1-5]. Even then, there is still a considerable proportion of men in their 80's and 90's who are in the "normal" range of testosterone values.[1-8] There is some uncertainty whether declines in testosterone are associated with the aging process itself, or with other age-related phenomena such a greater number of medical co-morbidities and lifestyle differences such as being less involved in physical activity.

Low testosterone levels have been associated with diabetes mellitus, metabolic syndrome, depression, impaired cognition, loss of muscle mass and bone mineral density.[8] There is little consensus in the literature regarding the implications of the decline of testosterone levels on health related outcomes; especially its link to mortality. The Rancho Bernardo study[1] was the first prospective, population based study to demonstrate an increased mortality for men in the lowest quartile of total testosterone levels. Two other prospective studies – the Caerphilly study[9] and the Massachusetts Male Aging Study (MMAS)[10] had not shown any association between low testosterone levels and early mortality. Recently, a prospective Swedish study[11] reported a positive association between serum testosterone and estradiol levels and mortality. Another study[12] reported that low concentrations of a combination of hormones, but not of bioavailable testosterone alone, were associated with mortality.

The objective of this study is to investigate the association between total and free testosterone levels with all-cause and cause-specific mortality within a large sample of older men from the Longitudinal Aging Study Amsterdam (LASA). Furthermore, we will investigate whether the possible association between low testosterone levels and mortality is influenced by the presence of chronic diseases, such as heart disease, diabetes mellitus and cancer.

Methods

The data for this study were collected as part of the LASA study - an ongoing interdisciplinary, longitudinal study into the predictors and consequences of changes in physical, cognitive, emotional and social functioning of older community dwelling people. The design and sampling procedure are described in detail elsewhere.[13] To summarise, the sample was drawn randomly from men and women aged 55 years and over, living in 11 municipalities in three

regions of the Netherlands. They were stratified by age, sex, urbanisation and expected 5 year mortality to ensure sufficient sample size for longitudinal analyses. People in the older age groups and men were over-sampled to take into account expected attrition rates from illness and death.[14]

The LASA study started in 1992/1993 with regular 3 yearly follow-ups and is still ongoing. In the first cycle, 3107 respondents underwent a baseline examination. Follow-up measurements were collected every three years and consisted of a main and a medical interview. Data were gathered in face-to-face interviews in the homes of the respondents by specially trained and intensively supervised interviewers. In the second data collection (1995/1996), 2204 persons (71%) completed the main interview. Attrition between these two cycles was mainly due to mortality. 417 (13.4%) of the original participants died.

For the current study, data from the first follow-up (1995/96) were used as a baseline and included male respondents who participated in the main and medical interview and were aged 65 years and older as of January 1, 1996. Respondents who were lost to follow-up between 1992/93 and 1995/96 were older, were more often current smokers, were more likely to be cognitively impaired, and to have depressive symptoms and had more chronic diseases at baseline compared to respondents with complete follow-up data. Blood samples were drawn in 1352 participants of whom 726 were men. Levels of sex hormones, Sex hormone-binding globulin (SHBG) and albumin were determined in 628 of these men. After exclusion of two persons with missing data and 19 persons who used steriods, 607 participants remained for the analyses.

Informed consent was obtained from all respondents and the ethical review board of the VU University Medical Center approved the study.

Assessment of hormones

Levels of total testosterone were obtained during the examination in 1995/1996 and kept frozen until determination. Total testosterone concentrations were measured by radio-immunoassay (Coat-A-Count, DPC, Los Angeles, USA), with an inter-assay CV of 11% at 2.6 nmol/L (74.9 ng/dL) and 7% at 11.5 nmol/L (331.4 ng/dL). The detection limit of total testosterone was 1 nmol/L (28.8 ng/dL). Calculated free testosterone was determined according to the method described by Vermeulen et al. taking the concentration of total testosterone, sex hormone-binding globulin (SHBG) and albumin into account.[15] SHBG concentrations were measured by an immunoradiometric assay (IRMA, Orion Diagnostica, Espoo, Finland) with an inter-assay CV of 6% at 10 nmol/L (0.25 ug/dL). The detection limit for SHBG was

6 nmol/L (0.15 ug/dL). Albumin concentrations were measured directly after blood collection using an automated analyser (Hitachi 747, Hitachi High Technologies Co, Tokyo, Japan) obtaining a CV of less than 2%.

Age

Age was measured in years as at 1st January 1996. It was analysed both as a continuous variable as well as in stratified groups. For respondents who died, the date of death was traced through death certificates from municipal registers through June1, 2007. Survival was computed as the date of death minus the date of the blood sampling or the end of follow-up minus the date of blood sampling, whichever came first.

Confounders

Potential confounders included physical factors such as age, body mass index (BMI), cognitive impairment, chronic illness and prior corticosteroid use; and lifestyle factors such as alcohol use, smoking, physical activity and level of education. BMI was calculated as weight (kg)/height (m^2). Body weight was measured without clothes and shoes using a calibrated balance beam scale. Height was measured using a stadiometer. Cognition was assessed with the mini-mental state examination (MMSE).[16] The MMSE by Folstein is a brief 30 point questionnaire that is used to assess cognition. Scores less than 24 indicate cognitive impairment. Level of education was assessed by asking the respondent for the highest education level completed, ranging from incomplete elementary school to university education. The answers were categorised into 3 groups. The first group comprised of al individuals who had education ranging from incomplete elementary education to lower vocational training. The second group had education ranging from general intermediate to general secondary education; and the final group consisted of anyone who completed more than general secondary school education. Alcohol use was dichotomised to those who consumed 0-7 drinks per week and those that consumed more than 7 drinks a week. Smoking status was categorised as never, former or current. The lifelong non-smokers were the reference group. Physical activity was measured with the LASA physical activity questionnaire (LAPAQ). The LAPAQ is a face-to-face questionnaire that includes frequency and duration of activities such a walking, bicycling, gardening, light and heavy household activities and a maximum of two sporting activities during the previous two weeks.[17] Chronic illnesses were assessed using self-report during the main interview in 1995/1996 and included pulmonary disease (asthma and chronic obstruc-

tive pulmonary disease), cardiac disease, diabetes mellitus, arthritis, stroke and peripheral atherosclerosis.

Statistical analysis

All statistical analyses were performed using SPSS Version 15. Baseline variables are described according to two age groups (65-75 years and 75+ years). For normally distributed continuous variables, the mean and standard deviation are reported, for skewed continuous variables, median values with the interquartile range are reported, while categorical measures are reported as percentages. Characteristics of younger and older men were compared using chi-square tests, Student t tests, or Mann-Whitney tests.

Multiple linear regression analyses were performed to examine the cross-sectional association between testosterone and age in 1995/6. Both age en total and free testosterone were analysed as continuous variables. In Model 1 results were adjusted for BMI, cognitive impairment, smoking, alcohol use, physical activity and education level. In Model 2 results were additionally adjusted for presence of pulmonary disease, cardiac disease, diabetes mellitus, cancer, stroke and peripheral atherosclerosis to examine the influence of presence of chronic diseases on the association between age and testosterone levels.

The second part of the study explored the relationship between total and free testosterone levels and all-cause and cardiovascular mortality using Cox regression analyses. Total and free testosterone levels were categorized in quartiles with the highest quartile as the reference category. In Model 1, the results were adjusted for age. In Model 2, results were additionally adjusted for BMI, cognitive impairment, depressive symptoms, smoking, alcohol use, physical activity and education level. In the final model (Model 3) results were additionally adjusted for chronic diseases to examine the influence of chronic diseases in the association between testosterone and mortality separately.

Results

Baseline Characteristics

The baseline characteristics of the cohort with regards to their hormonal parameters as well confounders were categorised according to two age groups (Table 1). The participants in the older age group had significantly lower total testosterone levels ($p=0.002$) and lower free testosterone levels compared to the younger age group ($p<0.001$). They also had higher

Table 1

LASA study characteristics stratified by age

Variables	Ages 65-75 years (n = 293)	Ages >75 years (n = 314)	P value
Age (years)	69.8 ± 2.7	81.1 ± 3.8	<0.001
BMI (kg/m2)	26.3 ± 3.3	25.8 ± 3.5	0.05
Total Testosterone (nmol/L)§	16.0 ± 4.9	14.7 ± 5.0	0.002
Free Testosterone (pmol/L)§	301.6 ± 83.2	253.1 ± 79.7	<0.001
SHBG (nmol/L)§*	37.8 (28.6 – 48.1)	43.4 (34.1 – 56.1)	<0.001
Cognitive impairment MMSE ≤23**	4.4	17.3	<0.001
Hart disease	29.0	35.7	0.08
Artery disease	11.6	12.4	0.76
Diabetes	4.4	7.3	0.13
Stroke	5.8	11.1	0.02
Cancer	8.2	10.5	0.33
CNSLD	15.0	19.1	0.18
Alcohol**			
None	12.3	16.6	
0-7 drinks/wk	45.4	51.6	
>7 drinks/wk	42.3	31.8	0.02
Smoking**			
Never	8.2	10.8	
Former	65.5	65.9	
Current	26.3	23.2	0.43
Physical activity (min/day)*	100.0 (53.6 – 162.9)	83.6 (39.1 – 154.5)	0.04
Education**			
Low	48.3	58.9	
Medium	36.0	25.2	
High	15.7	15.9	0.01

§ For conversion from metric to SI units –Divide by 0.0347 for total testosterone (ng/dL), by 34.7 for free testosterone (ng/dL), by 40 for SHBG (ug /dL).
All differences are measured in mean +/- standard deviation unless stated otherwise
*Result represents median [interquartile range]
**Result represents percentages
†Differences in mean values between the 3 age categories were examined with one way analysis of variance (ANOVA) tests for continuous variables, Kruskal-Wallis tests for continuous skewed variables and Pearson's Chi square tests for categorical variables.

SHBG levels (p<0.001). Older persons were more often cognitively impaired, more often experienced a stroke, consumed less alcohol, were less physically active, had a lower BMI and had a lower education level compared to younger persons.

Association between Testosterone levels and Age

Multiple linear regression analyses were carried out on the cross-sectional data collected in 1995/6 (Table 2). Increasing age was significantly associated with lower total testosterone levels in the unadjusted analyses (β -0.16, p<0.001). There was little change in the results when any of the confounders were added (Model 1 and 2). Increasing age was also significantly associated with lower free testosterone levels (β -0.32, p<0.01). Again, adding confounders into the model did not markedly change the results.

Testosterone levels and Mortality

Cox regression analyses were performed on the longitudinal mortality data collected as part of the LASA study. There were 373 deaths from all causes during the average of 10.9 ± 3.7 years of follow-up. Cause-specific deaths were only recorded for cardiovascular causes and until the year 2003. In that time period there were 83 deaths from cardiovascular causes. There was no statistically significant age-adjusted association between quartiles of total testosterone and all-cause mortality, regardless of the presence of other confounders (Table 3).

	Total T		
Age (years)	Unadjusted	Model 1†	Model 2††
	β	β	β
	-0.16**	-0.17**	-0.16**
	Free T		
Age (years)	Unadjusted	Model 1†	Model 2††
	β	β	β
	-0.32**	-0.31**	-0.31**

β represents standardized regression coefficient, **p<0.001
†Model 1 adjusted for BMI, cognitive impairment, smoking, alcohol use, physical activity and education
††Model 2 additionally adjusted for chronic diseases.

Table 2

Results of multiple linear regression analyses of total and free testosterone levels with age

For example, model 1 shows a hazard ratio for mortality was 1.02 (95% confidence interval (CI) 0.75 – 1.39) for men in the lowest quartile of total testosterone (<12.4 nmol/l) compared to men with levels of total testosterone >18.3 nmol/l (reference group). These results changed only slightly after additional adjustment for other confounders. Similar results were found for quartiles of free testosterone and mortality. Mortality was further subdivided into death from cardiovascular causes (83 events). There was no association between total or free testosterone levels and death from all cardiovascular causes (Table 4). For example, model 1 shows a hazard ratio for mortality of 1.25 (95% CI 0.64 – 2.47) for men in the lowest quartile of free testosterone (<227.4 pmol/l), p=0.52 compared to men with levels of free testosterone >329.2 pmol/l (reference group). After adjusted for other confounders including chronic diseases (model 3) the hazard ratio is 1.80 (95% 0.82 – 3.91) for men in the lowest quartile of free testosterone (p=0.14) compared to men in the reference group. To examine the possible influence of prevalent diseases on the association between testosterone levels and mortality, we calculated hazard ratios for mortality after excluding persons with prevalent cancer, heart disease or diabetes. Excluding prevalent diseases showed no major impact on mortality (data not shown).

Table 3

Total and Free Testosterone and all cause mortality risk

	Model 1†		Model 2††		Model 3†††	
	HR	p	HR	p	HR	p
Total Testosterone (nmol/l)						
<12.4	1.02 (0.75 – 1.39)	0.91	1.05 (0.76 – 1.43)	0.78	1.03 (0.75 – 1.41)	0.86
12.4 – 15.1	0.95 (0.70 – 1.29)	0.74	0.98 (0.72 – 1.34)	0.90	1.07 (0.78 – 1.47)	0.67
15.2 – 18.3	1.00 (0.73 – 1.38)	0.99	1.09 (0.77 – 1.53)	0.64	1.07 (0.76 – 1.51)	0.68
>18.3	Reference		Reference		Reference	
Free Testosterone (pmol/l)						
<227.4	1.19 (0.85 – 1.65)	0.32	1.17 (0.84 – 1.64)	0.35	1.18 (0.84 – 1.65)	0.34
227.5 – 275.3	1.28 (0.92 – 1.77)	0.14	1.23 (0.87 – 1.72)	0.24	1.21 (0.86 – 1.71)	0.27
275.4 – 329.2	0.93 (0.66 – 1.30)	0.66	0.90 (0.64 – 1.27)	0.55	0.95 (0.67 – 1.33)	0.76
>329.2	Reference		Reference		Reference	

HR= Hazards Ratio (95% Confidence interval)
†Model 1: adjusted for age
††Model 2: additionally adjusted for BMI, smoking, alcohol use, physical activity, education, cognitive impairment, depressive symptoms ††Model 3: additionally adjusted for chronic diseases.

Table 4

Total and Free Testosterone and cardiovascular deaths (83 events)

	Model 1†		Model 2††		Model 3†††	
	HR	p	HR	P	HR	p
Total Testosterone	1.03 (0.84 – 1.26)	0.78	1.11 (0.88 – 1.39)	0.40	1.03 (0.80 – 1.33)	0.81
<12.4	0.91 (0.51 – 1.63)	0.75	0.61 (0.31 – 1.23)	0.16	0.76 (0.36 – 1.60)	0.47
12.4 – 15.1	1.17 (0.65 – 2.11)	0.61	0.71 (0.36 – 1.37)	0.30	0.82 (0.41 – 1.64)	0.57
15.2 – 18.3	0.60 (0.30 – 1.21)	0.15	0.38 (0.18 – 0.82)	0.01	0.41 (0.18 – 0.90)	0.03
>18.3	Reference		Reference		Reference	
Free Testosterone	0.95 (0.78 – 1.17)	0.65	0.99 (0.79 – 1.24)	0.91	0.88 (0.68 – 1.14)	0.34
<227.4	1.25 (0.64 – 2.47)	0.52	1.30 (0.65 – 2.60)	0.46	1.80 (0.82 – 3.91)	0.14
227.5 – 275.3	1.30 (0.66 – 2.57)	0.44	1.24 (0.61 – 2.50)	0.56	1.48 (0.71 – 3.07)	0.29
275.4 – 329.2	0.94 (0.46 – 1.93)	0.86	1.05 (0.49 – 2.26)	0.90	1.29 (0.59 – 2.84)	0.52
>329.2	Reference		Reference		Reference	

HR= Hazards Ratio (95% Confidence interval)
†Model 1: adjusted for age
††Model 2: additionally adjusted for BMI, smoking, alcohol use, physical activity, education, cognitive impairment, depressive symptoms ††Model 3: additionally adjusted for chronic diseases.

Discussion

Our study extends the current body of evidence that suggests that there is a decrease in both total and free testosterone levels in men as they age. This was independent of obesity, medical co-morbidities, cognitive impairment and several lifestyle factors such as alcohol, smoking and physical activity. In our study, there was no statistically significant association found between total or free testosterone levels and mortality. After we subdivided mortality into death from cardiovascular causes, we still did not observe any associations between low testosterone levels and early mortality.

The LASA cohort represents a cohort of community dwelling older men who had numerous measurements including serum testosterone levels taken at the start of the study. They were not selected on the basis of any specific symptom or sign including those due to testosterone deficiency. Hence they were an ideal cohort to look at the spectrum of testosterone levels in men at different stages of the aging process.

The Rancho Bernardo study[1] was the first prospective, population based study to demonstrate an increased mortality for men in the lowest quartile of total testosterone levels. These results were unaffected when adjusted for co-morbidities and when deaths in the first 5 years were excluded. Only a few smaller studies until that point had showed this associa-

tion.[18, 19] Two other prospective studies – the Caerphilly study[9] and the Massachusetts Male Aging Study (MMAS)[10] had not shown any association between testosterone and mortality. One possible explanation cited was the 20 year age difference in median age between the study populations of the first two studies and the final study.[1, 20] However, our study brings this conclusion under question as our cohort had a median age of 75.4 years, which is similar to that of the Rancho Bernardo cohort (median age 73.6 years). Our sample size and demographics were similar and our sampling procedures comparable. The main difference was that our mean total testosterone level was much higher – 15.4 nmol/L (443.8 ng/dL) than theirs – 10.4 nmol/L (300ng/dL). The reason for this large difference is not clear as the populations studied appear similar. The mortality rate was similar in the Rancho Bernardo cohort (57.5 per 1000 person years) as compared to the LASA cohort (54.4 per 1000 person years). With 373 deaths among 628 men, our study was well powered to find an association if one existed. However, the difference in deaths from cardiovascular causes varied more markedly between the two cohorts with cardiovascular causes of death found in 49.9% (264/529) as opposed to 22.3% (83/373) of the LASA deaths. The reason for this discrepancy is not clear. While Rancho Bernardo had a higher prevalence of diabetes (15%) than LASA (6.2%), current smoking was more prevalent among the LASA participants (24.8%) compared to those in the Rancho Bernardo group (11%). Established cardiovascular disease had a similar prevalence in both groups (35% for the Rancho Bernardo study and 32.3% for the LASA study).

Recently, a prospective Swedish study[11] reported a positive association between serum testosterone and estradiol levels and mortality. Their demographics were similar to the LASA study with a mean age of 75 years and mean testosterone levels were comparable (total testosterone 450 ng/dL in the Swedish study compared to 443.8 ng/dL in LASA). The differences lay in the significantly shorter follow-up in this study of 4.5 years with a much lower mortality rate of 28.3 per 1000 person years and the method of measuring testosterone – mass spectroscopy in their study as opposed to radioimmunoassay in our study. Although this study did not rely on the combination with estradiol to explain the increase in mortality, adding estradiol did strengthen the link. Methodological differences such as assay variability may be another possible explanation. [21] Another smaller study[12] also looked at a combination of hormonal parameters in explaining the increase in mortality, suggesting the relationship between mortality and hormonal parameters may be more complex. The problems are further compounded for free testosterone levels, as its measurement is laborious and expensive. It has been suggested that the concentration of free testosterone can be accurately estimated by calculation[15] but this too needs to be standardised, as there are a number of different algorithms that can be used and there is significant variability between

these algorithms.[22] In the Caerphillly and MMAS studies the mean testosterone levels were similar to the levels in LASA taking into account the younger patient populations in those studies. In fact, the median total testosterone level of the Rancho Bernardo cohort (10.4 pmol/L) has been regarded by many as a cut-off for "normal" value in many studies.[23,24]

Two hypothesis regarding the association between low levels of testosterone and mortality can be proposed (4): 1) low testosterone levels cause or worsen disease and therefore cause death, or 2) a disease causes testosterone levels to decline and are therefore associated with death. In our study we investigated the first hypothesis by adjusting for chronic diseases. As the results did not markedly change when adjusting for chronic diseases, we conclude that chronic diseases in our study are not mediating the association between testosterone and mortality. We tested the second hypothesis by excluding men with certain chronic diseases from the analyses. This had no major impact on the association between testosterone levels and mortality.

With regards to cardiovascular mortality in particular, our study showed no association with testosterone levels. This is in keeping with several other prospective studies including the recent Swedish study. [9,10,12] The Rancho Bernardo study and a more recent nested case-control study[25] did however find an association between testosterone levels and cardiovascular mortality.

There are some limitations to our study. Testosterone was only measured once amongst our cohort. The sample was obtained between 0800h and 1100h to minimise variation. However, studies have shown that there is little benefit in repeating measurements[26] and diurnal variation is believed to be even less pronounced in older men.[27] No further testosterone measurements were taken subsequent to 1995/1996 making longitudinal analyses of testosterone data not possible. Our study population is from a largely Caucasian background and our findings may not apply to other ethnic communities. Unfortunately, we had no data on patients with prostate cancer or those who were undergoing testosterone deprivation or replacement therapy; however inclusion of these people would likely overestimate the associations with mortality rather than underestimate it.

In summary, our study showed a clear association between total and free testosterone levels and age but not with mortality. This was true regardless of adjustment for confounders including prevalent diseases. Further longitudinal studies are required to clarify if a link between testosterone and mortality does exist. Perhaps multiple rather than a single dysregulation of hormones should be investigated as a marker of poor health in older men. Future studies need to address this issue.

References

1. Laughlin GA, Barrett-Connor E, Bergstrom J. 2008 Low Testosterone and Mortality in Older men. The Rancho Bernardo Study. J Clin Endocrinol Metab. 93: 63-75.
2. Harman SM, Metter EJ, Tobin JD, Pearson J, Blackman MR. 2001 Longitudinal Effects of Aging on Serum Total and Free Testosterone Levels in Healthy Men Baltimore Longitudinal Study of Aging. J Clin Endocrinol Metab. 86: 724-731.
3. Andersson AM, Jensen TK, Juul A, Petersen JH, Jorgenson T, Skakkebaek NE. 2007. Secular Decline in Male Testosterone and Sex Hormone Binding Globulin Serum Levels in Danish Population Surveys. J Clin Endocrinol Metab. 92(12): 4696-4705.
4. Wu FCW, Tajar A, Pye SR, Finn JD, O'Neill TW, Bartfai G, Casanueva F, Forti G, Giwercman A, Huhtaniemi IT, Kula K, Punab M, Boonen S, Vanderschueren D. 2008. Hypothalamic-Pituitary-Testicular Disruptions in Older Men are Differentially Linked to Age and Modifiable Risk Factors: The European Male Aging Study. J Clin Endocrinol Metab. 93 (7): 2737-2745.
5. Travison TG, Araujo AB, Kupelian V, O'Donnell AB, McKinlay JB. 2007. The Relative Contributions of Aging, Health and Lifestyle Factors to Serum Testosterone Decline in Men. The Massachusetts Male Aging Study. J Clin Endocrinol Metab. 92(2): 549-555.
6. Muller M, den Tonkelaar I, Thijssen JHH, Grobbee DE, van der Schouw YT. 2003. Endogenous sex hormones in men aged 40-80 years. Eur J Endocrinol. 149: 583-589.
7. Araujo AB, Esche GR, Kupelian V, O'Donnell AB, Travison TG, Williams RE, Clark RV, McKinlay JB. 2007. Prevalence of Symptomatic Androgen Deficiency in Men. J Clin Endocrinol Metab. 92 (11): 4241-4247.
8. Matsumoto AM. 2002. Andropause: Clinical Implications of the Decline in Serum Testosterone Levels with Aging in Men. J Gerontol A Biol Sci Med Sci. 57A (2): M76-M99.
9. Smith GD, Ben-Shlomo Y, Beswick A, Yarnell J, Lightman S, Elwood P. 2005. Cortisol, Testosterone and Coronary Heart Disease. Prospectice Evidence From the Caerphilly Study. Circulation. 112: 332-340.
10. Araujo AB, Kupelian V, Page ST, Handelsman DJ, Bremner WJ, McKinlay JB. 2007. Sex Steroids and All-Cause and Cause-Specific Mortality in Men. The Massachusetts Male Aging Study. Arch Intern Med 167: 1252-1260.
11. Tivesten A, Vandenput L, Labrie F, Karlsson MK, Ljunggren O, Mellstrom D, Ohlsson C. 2009. Low serum testosterone and estradiol predict mortality in elderly men. J Clin Endocrinol Metab. 94(7): 2482-2488.

12. Maggio M, Lauretani F, Ceda GP, Bandinelli S, Ling SM, Metter EJ, Artoni A, Cazzato A, Ceresini G, Guralnik JM, Basaria S, Valenti G, Ferrucci L. 2007. Relationship between low levels of anabolic hormones and 6 year mortality in older men. The InCHIANTI Study. Arch Intern Med. 167(20): 2249-2254.
13. Deeg DJH, Knipscheer CPM, van Tilburg W. 1993. Autonomy and Well Being in the Aging Population: Concepts and Design of the Longitudinal Aging Study Amsterdam. Netherlands Institute of Gerontology. Bunnick, the Netherlands.
14. Deeg DJ, van Tilburg T, Smit JH, de Leeuw ED. 2002. Attrition in the Longitudinal Aging Study Amsterdam: the effect of differential inclusion in side studies. Journal of Clin Epidemiol. 55: 319-328.
15. Vermeulen A, Verdonck L, Kaufman JM. 1999. A critical evaluation of simple methods for the estimation of free testosterone in serum. J Clin Endocrinol Metab.84: 3666-3672
16. Folstein MF, Folstein SE, McHugh PR. 1975. Mini-mental state: a practical method for grading the cognitive state of patients for a clinician. J Psychiatr Res. 12:189-198.
17. Stel VS, Smit JH, Pluijm SM, Visser M, Deeg DJ, Lips P. 2004 Comparison of the LASA physical activity questionnaire with a 7-day diary and pedometer. J Clin Epidemiol. 57:252-258.
18. Shores MM, Moceri VM, Gruenewald DA, Brodkin KI, Matsumoto AM, Kivlahan DR. 2004. Low Testosterone Is Associated with Decreased Function and Increased Mortality Risk: A Preliminary Study of Men in a Geriatric Rehabilitation Unit. J Am Geriatr Soc. 52:2077-2081.
19. Shores MM, Matsumoto AM, Sloan KL, Kivlahan DR. 2006. Low Serum Testosterone and Mortality in Male Veterans. Arch Intern Med. 166:1660-1665.
20. Snyder PJ. 2008. Might Testosterone Actually Reduce Mortality? J Clin Endocrinol Metab. 93(1): 32-33.
21. van Uytfanghe K, Stockl D, Kaufman JM, Fiers T, De Leenheer A, Thienpont LM. 2005. Validation of 5 routine assays for serum free testosterone with a candidate reference measurement procedure based on ultrafiltration and isotope dilution-gas chromatography-mass spectrometry. Clin Biochem, 38(3): 253-261.
22. de Ronde W, van der Schouw YT, Pols HAP, Gooren LJG, Muller M, Grobbee DE, de Jong FH. 2006. Calculation of Bioavailable and Free Testosterone in Men: A Comparison of 5 Published Algorithms. Clin Chem, 52(9): 1777-1784.
23. Marberger M, Roehrborn CG, Marks LS, Wilson T, Rittmaster RS. 2006. Relationship among Serum Testosterone, Sexual Function, and Response to Treatment in Men Receiving Dutasteride for Benign Prostatic Hyperplasia. J Clin Endocrinol Metab 91(4): 1323-1328.

24. Gould DC, Petty R, Jacobs HS. 2000. For and against the male menopause – does it exist? Br Med J 320: 858-861.
25. Khaw KT, Dowsett M, Folkerd E, Bingham S, Wareham N, Luben R, Welch A, Day N. 2007. Endogenous testosterone and mortality due to all causes, cardiovascular disease and cancer in men. The EPIC-Norfolk Study. Circulation. 116:2694-2701.
26. Brambilla DJ, McKinlay SM, McKinlay JB, Weiss SR, Johannes CB, Crawford SL, Longcope C. 1996. Does Collecting Repeated Samples from Each Subject Improve the Precision of Estimated Steroid Hormone Levels? J Clin Epidemiol. 49(3):345-350.
27. Plymate SR, Tenover JS, Bremner WJ. 1989. Circadian Variation in Testosterone, Sex Hormone-Binding Globulin, and calculated non-Sex Hormone-Binding Globulin Bound Testosterone in Healthy Young and Elderly Men, J Androl. 10(5): 366-371.