Testosterone and frailty in elderly men

Mariëlle Emmelot-Vonk

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TESTOSTERONE AND FRAILTY IN ELDERLY MEN

Testosteron en frailty bij oudere mannen
(met een samenvatting in het Nederlands)

Proefschrift

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Chapter 1
General introduction

Currently 15-20% of the population in the Netherlands is aged more than 65 years, and the most rapidly growing segment of our population are those aged more than 85 years. A substantial proportion of these elderly people can be said to be frail. Frailty is "*a geriatric syndrome of decreased reserve and resistance to stressors, resulting from cumulative declines across multiple physiologic systems, causing vulnerability to adverse health outcomes including falls and fractures, disabilities, hospitalization, institutionalization and mortality*".[1] The incidence of frailty increases with age and will become more common as our population continues to grow old. 3% to 7% of persons aged over 65 years are frail; this percentage increases to 26% for persons older than 80 years and to 32% for those older than 90 years.[2-4]

Two physical changes associated with aging appear to be the major drivers of frailty, namely, loss of muscle mass (sarcopenia) and a reduction in bone mineral density.[4-6] Besides sarcopenia and a decrease of bone mineral density, also deterioration in other organs and systems, like the cardiovascular system, brain, immune system, metabolism and the hormonal system, contributes to frailty. [4,7-9]

It is generally considered that frailty, unlike the physiologic aging process, is in part reversible and amenable to interventions.[6] Therefore, it is important to expand our understanding of the mechanisms that play a role in the pathophysiology of sarcopenia and bone mineral density, so we can develop effective measures to prevent frailty in future.

The last decades more attention has been drawn to the potential importance of androgens in etiology, prevention and treatment of frailty. With aging there is a slow, but progressive decline in testosterone levels in men. The prevalence of men with low testosterone levels (e.g., total testosterone levels below 11.3 nmol/l) increases from 12% of men aged 50 years till up to 50% in men older than 80 years.[10]

Research has suggested that age-related androgen reduction plays a distinct role in the development of several aspects of frailty and aging.[8,11] The age-related decline in testosterone is associated with changes in body composition, like a decline in muscle mass and the development of visceral obesity.[12] The decline in muscle mass leads to a decline of muscle strength, while the pattern of fat distribution has important implications with regard to the risk of metabolic diseases and the development of cardiovascular disease. Low testosterone levels are associated with a higher incidence of the metabolic syndrome and carotid atherosclerosis, while higher testosterone levels seem to protect against

cardiovascular disease.[13-15] Besides lower muscle strength, there is also a decline of bone mineral density, as a result of which there is a higher risk of falls and fractures.[12] Lower testosterone levels are also associated with poorer cognitive function.[16] Because androgens are associated with muscle strength and with cognitive functioning, it is reasonable to expect that circulating androgen levels affect activities of daily living (ADL) as well. Although this evidence suggests that the age-related decline of testosterone is an important factor associated with several aspects of frailty, the causal role of declining testosterone levels with aging on these aspects has not been proven nor is it known to what extent reversal of symptoms is possible with androgen substitution.
The exact mechanism by which androgen levels affect muscle mass, bone density and, finally, frailty, is still not well understood. It has been hypothesized that androgens can act directly as an independent risk factor or indirectly via other risk factors, such as by promoting insulin resistance, obesity and chronic low-grade inflammation. Results from studies are conflicting, due to differences in study population, study design and small sample sizes.

The aim of this thesis is

1. to obtain more insight in the relation between levels of circulating androgens at one site and sarcopenia, bone mineral density and frailty at the other site, in men.

2. to assess the effects of testosterone supplementation on muscle mass and strength, bone mineral density and other aspects of aging/frailty in elderly men with low normal testosterone levels.

Outline of this thesis

In *chapter 2* the relation between endogenous sex hormone levels and muscle strength and bone mineral density in men are described. First, the associations between sex hormone levels, glucose metabolism, inflammation and muscle strength are investigated (*chapter 2.1*). Second, the relations between sex hormone levels, genetic variation and bone mineral density are presented (*chapter 2.2*).

In *chapter 3* the design and baseline characteristics of a double-blind, randomized, placebo-controlled testosterone supplementation study in elderly men with low normal testosterone levels will be described.

In *chapter 4* the results of this testosterone supplementation study are presented. In *chapter 4.1* the associations between testosterone concentrations and symptoms of testosterone deficiency are studied. Moreover, the effects of testosterone supplementation on the symptoms of testosterone deficiency are described. The effects of testosterone supplementation on functional mobility, body composition, lipids, bone mineral density, cognition and safety are shown in *chapter 4.2* and the effects on sexual function are presented in *chapter 4.3*.

In *chapter 5* the results of this thesis are discussed with a specific focus on the mechanism by which androgen levels affect muscle strength and bone density, and some recommendations are made for future research. A summary completes this thesis.

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Chapter 2.1
Determinants of muscle strength and physical performance in men

Abstract

Objective To examine the association of sex hormone levels, glucose metabolism, chronic low-grade inflammation and obesity with muscle strength and physical performance.
Design Cross-sectional study in 400 independently living men aged between 40 and 80 years.
Methods Total testosterone (TT), SHBG, estradiol and dehydroepiandrosterone-sulfate (DHEAS), high sensitive (hs) CRP, insulin and glucose were measured. Bio-available (BT) and free (FT) testosterone and insulin sensitivity were calculated. Isometric grip strength, leg extensor strength and physical performance score were measured. Fat mass and lean body mass were assessed with dual energy X-ray absorptiometry. Visceral fat was measured with ultrasonography. Linear regression analysis was used to asses associations.
Results In multivariate analyses, sex hormone levels were not associated with muscle strength and physical performance. Higher levels of insulin, glucose and hs-CRP, and fat mass were all associated with reduced muscle strength and physical performance.
Conclusion Glucose metabolism, chronic low-grade inflammation en obesity all play a role in the age-related decline of muscle strength and physical performance in men. Circulating testosterone levels have no direct effect on muscle strength and physical performance.

Introduction

In men, aging is associated with a decrease of muscle mass and muscle strength, known as sarcopenia. Muscle strength is a key factor in maintaining independence in the elderly. Decreased muscle strength is a risk factor for falls and fractures, frailty and functional limitations.[1,2] Moreover, reduced muscle strength confers greater risk of mortality.[3-5]

It is generally considered that the loss of muscle mass and strength is in part reversible and amenable to interventions. It is important to expand our understanding of the mechanisms that play a role in the pathophysiology of sarcopenia, to be able to develop effective preventive measures. Various biological mechanisms can be examined to explain the relation of loss of muscle strength with aging.

First, there are changes in sex hormone levels with aging in men. There is a gradual decline in serum levels of total testosterone (TT). Because of a concomitant rise in sex hormone binding globuline (SHBG) levels with aging, the concentrations of bio-available (BT) and free (FT) testosterone decline even more. Also, there is a decline in concentrations of the adrenal androgen dehydroepiandroesterone-sulfate (DHEAS)[6], while there is no age-related decline in estradiol concentrations. Sex hormones have well known anabolic effects on muscle tissue.

Secondly, aging is associated with changes in glucose metabolism. Age-related insulin resistance has been shown to contribute to impaired glucose handling in elderly persons.[7] Glucose is an important determinant of muscle function, as glucose uptake is necessary for adequate muscle contraction.

Third, aging is associated with an elevation in pro-inflammatory cytokines, which play a central role in the hepatic production of C-reactive protein (CRP) and other acute-phase proteins involved in the inflammatory response.[8] Inflammatory biomarkers may contribute to loss of muscle function via direct and indirect effects on muscle catabolism.[9]

Finally, normal aging is associated with a progressive increase in fat mass.[10] Body fat distribution also changes with age; with visceral abdominal fat increase and subcutaneous abdominal fat decrease.[1112] This can lead to loss of muscle strength through infiltration of fat within and around muscle.[13] Moreover, obese persons tend to be less physically active and this may further contribute to decreased muscle strength.[14]

Cross-sectional studies have suggested that there are associations between these four variables (sex hormones, glucose metabolism, inflammation and obesity) and muscle

strength, but the associations are not consistent and the associations are less clear for physical performance. We set out to examine the associations of sex hormone levels, glucose metabolism, chronic low-grade inflammation and obesity with muscle strength and physical performance in a large, population-based random sample of men.

Subjects and Methods

Study procedure

The study is a cross-sectional study in 400 independently living men aged 40 to 80 years. Details of the study design, recruitment, and procedures have been published.[15,16] In brief, participants filled in questionnaires on lifestyle and dietary intake, visited the study center twice for physical examinations and fasting blood samples were collected. All participants gave written informed consent before enrollment in the study and the institutional review board of the University Medical Center Utrecht approved the study. Data collection took place between March 2001 and April 2002.

Procedure

During the visit medical histories were obtained, including a self-reported physician diagnosis of stroke, coronary heart disease, or diabetes mellitus. Participants were asked about current use of medications; these reports were checked by examining labels of drugs brought to the clinic. Furthermore, smoking history and alcohol consumption were assessed. One pack-year was taken to be equivalent to the consumption of 20 cigarettes per day for 1 year. Peripheral blood pressure was measured twice at the right brachial artery with a semi-automated device (Dynamap, GE HealthCare, Tampa, FL). The average of the two measurements of systolic and diastolic blood pressure was used for analysis and further calculation. Physical activity was assessed using the Voorrips-questionnaire, a questionnaire validated in an elderly population. Low scores represent low physical activity.[17]

Muscle strength

Isometric grip strength (IGS) was measured using an adjustable hand held dynamometer (JAMAR dynamometer) at the non-dominant hand. The subjects were seated with their shoulder adducted and neutrally rotated. The dynamometer was held freely, without support. The elbow was flexed at 90°, and care was taken that it did not touch the trunk. The forearm was in a neutral position, and the wrist was held at between 0° and 30° dorsi-flexion and between 0° and 15° ulnar deviation. The subjects were told to put maximal force on the dynamometer. The maximal value of three trials was noted in kilogram.
Leg or knee extensor strength (LES) was measured using the Hoggan MicroFET hand-held-dynamometer. The measurement required that the participant in a seated position with folded arms gives a maximal push to a hand-held dynamometer with successively a stretched right or left leg. The seat had no arms and a low back. The forces generated in the leg were contained between the buttocks and the sole of the foot. The maximal value of three trials for the right or left leg, whichever is the largest, was noted in Newton.

Physical performance

The physical performance score was assessed as described by Guralnik et al[18], including measurements of standing balance, walking speed, and ability to rise from a chair. Three tests for standing balance were considered in hierarchical difficulty in assigning a single score of 0-4 for standing balance. For the 8-ft (2.4m) walk and five times repeated chair stand, those who could not complete the task were assigned a score of 0. Those completing the task were assigned scores of 1-4, corresponding to the quartiles of time needed to complete the task, with the fastest times scoring as 4. Summing the category scores for the three scales created the summary physical performance score (0-12).

Body composition

Height and weight were measured in standing position without shoes. Body Mass Index (BMI) was calculated as the weight in kilograms divided by the square of height in meters. Total body composition measurements were performed with dual energy X-ray absorptiometry (DEXA) using a Hologic QDR1000 (Hologic Europe, Zaventem, Belgium). Calculations were made regarding fat-mass and lean body mass. Quality assurance, including calibration was performed routinely every morning for DEXA, using the standard provided by the manufacturer.

Ultrasonography of visceral fat was performed in all subjects with an HDI 3000 (Philips Medical Systems, Eindhoven, The Netherlands) using a C 4-2 transducer. For all images the transducer was placed on a straight line drawn between the left and right midpoint of lower rib and iliac crest. Measurements were made at the end of quiet expiration, applying minimal pressure without displacement of intra-abdominal contents as observed by ultrasound image. Visceral fat comprises the distance between peritoneum and lumbar spine. We measured distances from three different angles (medial, left and right). The mean of the three different measurements in cm was used for analyses.

Laboratory measurements

Fasting venous blood samples were obtained. Platelet free serum was obtained by centrifugation and was immediately stored in -20°C. An automatic enzymatic procedure was used to determine serum total cholesterol (Synchron LX Systems; Beckman Coulter). Glucose levels were assessed using a GlucoTouch reflectometer (LifeScan, Inc., Benelux), a reagent-strip glucose oxidase method. Venous whole blood was immediately applied to the test strip. Fasting plasma insulin levels were measured using commercially available assays. To assess insulin sensitivity in the subjects, we calculated the quantitative insulin sensitivity check index (QUICKI). QUICKI was calculated using: QUICKI = 1/[log (fasting insulin (mU/l)) + log (fasting glucose (mg/dl))]. We used QUICKI to measure insulin sensitivity instead of the hyperinsulinemic euglycemic clamp, which is the gold standard for measuring insulin resistance. However, this measurements has been validated and proved to be strongly correlated with insulin resistance measured by clamp (correlation coefficients of 0.81). High sensitivity C-reactive protein (hs-CRP) was measured using a Behring Nefelmeter II (Dade Behring, Liederbach, Germany). The lower limit of detection was 0.175 mg/l and the inter-assay variation was 2.4%. Hs-CRP levels > 10 mg/l can be taken as evidence of active inflammation processes (e.g. infection, trauma), therefore subjects with hs-CRP>10 mg/l were excluded (n=17 (4.2%)).

Hormone measurements

Levels of steroid were measured in serum. Total testosterone was measured after diethylether extraction using an in house competitive radioimmunoassay employing a polyclonal antitestosteron-antibody (Dr Pratt AZG 3290). [1α, 2α-^{3}H]-Testosteron (DuPont,

Nederland B.V.) was used as a tracer following chromatographic vertification of its purity. The lower limit of detection was 0.24 nmol/L and inter-assay variation was 6.0; 5.4 and 8.6% at 2.1; 5.6 and 23 nmol/L respectively (n=85). Sex Hormone Binding Globuline (SHBG) was measured using an immunometric technique (Diagnostic Products Corporation, Los Angeles, USA). The lower limit of detection was 5 nmol/L and inter-assay variation was 6.1; 4.9 and 6.9% at 11.6; 36 and 93 nmol/L respectively (n=30). DHEAS was measured using an immunometric technique on an Advantage Chemiluminescense System (Nichols Institute Diagnostics, San Juan Capistrano, USA). The lower limit of detection was 0.1 μmol/L and inter-assay variation was 5.2; 5.6 and 4.2% at 1.0; 4.9 and 14.2 μmol/L respectively (n = 19). Free and Bio-available testosterone were calculated from SHBG and total testosterone using the method of Vermeulen et al.[19] Total estradiol (E2) was measured after diethylether extraction and Sephadex chromatography using an in house competitive radioimmunoassay employing a polyclonal anti-estradiol-antibody (Dr F de Jong, Erasmus MC, Rotterdam, The Netherlands). [2,4,6,7-^{3}H]-Estradiol (Amersham, Nederland B.V.) was used as a tracer following chromatographic verification of its purity. The lower limit of detection was 20 pmol/L (2 mL sample) and inter-assay variation was 10.0 and 3.1% at 81 and 660 pmol/L respectively (n=24, resp. 17).

Data analysis

Distributions of anthropometric measures, lifestyle characteristics, and sex hormone levels were expressed as mean and standard deviation for normally distributed continuous variables and frequency and percentage for categorical variables.

Because the distribution of hs-CRP levels was highly skewed, hs-CRP levels were transformed by calculating the natural logarithm.

Relations between variables were assessed using linear regression for continuous variables and described as the linear regression coefficient (β) and its standard error (SE). Multiple regression analyses were used to adjust for age, smoking, alcohol, physical activity and total lean body mass, as a marker of muscle mass.

The level of statistical significance was set at $P < 0.05$ in all analyses. Analyses were performed with the SPSS statistical software package, version 11.0 (SPSS Inc, Chicago, Ill)

Results

The characteristics of the participants are shown in *table 1*. The mean age of the participants was 60.2 years (SD 11.3). Only 8% of the participants had the maximum score of 12 points on the physical performance score. (data not shown)

Table 1 *Characteristics of the participants*

	Mean (SD) N=400
Age (years)	60.2 (11.3)
Body mass index (kg/m2)	26.3 (3.5)
Smoking (packyears)	20.6 (16.6)
Alcohol consumption (units/week)	12.9 (13.3)
Physical Activity (Voorrips score)	18.1 (7.5)
Hormone levels:	
- Total Testosterone (nmol/L)	18.5 (5.3)
- Sex hormone binding globuline (nmol/L)	40.6 (14.5)
- Free testosterone (pmol/L)	354.2 (98.1)
- Bioavailable testosterone (nmol/L)	8.1 (2.3)
- Dehydroepiandrosterone-sulfate (µmol/L)	6.7 (3.3)
- Estradiol (pmol/L)	91.3 (22.8)
Muscle strength:	
- Isometric grip strength (kg)	43.3 (26.3)
- Leg extensor strength (Newton)	393.8 (79.5)
Physical performance score (0-12)	9.0 (1.8)
- standing balance score (0-4)	3.8 (0.6)
- walking speed score (0-4)	2.7 (0.8)
- chair stand score (0-4)	2.5 (1.1)
Glucose Metabolism:	
- glucose (mmol/L)	6.0 (1.5)
- insulin (mIU/L)	8.4 (5.9)
- insulin sensitivity (QUICKI)	0.35 (0.04)
Chronic Inflammation	
- hs-CRP (mg/L)	3.2 (8.3)
Body Composition:	
- total fat mass (kg)	17.1 (5.5)
- total lean mass (kg)	61.6 (7.3)
- total body mass (kg)	81.4 (11.7)
- percentage total body fat (%)	20.6 (4.2)
- visceral fat (cm)	7.5 (2.2)
	Number (%)
Presence of chronic disease	194 (48.5)
Presence of diabetes	21 (5.3)
Presence of cardiovascular disease	68 (17)

Although in univariate analyses higher levels of free and bio-available testosterone and lower levels of SHBG were associated with larger muscle strength and better physical performance, after correction for age, smoking, alcohol, lean body mass and physical

activity, there were no statistically significant associations anymore between sex hormone levels and muscle strength and physical performance (*Table 2*).

Table 2 *The association between muscle strength and physical performance, and hormone levels.* Multivariate analysis adjusted for age (years), smoking (packyears), alcohol (units/week), total lean mass (kg) and physical activity (voorrips score).

	Isometric grip strength (kg)		Leg extensor strength (N)		Physical performance Score		Standing balance		Walking speed		Chair stand	
	β (SE)	P-value	β (SE)	P-value	β (SE)	P-value	β (SE)	P-value	β (SE)	P-value	β (SE)	P-value
Total testosterone (nmol/L)												
Crude	0.13 (0.1)	0.12	0.50 (0.8)	0.52	0.04(0.0)	0.004	0.01 (0.0)	0.37	0.01 (0.0)	0.07	0.03 (0.0)	0.02
Adjusted	0.06 (0.1)	0.40	-0.09 (0.2)	0.90	0.01 (0.0)	0.39	0.00 (0.0)	0.92	0.00 (0.0)	0.72	0.01 (0.0)	0.42
SHBG (nmol/L)												
Crude	-0.11 (0.0)	<0.001	-1.49 (0.3)	<0.001	-0.02 (0.0)	0.02	-0.00 (0.0)	0.21	-0.00 (0.0)	0.49	-0.01(0.0)	0.004
Adjusted	0.02 (0.0)	0.47	-0.46 (0.3)	0.09	0.00 (0.0)	0.94	0.00 (0.0)	0.42	0.00 (0.0)	0.86	-0.00 (0.0)	0.52
Free testosterone (pmol/L)												
Crude	0.02 (0.0)	<0.001	0.22 (0.0)	<0.001	0.01 (0.3)	<0.001	0.001 (0.0)	0.04	0.001 (0.0)	0.01	0.003 (0.0)	<0.001
Adjusted	0.00 (0.0)	0.71	0.05 (0.0)	0.23	0.00 (0.0)	0.30	0.00 (0.0)	0.40	0.00 (0.0)	0.81	0.00 (0.0)	0.13
Bio-available testosterone (nmol/L)												
Crude	0.96 (0.2)	<0.001	9.62 (1.7)	<0.001	0.22 (0.0)	<0.001	0.03 (0.0)	0.04	0.05 (0.0)	0.01	0.13 (0.0)	<0.001
Adjusted	0.07 (0.2)	0.71	2.10 (1.8)	0.23	0.04 (0.0)	0.30	-0.01 (0.0)	0.40	0.01 (0.0)	0.81	0.04 (0.0)	0.13
DHEAS (µmol/L)												
Crude	0.64 (0.1)	<0.001	6.52 (1.2)	<0.001	0.09 (0.0)	<0.001	0.04 (0.0)	<0.001	0.01 (0.0)	0.38	0.05 (0.0)	0.005
Adjusted	-0.01 (0.1)	0.95	1.05 (1.2)	0.40	-0.05 (0.0)	0.10	0.01 (0.0)	0.40	-0.02 (0.0)	0.14	-0.03 (0.0)	0.10
Estradiol (pmol/L)												
Crude	0.02 (0.0)	0.34	0.18 (0.2)	0.31	-0.00 (0.0)	0.28	-0.00 (0.0)	0.09	-0.00 (0.0)	0.13	0.00 (0.0)	0.85
Adjusted	-0.01 (0.0)	0.75	-0.02 (0.2)	0.91	-0.00 (0.0)	0.85	-0.00 (0.0)	0.09	-0.00 (0.0)	0.07	0.00 (0.0)	0.35

Abbreviations: β, linear regression coefficient; SE, standard error

Table 3 shows that after correction for age, smoking, alcohol, lean body mass and physical activity, higher glucose and insulin concentrations were associated with a reduced muscle strength (isometric grip strength β -0.83; p=0.001 and β -0.31; p<0.001, respectively; and leg extensor strength β -1.45, p=0.02 for insulin, while for glucose the association was not statistically significant). Higher glucose and insulin concentrations were also associated with lower physical performance scores (β -0.12; p=0.04 and β -0.04; p=0.01, respectively). A higher insulin sensitivity is associated with an increase in isometric grip

strength (β 42.37; p<0.001) and a higher physical performance score (β 5.46; p=0.03). Increased levels of natural log hs-CRP are associated with a decrease in muscle strength (isometric grip strength β -1.74; p=0.045), but not with physical performance. A higher fat mass was associated with a decrease in muscle strength and a lower physical performance score (isometric grip strength ß -0.00; p<0.001, physical performance score ß -0.00; p=0.04). Also, more visceral fat was associated with a decrease in muscle strength (isometric grip strength ß -0.73; p<0.001).

Table 3 *The association between muscle strength and physical performance, and glucose metabolism and chronic inflammation markers. Variables are adjusted for age (years), smoking (packyears), alcohol (units/week), total lean mass (kg) and physical activity (Voorrips score).*

	Isometric grip strength		Leg extensor strength		Physical performance Score		Standing balance		Walking speed		Chair stand	
	β (SE)	P-value	β (SE)	P-value	β (SE)	P-value	β (SE)	P-value	β (SE)	P-value	β (SE)	P-value
Glucose (mmol/L)	-0.83 (0.2)	0.001	1.89 (2.5)	0.45	-0.12 (0.1)	0.04	-0.01 (0.0)	0.80	-0.08 (0.0)	0.01	-0.04 (0.0)	0.29
Insulin (mIU/L)	-0.31 (0.1)	<0.001	-1.45 (0.6)	0.02	-0.04 (0.0)	0.01	-0.01 (0.0)	0.02	-0.01 (0.28)	0.28	-0.02 (0.0)	0.07
Insulin sensitivity (quicki)	42.37 (10.4)	<0.001	85.74 (105.4)	0.42	5.46 (2.5)	0.03	1.64 (0.9)	0.055	2.17 (1.3)	0.09	1.75 (1.7)	0.29
Log hs-crp	-1.74 (0.9)	0.045	4.59 (8.7)	0.60	-0.28 (0.2)	0.17	-0.05 (0.1)	0.49	-0.12 (0.1)	0.24	-0.06 (0.1)	0.68
Fat mass (kg)	-0.00 (0.0)	<0.001	0.00 (0.0)	0.84	-0.00 (0.0)	0.04	-0.00 (0.0)	0.16	-0.00 (0.0)	0.13	-0.00 (0.0)	0.35
Visceral fat (cm)	-0.73 (0.2)	<0.001	-1.34 (1.8)	0.47	-0.05 (0.0)	0.23	0.01 (0.0)	0.54	-0.04 (0.0)	0.09	-0.01 (0.0)	0.73

Abbreviations: β, linear regression coefficient; SE, standard error

Discussion

We found that sex hormone levels and SHBG were not related to muscle strength and physical function after correction for confounders. Higher levels of glucose, insulin and hs-CRP and excess fat mass and visceral fat were associated with reduced muscle strength and/or worse physical performance, while insulin sensitivity showed opposite associations. Most observational studies in men show that age-related declines in androgen levels affect muscle strength and physical performance in men.[20-24] However not all studies are in agreement. Some did not show an association between testosterone level and muscle strength or physical performance[25,26], comparable with our study, or reported a positive association only with hormone levels below certain threshold values.[27] Moreover, recent data from a longitudinal study show that lower testosterone levels are not associated with a 3-year decline in muscle strength or physical performance.[28] Few studies have examined

the effect of estradiol on muscle strength and physical function in men, but consistent with our study, the available data show no association between estradiol level and muscle strength or physical performance in men.[21-24]

The results of the few studies focusing on the relation between glucose metabolism and muscle strength are in line with our findings of higher levels of glucose and insulin being associated with less muscle strength and worse physical performance, in men with diabetes mellitus[29,30]and without diabetes mellitus[31] ,although one study did not confirm this[32.]

Recent data from a longitudinal study show that type 2 diabetes mellitus is associated with accelerated loss of muscle strength.[33]

Several previous studies suggested the presence of a negative effect of chronic low-grade inflammation, measured with CRP, interleukin-6 and tumor necrosis factor-alpha, on muscle strength[34-37,] comparable with our study. The association is consistent across multiple comorbidities.[38] Longitudinal studies have confirmed that higher levels of inflammation levels are associated with a loss of muscle strength loss[39], although not all longitudinal studies could confirm this effect.[40]

Lower muscle strength is also associated with a higher fat mass and obesity (sarcopenic obesity), in this study and in other studies.[4142,43] Longitudinal studies have confirmed this association.[44] Less is known about the relation between fat mass and physical performance. In our study, we found an inverse association between fat mass and physical performance.

Based on the recent literature, there is a growing evidence that insulin resistance, inflammation and obesity are not only related to muscle strength and physical performance, but also are strongly related to each other. Cross-sectional studies have shown that pro-inflammatory cytokines are positively associated with fat mass, obesity and insulin resistance[35,45-49] In adipose tissue, either adipocytes directly or infiltrating macrophages produce pro-inflammatory cytokines, such as interleukin-6 and tumor necrosis factor-α and adipokines, such as leptin and adiponectin, that up-regulate the inflammatory response[50-52],which in turn may contribute to a decline of muscle mass and strength.[35] So, increases in fat mass, particularly visceral fat, may lead to increased secretion of a number of pro-inflammatory cytokine.[5354] A growing body of evidence has shown that an increase of cytokines contribute to insulin resistance.[545556] As we all well know, obesity is also associated with insulin resistance. Some studies in animals and humans have found that inflammatory molecules mediate obesity-related insulin resistance through a cross-talk

between cytokine receptors and insulin receptor signaling pathways.[45,57] Unfortunately, the cross-sectional design of our study does not allow a differentiation between cause and effect. Most of the factors are statistically related, but the design does not allow to decide on the direction of the associations. Therefore, no definitive conclusions can be drawn concerning the physiological pathway(s).

Besides the cross-sectional design, this study has another limitation. Single measurements of hormones levels may not reflect the average level of that hormone over an extended period of time. These limitations must be balanced against the strengths of this study. One of the major advantages of this study is that we were able to measure overall physical performance and activity instead of only muscle strength. A second major advantage of the study is that it was conducted in a population-based selection of a large number of men across a broad range of age.

In summary, glucose metabolism, chronic low-grade inflammation and obesity all play a role in the age-related decline of muscle strength and physical performance in men, while testosterone has no direct effect on muscle strength and physical performance.

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Chapter 2.2

Association between sex hormone levels, bone mineral density and gene polymorphisms in men

Abstract

Objective: There is growing evidence that estrogens are the most important sex hormones in the development and maintenance of the male skeleton. Genetic variation in the estrogen receptor genes (ER) and the aromatase gene has been suggested as determinants of bone mineral density (BMD), but results are inconsistent. We assessed the association between sex hormone levels, ERα, ERβ and Cyp19 aromatase polymorphisms and bone mass in men.

Design: Cross-sectional study including 400 independently living men between 40 and 80 years old.

Methods: Serum levels of total testosterone (TT), SHBG, estradiol (E_2) and dehydroepiandrosterone-sulfate (DHEAS) were measured. Bio-available (BT) and free (FT) testosterone were calculated. BMD was measured by dual energy x-ray absorptiometry. Two common variants in ERα gene (rs2234693 and rs9340799), one in ERβ gene (rs4986938), and one in CYP19 (aromatase) gene (rs10046) were genotyped. Multiple linear regression analysis was used to adjust for age, smoking, alcohol, physical activity and body mass index.

Results: Higher E_2 and DHEAS levels were associated with higher BMD, while there were no statistically significant associations between TT and BMD. Per allele of the CYP19 rs10046 SNP E_2 levels were 4.51 pmol/l lower (p=0.01). None of the ERα or β, and CYP19 variants were associated with BMD.

Conclusion: These results show that estradiol is more important for bone metabolism than testosterone. Genetic variation in CYP19 aromatase gene is associated with estradiol levels, but not with BMD.

Introduction

Unlike common perception, osteoporosis is not a problem confined to women only. Also in men it is a frequent problem and an important clinical issue. With advancing age, men lose bone mineral density (BMD) at a rate of up to 1% per year[1], which leads to an increased risk of fractures. One third of all fractures occur in men. Osteoporotic fractures cause significant morbidity and excess mortality.

Sex steroid hormones are important regulators of bone physiology in men and the fall in their circulating levels with aging represents a major factor in the pathogenesis of osteoporosis. There is growing evidence that estrogens (E_2) are the most important sex hormones in the development and maintenance of the male skeleton. Several studies have reported positive relations between serum E_2 concentrations and bone mineral density or bone turnover markers in men.[2,3] A small fraction of estradiol, the major circulating form of estrogen, is produced directly by the testis and the main source of estrogens is peripheral conversion of testosterone and adrenal sex steroids, like dehydroepiandrosterone-sulfate (DHEAS), by the aromatase enzyme. Estrogen exerts its effects on bone primarily via the estrogen receptors alpha (ERα or type 1) and beta (ERβ or type 2).[4]

Several genetic variants in the estrogen receptor gene and the aromatase gene have been described and associations of these polymorphisms with BMD have been reported, but results are inconsistent. Moreover, most studies focused on women and there are few studies focusing on men.

Therefore, the purpose of the present cross-sectional study of 400 independently living men between 40 and 80 years old is to assess the association of sex hormone levels and these gene polymorphisms with bone mass in men.

Subjects and Methods

Study population

The study is a cross-sectional study in 400 independently living men aged 40 to 80 years. Details of the study design, recruitment, and procedures have been published.[5,6] In brief, participants filled in questionnaires on lifestyle and dietary intake, visited the study center twice for physical examinations and fasting blood samples were collected. All participants gave written informed consent before enrollment in the study and the institutional review

board of the University Medical Center Utrecht approved the study. Data collection took place between March 2001 and April 2002.

Procedure

During the visits, smoking history and alcohol consumption were assessed. One pack-year was taken to be equivalent to the consumption of 20 cigarettes per day for 1 year. Height and weight were measured in standing position without shoes. Body Mass Index (BMI) was calculated as the weight in kilograms divided by the square of height in meters. Physical activity was assessed using the Voorrips questionnaire, a questionnaire validated in an elderly population. Low scores represent low physical activity.[7]

Bone mineral density

Bone mineral density (BMD) was measured with dual energy X-ray absorptiometry (DEXA) using a Hologic QDR1000 (Hologic Europe, Zaventem, Belgium). Scanning was performed according to the instructions of the manufacturer. BMD was measured of lumbar spine, pelvic and total body. Quality assurance, including calibration was performed routinely every morning for DEXA, using the standard provided by the manufacturer.

Hormone determinations

Fasting venous blood samples were obtained. Platelet free serum was obtained by centrifugation and was immediately stored in -20°C. Levels of steroid were measured in serum. Total testosterone was measured after diethylether extraction using an in house competitive radioimmunoassay employing a polyclonal antitestosteron-antibody (Dr Pratt AZG 3290). [1α, 2α-^{3}H]-Testosteron (DuPont, Nederland B.V.) was used as a tracer following chromatographic vertification of its purity. The lower limit of detection was 0.24 nmol/L and inter-assay variation was 6.0; 5.4 and 8.6% at 2.1; 5.6 and 23 nmol/L respectively (n=85). SHBG was measured using an immunometric technique (Diagnostic Products Corporation, Los Angeles, USA). The lower limit of detection was 5 nmol/L and inter-assay variation was 6.1; 4.9 and 6.9% at 11.6; 36 and 93 nmol/L respectively (n=30). DHEAS was measured using an immunometric technique on an Advantage Chemiluminescense System (Nichols Institute Diagnostics, San Juan Capistrano, USA). The lower limit of detection was 0.1 µmol/L and inter-assay variation was 5.2; 5.6 and

4.2% at 1.0; 4.9 and 14.2 µmol/L respectively (n = 19). Free and Bio-available testosterone was calculated from SHBG and total testosterone using the method of Vermeulen et al.[8] Total estradiol (E2) was measured after diethylether extraction and Sephadex chromatography using an in house competitive radioimmunoassay employing a polyclonal anti-estradiol-antibody (Dr F de Jong, Erasmus MC, Rotterdam, The Netherlands). [2,4,6,7-^{3}H]-Estradiol (Amersham, Nederland B.V.) was used as a tracer following chromatographic verification of its purity. The lower limit of detection was 20 pmol/L (2 mL sample) and inter-assay variation was 10.0 and 3.1% at 81 and 660 pmol/L respectively (n=24, resp. 17).

DNA analysis

All participants were genotyped for rs2234693 and rs9340799 in ERα, rs4986938 in ERb, and rs10046 in CYP19 using the TaqMan allelic discrimination assay (Applied Biosystems Inc [ABI], Nieuwerkerk aan den IJssel, the Netherlands). Primer and probe sequences were optimized using the single nucleotide polymorphism (SNP) assay-by-design service of ABI; details are available at http://store.appliedbiosystems.com. Reactions were performed with the TaqMan PRISM 7900HT Sequence Detection System (ABI), with 384 wells. It was not possible to genotype all the SNPs in all men. For 2 men DNA was not available. In the remaining 398 for rs2234693 27 were missing, for rs9340799 20 were missing, for rs4986938 31 were missing, and for rs10046 32 were missing. All four SNPs were in Hardy-Weinberg equilibrium (p-values > 0.13).

Data analysis

Distributions of anthropometric measures, lifestyle characteristics, and sex hormone levels were expressed as mean and standard deviation for normally distributed continuous variables and frequency and percentage for categorical variables.

Relations between variables were assessed using linear regression for continuous variables and described as the linear regression coefficient (β) and standard error (SE). Multiple regression analysis were used to adjust for age, smoking, alcohol, physical activity and BMI. To analyze the modifying effect of genetic variation on the association between hormone levels and BMD, interaction terms were included in the linear regression model including also the individual terms.

The level of statistical significance was set at $P < 0.05$ in all analyses. Analyses were performed with the SPSS statistical software package, version 15.0 (SPSS Inc, Chicago, Ill)

Results

The characteristics of the participants are shown in *table 1*. The mean age of the participants was 60.2 years (SD 11.3). Only 2% of the men had a medical history of osteoporosis, and 33.8% of the men were known to have had a fracture.

In multivariate analyses, after adjustment for age, smoking, alcohol, physical activity en BMI, higher levels of estradiol were associated with higher lumbar spine BMD (β 1.17 * 10^{-3} (SE 0.4 * 10^{-3}) per pmol/l; p=0.006) and pelvic BMD (β 0.78 * 10^{-3} (SE 0.2 * 10^{-3}) per pmol/l; p=0.001). Higher levels of DHEAS were associated with higher total body BMD (β 3.11 * 10^{-3} (SE 1.5 * 10^{-3}) per µmol/l; p=0.04). There were no statistically significant associations between testosterone levels and BMD (*table 2*).

Table 2 *The association between sex hormone levels and BMD. Difference in BMD per unit increase in hormone levels Variables are adjusted for age, smoking (packyears), alcohol (units/week), physical activity (Voorrips) and BMI (kg/m2)*

	BMD -total ß (SE)	p-value	BMD – lumbar spine ß (SE)	P-value	BMD - pelvic ß (SE)	P-value
Total testosterone (nmol/L)	$-0.27*10^{-3}$ (0.8)	0.75	$2.67*10^{-3}$ (2.0)	0.19	$1.57*10^{-3}$ (1.1)	0.16
Free testosterone (pmol/L)	$0.04*10^{-3}$ (0.0)	0.43	$0.18*10^{-3}$ (0.1)	0.14	$0.10*10^{-3}$ (0.1)	0.11
Bioavailable Testosterone (nmol/L)	$1.69*10^{-3}$ (2.1)	0.43	$7.62*10^{-3}$ (5.1)	0.14	$4.46*10^{-3}$ (2.8)	0.11
DHEAS (µmol/L)	$3.11*10^{-3}$ (1.5)	0.04	$2.43*10^{-3}$ (3.6)	0.50	$2.82*10^{-3}$ (2.0)	0.15
Estradiol (pmol/L)	$0.21*10^{-3}$ (0.2)	0.24	$1.17*10^{-3}$ (0.4)	0.006	$0.78*10^{-3}$ (0.2)	0.001
SHBG (nmol/L)	$-0.37*10^{-3}$ (0.3)	0.25	$0.37*10^{-3}$ (0.8)	0.63	$0.28*10^{-3}$ (0.4)	0.50

Abbreviations: β, linear regression coefficient; SE, standard error

Table 1 *Characteristics of the participants*

	Mean (SD) N=400
Age (years)	60.2 (11.3)
Body mass index (kg/m2)	26.3 (3.5)
Smoking (packyears)	20.6 (16.6)
Alcohol consumption (units/week)	12.9 (13.3)
Voorrips total score	18.1 (7.5)
Hormone levels:	
- Total Testosterone (nmol/l)	18.5 (5.3)
- SHBG (nmol/l)	40.6 (14.5)
- Free testosterone (pmol/l)	354.2 (98.1)
- Bioavailable testosterone (nmol/l)	8.1 (2.3)
- DHEAS (umol/l)	6.7 (3.3)
- Estradiol (pmol/l)	91.3 (22.8)
Bone mineral density (g/cm2) :	
- total body	1.14 (0.1)
- lumbar spine	1.02 (0.2)
- pelvis	0.98 (0.1)
	Number (%)
ERα rs2234693	
- homozygoot minor allele	152 (41)
- heterozygoot major allele	160 (43.1)
- homozygoot major allele	59 (14.7)
ERα rs9340799	
- homozygoot minor allele	96 (23.9)
- heterozygoot major allele	185 (46.1)
- homozygoot major allele	97 (24.2)
ERβ rs4986938	
- homozygoot minor allele	140 (34.9)
- heterozygoot major allele	183 (45.6)
- homozygoot major allele	44 (11.0)
CYP19 rs10046	
- homozygoot minor allele	93 (23.2)
- heterozygoot major allele	189 (47.1)
- homozygoot major allele	84 (20.9)
Presence of chronic disease	194 (48.5)
History of fractures	135 (33.8)
- fracture of hip	8 (2.0)
- fracture of wrist	32 (8.0)
- vertebral fracture	3 (0.8)
- fracture of leg (except hip)	25 (18.1)
- other fractures	98 (24.5)
Osteoporosis in medical history	8 (2.0)

None of the ERα or β, and CYP19 SNPs were associated with BMD. (*table 3*)

Table 3 *Association between gene polymorphisms and BMD. Difference in BMD per 1 allele of SNP. Variables are adjusted for age, smoking (packyears), alcohol (units/week), physical activity (Voorrips) and BMI (kg/m2)*

	BMD total body ß (SE)	p-value	BMD lumbar spine ß (SE)	P-value	BMD pelvic ß (SE)	P-value
ERα rs2234693	$8.38*10^{-3}$ (6.0)	0.16	$6.97*10^{-3}$ (14.4)	0.63	$12.57*10^{-3}$ (7.7)	0.11
ERα rs9340799	$-1.72*10^{-3}$ (5.9)	0.77	$15.53*10^{-3}$ (14.1)	0.27	$-0.84*10^{-3}$ (7.7)	0.91
ERβ rs4986938	$3.62*10^{-3}$ (6.6)	0.58	$6.36*10^{-3}$ (15.7)	0.69	$4.59*10^{-3}$ (8.5)	0.59
CYP19 rs10046	$-4.38*10^{-3}$ (6.2)	0.48	$-4.67*10^{-3}$ (14.9)	0.75	$-3.60*10^{-3}$ (8.1)	0.66

Abbreviations: β, linear regression coefficient; SE, standard error

Per additional allele of CYP19 rs10046 the estradiol concentration was -4.51 pmol/l (SE 1.7; p=0.01) lower. (*table 4*) There were no associations between any of the ERα or ERβ gene polymorphisms and sex hormone levels.

Table 4 *Association between gene polymorphisms and hormone levels. Difference in hormone level per allele. Variables are adjusted for age, smoking (packyears), alcohol (units/week), physical activity (Voorrips) and BMI (kg/m2)*

	Estradiol (pmol/L)		Total Testosterone (nmol/L)		SHBG (nmol/L)		Free Testosterone (pmol/L)		Bio-available Testosterone (nmol/L)		DHEAS (µmol/L)	
	ß (SE)	p-value	ß (SE)	P-value	ß (SE)	P-value	ß (SE)	P-value	ß (SE)	P-value	ß (SE)	P-value
ERα rs2234693	-0.64 (1.7)	0.70	-0.17 (0.4)	0.63	-0.60 (0.9)	0.53	0.09 (6.2)	0.99	0.00 (0.1)	0.99	0.06 (0.2)	0.78
ERα rs9340799	1.48 (1.6)	0.37	0.24 (0.3)	0.50	0.92 (0.9)	0.33	0.14 (6.1)	0.98	0.00 (0.1)	0.98	0.26 (0.2)	0.19
ERβ rs4986938	0.40 (1.8)	0.83	-0.56 (0.4)	0.15	-1.85 (1.0)	0.07	-3.29 (6.7)	0.63	-0.08 (0.2)	0.63	-0.16 (0.2)	0.46
CYP19 rs10046	-4.51 (1.7)	0.01	0.12 (0.4)	0.75	0.67 (1.0)	0.49	-1.45 (6.4)	0.82	-0.03 (0.1)	0.82	-0.06 (0.2)	0.78

Abbreviations: β, linear regression coefficient; SE, standard error

There was a suggestion that CYP19 rs10046 SNP modified the association between estradiol and BMD. For men homozygous for the CYP19 rs10046 minor allele , higher estradiol levels were associated with higher lumbar spine BMD (β $1.96 * 10^{-3}$ (SE $0.9 * 10^{-3}$) per pmol/l; p=0.03), and for men heterozygous for CYP19 rs10046 higher estradiol levels were associated with higher pelvic BMD (β $0.70 * 10^{-3}$ (SE $0.3 * 10^{-3}$) per pmol/l; p=0.004), however, p-values for interaction were far from significant (P-interaction=0.37,

and P-interaction=0.70, respectively) (*table 5*). ERα and ERβ SNPs did also not modify associations between any of the hormone levels and BMD (data not shown).

Table 5 *Associations between estradiol and BMD per CYP19 genotype. Associations were adjusted for age (years), smoking (packyears), alcohol (units/week), physical activity (Voorrips) and BMI (kg/m2)). Difference in BMD per unit increase in estradiol.*

A *CYP19 homozygoot minor allele (N=89)*

	Estradiol (pmol/L) ß (SE)	p-value
BMD total body	$0.25 *10^{-3}$ (0.4)	0.56
BMD lumbar spine	$1.96 *10^{-3}$ (0.9)	0.03
BMD pelvic	$0.77 *10^{-3}$ (0.6)	0.18

B *CYP19 heterozygoot major allele (N=181)*

	Estradiol (pmol/L) ß (SE)	p-value
BMD total body	$0.07 *10^{-3}$ (0.3)	0.81
BMD lumbar spine	$1.02*10^{-3}$ (0.7)	0.14
BMD pelvic	$0.70*10^{-3}$ (0.3)	0.03

C *CYP19 homozygoot major allele (N=81)*

	Estradiol (pmol/L) ß (SE)	p-value
BMD total body	$0.22 *10^{-3}$ (0.4)	0.60
BMD lumbar spine	$0.75 *10^{-3}$ (0.9)	0.41
BMD pelvic	$0.47 *10^{-3}$ (0.6)	0.41

Abbreviations: β, linear regression coefficient; SE, standard error

Discussion

The results of this study showed that in independently living men between 40 and 80 years of age higher E2 and DHEAS levels were associated with higher BMD at various sites , whereas testosterone was not associated with BMD. Although the CYP19 gene polymorphism was associated with lower levels of estradiol, there was no association between BMD and any of the SNPs we have tested. Furthermore, none of the SNPs modified associations between hormone levels and BMD.

These findings supports growing evidence on the important biological role of estradiol in stead of total testosterone in bone health of men. Case-reports of ER-negative[9] and aromatase deficient[10,11] men have shown that adequate estradiol is critical for bone development in young men, even when testosterone levels are adequate. In addition,

several epidemiological studies showed that in elderly males serum estradiol levels were correlated with BMD or bone turnover markers more strongly than testosterone concentrations, suggesting a key role of estrogen not only for skeletal maturation but also for the maintenance of bone mass.[3,12-14] This latter hypothesis has been clearly addressed by a recent intervention study[15], where estradiol- and testosterone supplements were given to elderly men with eliminated endogenous estradiol- and testosterone levels to investigate the relative contributions of those two hormones in bone metabolism. The results of that study showed that estradiol is the dominant sex hormone regulating bone resorption, and estradiol and testosterone are both important for the maintenance of bone formation.

Much less is known on low DHEAS levels in their contribution to the decrease of BMD with aging in men. DHEAS may have an effect on bone through the conversion of DHEA to active androgens and estrogens in peripheral tissues, enhancement of intrinsic growth factor (IGF)-1 bioactivity and regulation of bone-specific enzymatic activity.[16,17] Previous cross-sectional studies have shown correlations between serum DHEAS and markers of bone-turnover[18,19], and between serum DHEAS and BMD[20] in men, but the results are not consistent. However, some of the studies with negative results were too small to have adequate statistical power.[21] Our study did find a a higher BMD in men with higher DHEAS levels, even after adjustment for multiple potential confounding factors.

Family and twin studies have shown that heritability could explain up to 50-80% of the variation of BMD.[22,23] In view of the recognized role that estrogens play in bone metabolism in men, the CYP19 aromatase gene and the estrogen receptor gene represents two important candidates to be evaluated in an attempt to elucidate the genetic background of male osteoporosis. Several polymorphic regions have been detected in the human CYP19 aromatase gene and the estrogen receptor α and β genes.[24,25] Previous studies looking into the association between these SNPs and BMD have shown inconsistent results. Most studies focused on post-menopausal women, in whom associations have been reported several times.[26-28] The few studies focusing on men did typically not show an association [26,27,29-31], comparable with our study. However, some of the studies have shown an association between BMD and the estradiol receptor gene polymorphism[32-35] and the CYP19 aromatase gene polymorphism[36-39] The discrepancy between the studies may be due to limited sample sizes, differences between populations (race, age, ethnic or environmental differences) and differences in the polymorphisms that were studied.

It is not surprising that CYP19 aromatase gene polymorphism is associated with lower estradiol concentrations, because the aromatization of androgenic precursors into estrogens is responsible for the synthesis of virtually all estrogens in postmenopausal women and in men. It has been reported that the rs10046 SNP, a C/T single nucleotide polymorphism in the 3'-untranslated region (3'UTR) of CYP19 gene affects dispositions of estradiol and testosterone.[40] Also other studies have reported on this association in men.[36,41] The question is whether this gene polymorphism can play a role in the association between estradiol and BMD in men. Our data suggest that the association between estradiol and BMD is modified by the CYP19 aromatase gene polymorphism. This modification was, however, not statistically significant which could reflect that the number of men in our study was still too small to have enough statistical power to precisely estimate the presence of interaction. Even larger scale studies are necessary to give a definitive answer.

Our study has some limitations. First, like those of other cross-sectional studies, our findings of associations cannot be taken as definitive evidence of a causal relationship. Single measurements of hormones levels may not reflect the average level of that hormone over an extended period of time. Moreover, serum hormone levels may not reflect levels of hormones locally in the bone that promote bone growth. It has been suggested that only a fraction of the androgens that are synthesized locally diffuse into the circulation; it is not clear whether the same is true for estrogens.[42] Because participants in our study are part of a population-based study, there is a potential for selection bias that can occur in all age groups; healthier subjects are more likely to respond and participate in this type of study. Such a bias may underestimate the reported associations. Most of the studies that investigated the association between sex hormone levels and BMD, used the BMD measured at lumbar spine and hip, instead of the pelvic bone. So, the results are difficult to compare with other studies. Finally, the results from participants of a Caucasian population may not be generalizable to study populations of different ethnical and environmental background. These limitations must be balanced against the strengths of this study. One of the major advantages of this study is that it was population based and conducted in a large number of men across a broad range of age. Another issue that needs to be considered is that total testosterone was measured using a radioimmunoassay with extraction method. Compared with commercially available direct assays this method is more reliable and reduces the cross-reaction of assay antibodies with other hormone-like substances, that can

lead to random and systematic errors.[8] Another putative predictor of level of testosterone is time of blood sampling.[43] In this study, blood samples were obtained between 0800h and 1000h, which is necessary to obtain reliable measurements due to the possible daily variation of androgens.[44]

In summary, our findings and those of others suggest that low estradiol levels are of a larger importance than low total testosterone levels to explain low BMD in men. However, also low DHEAS levels seem to play a role. Our findings do not support a major role for genetic variations in the CYP 19 aromatase, estrogen receptor α and β genes, in explaining differences in BMD in men, although the CYP19 rs10046 SNP was associated with lower E_2 levels.

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Chapter 3

Doubel blind, randomized, placebo-controlled trial on the effects of testosterone supplementation in elderly men with moderate to low testosterone levels: design and baseline characteristics [ISRCTN23688581]

Abstract

In ageing men testosterone levels decline, while cognitive function, muscle and bone mass, sexual hair growth, libido and sexual activity decline and the risk of cardiovascular diseases increase. We set up a double-blind, randomized placebo-controlled trial to investigate the effects of testosterone supplementation on functional mobility, quality of life, body composition, cognitive function, vascular function and risk factors, and bone mineral density in older hypogonadal men.

We recruited 237 men with serum testosterone levels below 13.7 nmol/L and ages 60-80 years. They were randomized to either four capsules of 40 mg testosterone undecanoate (TU) or placebo daily for 26 weeks. Primary endpoints are functional mobility and quality of life. Secondary endpoints are body composition, cognitive function, aortic stiffness and cardiovascular risk factors and bone mineral density. Effects on prostate, liver and hematological parameters will be studied with respect to safety.

Measure of effect will be the difference in change from baseline visit to final visit between TU and placebo. We will study whether the effect of TU differs across subgroups of baseline waist girth (< 100 cm vs. ≥ 100 cm; testosterone level (<12 versus ≥ 12 nmol/L), age (< median versus ≥ median), and level of outcome under study (< median versus ≥ median).

At baseline, mean age, BMI and testosterone levels were 67 years, 27 kg/m^2 and 10.72 nmol/L, respectively.

Introduction

In men after the age of 30-40, testosterone production gradually declines which continuously persists into old age.[1,2] Ageing in men is accompanied by a decrease in muscle and bone mass, cognitive changes and decreased libido and sexual activity, all of which have been suggested to be related to the decrease in testosterone production.[3] Recent research has provided evidence that androgens play distinct roles in aspects of bone metabolism[4], body composition such as muscle and fat mass distribution[5,6,6], cognitive functioning[7], well-being[8], cardiovascular diseases[9], prostate hyperplasia[10,11] and in aspects of sexual behavior[12]. Since androgens are associated with muscle function and with cognitive functioning, it is reasonable to expect androgens to be related to activities of daily living (ADL) as well.[13] The association of lower testosterone levels with age-related conditions and the steady androgen levels decline with aging stimulated further investigations to test usefulness and safety of androgen supplementation.

There are varying degrees of evidence regarding potential risks and benefits of testosterone treatment in older men.[14] The results of the Women Health Initiative (WHI) randomized trial raised concerns about the risk-benefit ratio of hormonal treatment.[15] Concerns regarding the risks have focused primarily on the potential for increased incidence of prostatic cancer, benign prostatic hyperplasia[16], urinary obstruction[17], gynecomastia[18], sleep apnea[19] and polycythemia[20]. Although androgens are necessary for the development and normal function of the human prostate, the role of testosterone in the progression of prostate cancer and benign prostatic hyperplasia is not yet clear. Epidemiological studies have been unable to relate the occurrence of benign prostatic hyperplasia and prostate cancer to androgens.[21,22] The current opinion is that androgens are not causal but permissive for the development of these diseases. Withdrawal of androgens leads to objective response rates of metastatic lesions and of the primary tumor.[23] Recently, the issue whether physiological levels of androgens are associated with prostate cancer risk seems to be adequately refuted by the quantitative assessment of the current evidence.[22] In spite of the circumstantial scientific suggesting potential risks and limited support for benefits, testosterone use has become increasingly popular in men of all ages.

The levels at which testosterone therapy might be indicated in subjects with particularly low circulating levels are also unclear. It is uncertain whether men who are at the lower end of the normal range of testosterone production would benefit from treatment. Therefore, we

set up a randomized trial with testosterone undecanoate. The key objectives of the study are to treat men aged 60 years and over, with low to low-normal testosterone levels, with testosterone undecanoate during six months and to study the effect of this treatment on functional mobility, quality of life, body composition, cognitive function, vascular ageing and bone mineral density. Safety assessments will performed by measurements of the prostate, liver enzymes and hematological parameters.

Testosterone undecanoate, the oral androgen to be used in this intervention study, did not lead to signs of prostate tumors in men who were followed-up for a minimum of 10 years.[24] With the androgens used in this study androgen levels do not rise above normal. Most benefits are expected on muscle strength and cognitive functioning. These areas are of major importance in determining the ability of living independently at old age, and therefore elderly men are a potential subgroup that could benefit from androgen supplementation.

Design and methods

The study is a single center randomized, placebo-controlled, double-blind trial to assess the effects of supplementation with testosterone undecanoate on functional mobility, quality of life, body composition, cognitive function, vascular function and risk factors, and bone mineral density in men, aged 60-80 years. The planned number of study subjects is 240. After completion of the baseline measurements subjects are randomized to four capsules of 40 mg testosterone undecanoate (TU) or placebo daily for 26 weeks. The Institutional Review Board of the University Medical Center Utrecht approved the study protocol. All participants gave written informed consent at screening visit.

Study population

The study was designed so that the study population would comprise men in the lower half of the population distribution of testosterone levels. Therefore, the exclusion criteria were mainly limited to criteria indicating contraindications for testosterone, a high probability of experiencing serious side effects, or a low likelihood of completing the study.

In and exclusion criteria

Participants in this trial were healthy men aged 60 to 80 years (at the moment of inclusion), who lived in Utrecht and vicinity and had a low normal testosterone concentration. Detailed information and definitions of the exclusion criteria are displayed in *Table 1*. Men could not be enrolled when they had a history of recent severe myocardial infarction or cerebrovascular accident (< 6 months), cardiac failure, unless medically treated and not symptomatic, history or presence of any malignancy within the past 5 years, except for non-melanoma skin cancer, subjects with ever history of testosterone (any hormone) dependent tumors (especially prostate or breast cancer), serious liver disease, serious renal disease, hematological abnormalities, epilepsy (or the use of anti-epileptic medication) or migraine more than once a month, diabetes mellitus, presence of any disease or condition that is clinically relevant and which might result in premature discontinuation, according to the opinion of the investigator, corticosteroid use, use of testosterone esters and alike substances within the past 60 days, increased age-specified-PSA levels and prostate hypertrophy in medical history.

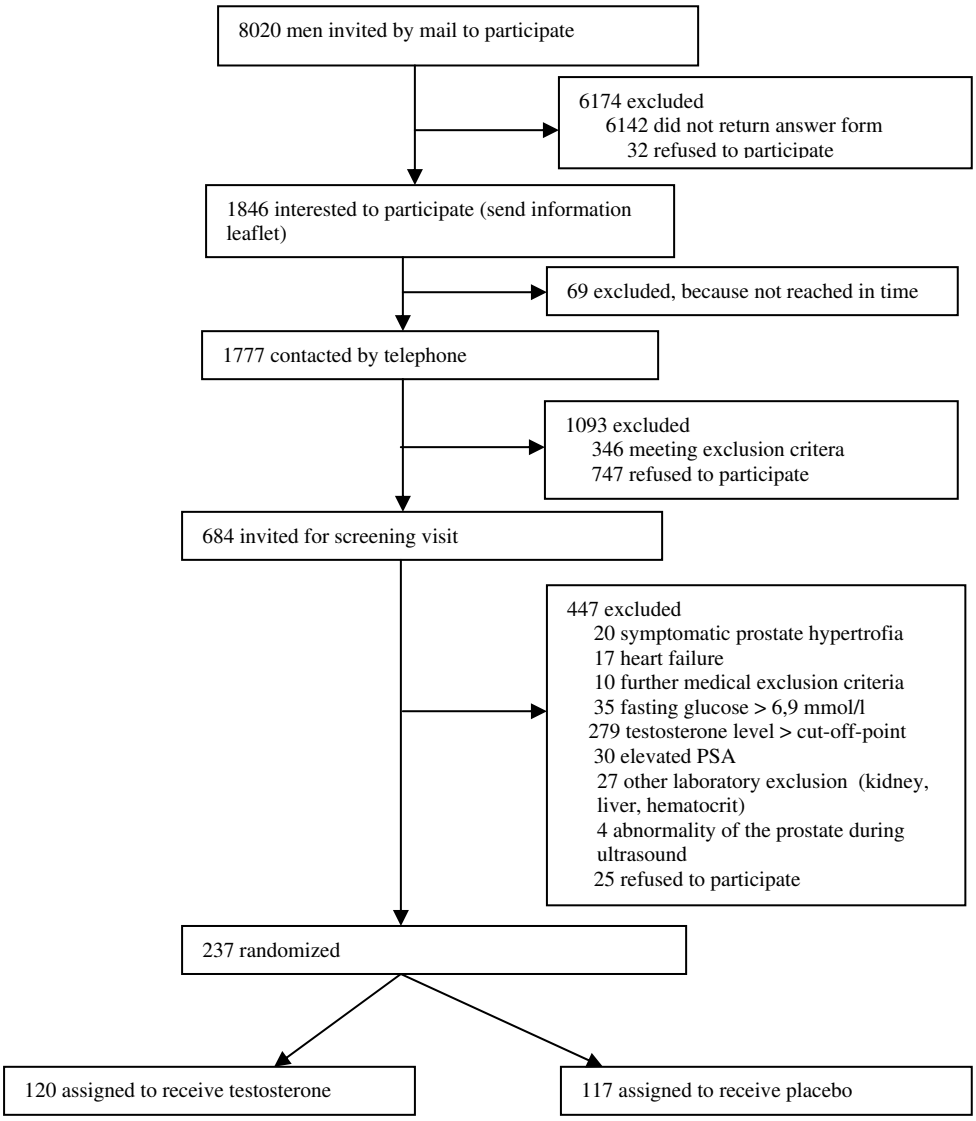
Recruitment

Recruitment started in November 1st, 2003. Participants were recruited by direct mailing to 8020 men aged 60 to 80 years whose addresses were randomly selected by the municipal register of Utrecht. 1,846 men expressed interest in the study by sending in a response-card. Men who responded were explained the aim of the study and any questions were answered. Also an information leaflet was mailed. In this leaflet explanation was given regarding the study and the in – and exclusion criteria protocol. After one week of evaluation time 1,777 men were contacted by telephone and invited to the information and screening visit. Of these, 1030 responded positively and 747 refused to participate. Also most of the exclusion criteria were checked by telephone. Of those 1030 men, 346 were excluded because of one or multiple exclusion criteria (*Figure 1*).

Table 1. *Exclusion criteria*

Disease / Condition	*Definition*
Recent severe myocardial infarction or cerebrovascular accident	< 6 months
Cardiac failure, unless medically treated and not symptomatic	
History or presence of any malignancy within the past 5 years, except for non-melanoma skin cancer. Subjects with ever history of testosterone (any hormone) dependent tumors.	
Serious liver disease	ASAT, ALAT, AF, γGT >3 times upper limit of reference value (ASAT: 15-45 U/L; ALAT: 10-50 U/L; AF: 40-130 U/L ; γGT: 15-70 U/L: Central Laboratory, UMC Utrecht)
Serious renal disease	Serum creatinine levels > 180 mol/L)
Epilepsy (or the use of anti-epileptic medication) or migraine > once a month	
Diabetes mellitus	Diagnosed by physician or fasting glucose level of 6.9 mmol/L or higher (capillary)
Presence of any disease or condition that is clinically relevant and which might result in premature discontinuation, according to the opinion of the investigator.	
Medication	*Definition*
Corticosteroid use	Orally: <6 months ago in dosage >7.5 mg a day, with the exception of short bouts of prednisone for the period of 7 days. Inhalation: <6 months ago in the dosage of >800 g a day) Use of testosterone esters and alike substances within the past 60 days
Conditions for which increase of androgen-like substances are contra-indicated	PSA levels: age 60-69 years >4.5 µg/l;70 years and over >6.5 µg/L Prostate hypertrophy in medical history Renal, liver function abnormalities or hematological abnormalities (Hb <7; Ht >0.50) Prostate or breast cancer

FIGURE 1. *Participants Flow Diagram*



Information and screening visit

The selected 684 participants were asked to attend the ambulatory clinic of the Julius Center for Health Sciences and Primary Care of the University Medical Center Utrecht, The Netherlands for more information about study and screening. During this visit, after answering remaining questions, the informed consent form was signed. The participants filled in two questionnaires; the Androgen Deficiency in Ageing Males (ADAM) questionnaire and the Ageing Males' Symptoms rating scale (AMS). Remaining possible exclusion criteria were checked through a medical history and blood examination in the following order: fasting glucose level (capillary) of ≥ 6.9 mmol/L, (n=35 exclusions), testosterone level higher than the 50th percentile of the study population-based testosterone distribution (n=279 exclusions), elevated PSA level (age 60-69 years ≥ 4.5 µg/L; 70 years and above ≥ 6.5 µg/L (n=30 exclusions), and serious liver- (>3 times the upper limit of reference value) or renal diseases (creatinine > 180 µmol/L) or hematological abnormalities (hemoglobin ≤ 7.0 mmol/L, hematocrit ≥ 0.50) (n=27 exclusions). There were still 47 participants who met one of the other exclusion criteria and 25 participants who did not want to start intervention for other reasons. If participants did not match the study-profile due to one or more exclusion criteria, appropriate steps were undertaken to refer (if necessary) the participant to his general practitioner (n= 96). Major reasons for exclusion were testosterone level > cut-of-point, fasting glucose > 6.9 mmol/L or an elevated PSA level. Also, a fasting blood sample for a specific panel of laboratory assessments (including a spare DNA blood sample) was taken. Finally, 241 men proceeded to the randomization visit.

Randomization visit

The randomization visit assessment was conducted when all lab results were known and always within 4 weeks from the information and screening visit. First, digital rectal examination and trans-rectal ultrasound of prostate were performed. If there was any suspicion regarding prostate pathology, appropriate steps were undertaken to refer the participant to its general practitioner or consulting urologist. Four men were excluded for this reason. If the rectal ultrasound did not show any signs of pathology the participant continued the following baseline measurements: medical history, family medical history, vital signs (blood pressure , pulse), physical examination, anthropometry measurements

(height and body weight, waist- and hip circumference, upper leg-, arm- and calf circumference, sagittal abdominal diameter), International Prostate Symptom Score (IPSS) questionnaire, functional mobility measurements, bone mineral density measurements via DEXA scan, health related quality of life questionnaires and utilities instrument, measuring cognition (15-words test, Digit symbol Substitution test, Concept Shifting Task test, the Benton Judgment of Line Orientation test and the Shephard rotation task), full body DEXA scan (lean body mass, fat free and fat mass), Pulse Wave Velocity (PWV), abdominal ultrasound for fat distribution.

Finally 237 men were eligible for randomization. These subjects were randomly assigned to the intervention or the placebo group. A randomization list was computer-generated by Organon NV, Oss, The Netherlands. One box with active medication and one box with placebo medication were delivered at the UMC Utrecht Pharmacy with the randomization list. Pharmacy personnel labeled the jars for the participants and provided the study medication upon prescription of the trial physician. Randomization numbers were assigned to the subjects in orders of enrolment into the trial.

Intervention

The intervention consisted of four capsules of 40 mg testosterone undecanoate (Andriol ® Testocaps®) provided by Organon NV, Oss, the Netherlands. The placebo was an identically looking and tasting capsule. The duration of the intervention was 26 weeks in which participants had to take the supplement on a daily basis. If for one reason unbinding should be necessary during the course of the trial, a backup hospital pharmacist was available who was informed about the trial but not involved.

Control phone call (6 weeks)

All participants were called at 6 weeks after randomization. At 6 weeks participants were asked about: Medical history update, including co-medication and adverse events. Also, they filled in the IPSS questionnaire.

Control visit (13 weeks)

During the intervention period of 26 weeks, subjects were asked to visit our clinic at 13 weeks after randomization. At 13 weeks the following measurement were done: Medical history update, including co-medication, adverse events, vital signs, (blood pressure, pulse), digital rectal examination, IPSS questionnaire and laboratory measurements (PSA, hematology, liver and renal functions, spare blood sampling for additional investigations).

Final visit (26 weeks)

The final visit took place after 26 weeks of intervention. At the final visit, all tests carried out at base-line were repeated following the same procedures.

End point measurements

An overview of visits and measurements is shown in *Tables 2 and 3*, respectively. Endpoints were assessed at baseline and after 26 weeks. All the assessments took place at the baseline randomization visit were repeated at 26 weeks.

Table 2. *Baseline characteristics of participants*

	Testosterone (n=120)	Placebo (n=117)
Age (yr)	67.3 ± 5.1	67.5 ± 5.0
Weight (Kg)	86.1 ± 13.3	84.4 ± 13.6
Body Mass Index (kg/m²)	27.54 ± 3.85	27.20 ± 3.90
Smokers	21 (17.5)	15 (12.8)
Alcohol users	99 (82.5)	85 (77.3)
Hypertension	75 (62.5)	69 (59)
Systolic Blood Pressure (mm/Hg)	156.2 ± 23.2	151.1 ± 22.6
Diastolic Blood Pressure (mm/Hg)	89.8 ± 12.0	86.8 ± 11.7
Pulse Pressure (mm/Hg)	66.4 ± 15.9	64.3 ±15.2
Pulse Wave Velocity (m/s)	10.00 ± 2.51	9.53 ± 2.66
Total Testosterone (nmol/L)	10.93 ± 2.06	10.50 ± 1.89
Free Testosterone (nmol/L)	0.22 ± 0.05	0.21 ± 0.05
Bioavailable Testosterone (nmol/L)	5.23 ± 1.15	5.04 ± 1.20
SHBG (nmol/L)	33.17 ± 10.59	32.90 ± 10.38
Albumin (g/L)	43.94 ± 2.31	43.80 ± 2.38
Cholestrol (mmol/L)	5.61 ± 0.99	5.50 ± 0.97
HDL (mmol/L)	1.16 ± 0.28	1.16 ± 0.29
LDL (mmol/L)	3.92 ± 0.91	3.80 ± 0.87
Insuline (mIU/L)	10.14 ± 9.50	8.73 ±5.41
C-reactive proteine (mg/L)	4.27 ± 6.56	4.09 ± 6.97
PSA (µg/L)	1.54 ± 1.1	1.63 ± 1.1
Creatinine (µmol/L)	93.35 ± 18.0	93.72 ± 15.2
ASAT (U/L)	22.98 ±8.1	24.21 ± 12.2
ALAT (U/L)	26.36 ± 11.0	26.74 ± 13.6
AF (U/L)	71.57 ± 19.2	69.91 ± 17.9
GGT (U/L)	29.51 ± 15.751	30.18 ± 19.9
Hemoglobine (mmol/L)	9.18 ± 0.5	9.14 ± 0.6
Hematocrit (%)	0.45 ± 0.0	0.45 ± 0.0
Prostate Volume (ultra sound) (cc)	28.20 ± 12.4	27.6 ± 9.8
IPSS	6.38 ± 5	6.50 ± 4.8

Values are means or numbers with S.D. or percentage in parentheses.

Table 3. *Overview of the visits and measurements*

Assessment	Information visit and inclusion criteria	Screening and randomization visit	Phone call 6 weeks	Visit 13 weeks	Final visit 26 weeks
	V1	V2		V3	V4
Check in & exclusion criteria	X	X			
Informed consent	X				
Medical history /update	X	X	X	X	X
Physical examination		X			X
Vital signs		X		X	X
Anthropometry		X			X
Blood sample	X			X	X
Glucose	X				X
DNA blood sample	X				
Testosterone	X				X
PSA	X			X	X
Liver functions	X			X	X
Renal functions	X			X	X
Hematology	X			X	X
Spare blood	X			X	X
Rectal Ultrasound		X			X
Rectal Toucher		X		X	X
DEXA full body		X			X
DEXA bone mineral density		X			X
Ultrasound abdominal fat mass		X			X
Pulse wave velocity		X			X
Functional mobility		X			X
Cognition tests		X			X
Quality of Life		X			X
ADAM/AMS questionnaire	X				X
Sexual functioning questionnaire		X			X
IPSS questionnaire		X	X	X	X
Randomization		X			

Functional mobility measurement

We assessed the functional mobility by the use of timed "Get Up and Go" test and a questionnaire for the ability to perform activities of daily life the Stanford Health Assessment Questionnaire (HAQ).[25,26] Furthermore, skeletal muscle strength was assessed measuring handgrip strength and isometric knee strength.[27,28]

During the timed "Get Up and Go" test, the time taken by an individual to rise from a standard chair, walk three meters, turn around, return and sit down again was measured. The subject was requested to sit with his back against the chair and arms resting on the chair and performs the test three times. The fastest time was recorded in seconds.

The Stanford Health Assessment Questionnaire (HAQ) has been widely used to measure functional status and includes 24 questions grouped into 8 categories of 2, 3 or 4 ADL's. The categories were dressing, arising, eating, walking, hygiene, reaching, gripping, and others. Participants responded to these questions by checking the level of difficulty from 0 (without any difficulty) till 3 (unable to do). If participants needed help from another person or assistive devices for each of the ADL's the score raises automatically to 2 (with much difficulty).

Handgrip strength was measured with the JAMAR® dynamometer. The size of the grip was set so that the participant felt comfortable. The participant was in standing position his shoulder was adducted and neutrally rotated; the arm was vertical and the wrist in a neutral position. The participant squeezed the grip with maximal strength, alternating the left and right hand. The unit was automatically recorded the highest force exerted. Each test was repeated at least 5 times until no further improvements were seen. The best measure, recorded in kilograms, was used for analysis.

Isometric knee extensor strength was measured with a hand-held dynamometer. The participant was in a seated position at a mat-table with the hip flexed to 90 degrees, the knee stretched to 180 degrees and the legs dependent. The dynamometer was applied perpendicularly to each lower extremity just proximal to the malleoli. Participants were instructed to take a second or two to come to come to maximum effort and to then push as hard as possible during another three seconds, while the investigator was giving counterforce. Each test was repeated five times, and if the examiner was not confident that a maximal effort was reached one more effort was made. The best measure, recorded in Newton, was used for analysis.

Quality of life and well-being measurement

Quality of Life and well being was measured by the Short Form-36 Health Survey as a generic QoL questionnaire (SF-36) and the Herschbach-questionnaire as a hormone specific questionnaire. The SF-36 is a questionnaire consisting of questions regarding general health, ability to perform physical activity and work, emotional problems and assessment of his own health.[29] The Herschbach questionnaire is a questionnaire translated from the questionnaire "Fragen zur Lebenszufriedenheit" (FLZ) according to the method described by Henrich and Herschbach. The questionnaire is divided in a "general" and a "health" section, each including eight items. All items have been evaluated on a 5-point scale according to their individual importance (I) and degree of satisfaction (S). As a measure of evaluation, a combination of importance and satisfaction (I-1)*(S*2-5) will be used. In addition the sum of the combination values will be calculated for each section.[30]

Sexual behavior and erectile dysfunction measurement

The 'Eleven questions about sexual functioning (ESF) questionnaire, developed by the National Institute for Social Sexual Research (Rutgers Nisso Group, Utrecht, The Netherlands), has been used to assess sexual well being. The questionnaire has 11 questions measuring sexual drive (two questions); erectile function (three) and ejaculatory function (two), as well as assessing problems with sex drive, erections, or ejaculation (three); and overall satisfaction with sex life (one). Each question is scored on a scale of 0-4, with higher scores indicating better functioning.

The Androgen Deficiency in Ageing Males (ADAM) and the Ageing Males' Symptoms rating scale (AMS) questionnaires have been administered as well. The ADAM questionnaire contains 10 questions regarding the age-related decline in androgens. All questions should be answered yes or no. A positive questionnaire result, indicating an androgen deficient state, is defined as a 'yes' answer to question 1 or 7 or any 3 other questions.[31] AMS-questionnaire (Ageing Males' Symptoms rating scale) is a 17-question questionnaire investigating age related health complaints divided in three dimensions (psychological, somatovegetative and sexual) of each 5 questions. Each question can be scored from 1 (no symptoms) to 5 (very severe symptoms), so a dimension can score from 5 to 25 points. Within each dimension, cumulative scores indicate the severity of the

complaints on each territory; also the cumulative of all dimensions indicate an overall view of Ageing Males' Symptoms. Classification range spreads from no impairment at all to severe impairment.[32]

Body composition measurement

Body Composition was assessed by anthropometry (body mass index (BMI), waist and hip girth, upper arm-, upper leg and calf circumference and sagittal abdominal diameter), full body DEXA scan (lean body mass, fat free- and fat mass) and ultrasound of the abdominal fat mass.

BMI was calculated as the weight in kilograms divided by the square of the height in meters, after taking of coat, sweaters and shoes. All circumference measurements were done with a standard household centimeter. Waist circumference was measured at the level of midway the distance between the lower rib and iliac crest, after normal expiration without pressure of the centimeter at the skin. The hip circumference was measured at the level of the greater trochanter. The upper arm circumference was measured at the non-dominant arm at the level of midway between the tip of the acromnion and the olecranon. The thigh circumference was measured just below the gluteal fold of the left leg. Calf circumference was measured at the level of the largest circumference of the left calf. The Sagittal abdominal diameter (SAD) was measured using a Holtain-Kahn (abdominal calliper (Holtain ltd., Crosswell, UK) which allows a direct reading of the distance between the subjects back and the front of the subjects' abdomen. With subject in supine position a mark was made halfway between the left and right iliac crest. The lower arm of the caliper was inserted underneath the subjects back and the upper arm was adjusted until touching the abdominal wall at the level of the mid-abdominal mark. The measurement was taken with a resting and at the end of a normal expiration. The distance between the subjects back and abdominal wall was measured on a centimeter scale and round off to the nearest 0.1 cm. Since with the abdominal subcutaneous fat tends to slip along the flanks, when the subject is in supine position, the SAD is an indirect measurement of the amount of visceral fat mass.

Total body composition was measured with dual energy X-ray absorptiometry (DEXA) using a Lunar prodigy® DEXA instrument. Scanning was performed according to the instructions of the manufacturer. After placement of the subject on the table, there was

scanning of the whole subject from dorsal to ventral. Both legs and feet were endorotated and fixed to on another. Calculations were made regarding fat-mass, fat-free mass and lean body mass.[33]

Abdominal ultrasonography was performed in all abdominal obese subjects with an Ultramark 9®. The distances between the posterior edge of the abdominal muscles and the lumbar spine or psoas muscles were measured using electronical callipers. For all images the transducer was placed on a straight line drawn between the left and right mid-point of lower rib and iliac crest. The middle was marked 10 cm from the left and right side. Distances were measured from three different angles: medial, left and right for intra-abdominal fat mass and medial for subcutaneous fat mass. Measurements were made at the end of quiet expiration, applying minimal pressure without displacement of intra-abdominal contents as observed by ultrasound image.[34]

Cognitive function measurement

Cognitive function was measured as follows. Verbal memory was tested with the Dutch version of the Rey Auditory Verbal Learning Task. This is a test for long-term memory retention. Fifteen words were read to the subject, who was required to report as many words as he could remember immediately after presentation. After a delay of 15 min (in which another test, the Benton Judgment of Line Orientation, was administered), the subject was asked to recall as many words as possible from memory.[35]

Mental processing speed was tested with the “Digit Symbol Substitution test”. This is a sub-test from the Wechsler Adult Intelligence Scale (WAIS) that covers general knowledge. It measures cognitive and perceptual-motor processing speed. The subject was given a code that pairs symbols with digits. The test consists of matching as many series of digits to their corresponding symbols as possible in 90 seconds.[36]

The trail making test was used to test planning of movement, vasomotor tracking, and processing speed. In this test, pseudo-randomly placed circles with numbers (Trail Making A1), with letters (Trail Making A2), and with both numbers and letters (Trail Making B) have to be connected with a line as fast as possible in a fixed order. In the event of error, the subjects were immediately informed and asked to restart from the point of error: this was done with the timer left running. The time taken to complete the trail without error was recorded.[37]

The "Benton Judgment of Line Orientation test" was used to measure visual-spatial skills. This test measures basic perceptual processes contributing to extra-personal spatial perception. The test requires the subject to identify which 2 of 11 lines presented in a semicircular array have the same orientation in two-dimensional space as two-target lines.[38] The visospatial performance was assessed by the Vandenberg and Kuse adaptation of Shepard and Metzler's three-dimensional mental rotations test.[39] This is the cognitive task that has been most consistently associated with testosterone levels. The test was consisted of 20 items in which the subject was presented with a three-dimensional geometric target line drawing and four test drawings, and was required to indicate which two of four test drawings depict the target drawing in rotated positions. Two parallel test versions were made by taking the odd and even items on time 1 (baseline) and time 2 (after intervention) respectively (10 items for each test). These parallel versions have been shown to correlate strongly with each other and to have a high reliability. Subjects were instructed to "work as quickly as possible, but do not sacrifice accuracy for speed". They were allowed 10 minutes to complete the test.

Aortic stiffness and cardiovascular risk factors measurements

Total cholesterol, HDL cholesterol and triglyceride were measured by a timed endpoint method (Synchron LX®, Beckman Coulter, Fullteron, California, USA).[40] LDL was calculated with the Friedewald equation.[41] Insulin was measured by a solid-phase two site chemiliminesent immunometric assay (IMMULITE 2000, Diagnostic Products Corporation, Los Angeles, California, USA). Serum levels of highly sensitive CRP were measured using a near-infrared particle immunoassay of the Synchron LX System (Synchron LX®, Beckman Coulter, Fullteron, California, USA).

Systolic and diastolic blood pressures and pulse were measured in duplicate at the dominant arm with the subjects in sitting position after 5 minutes of rest with an automated and calibrated oscillometric device (Omron Healthcare Europe, Hoofddorp, The Netherlands). Subsequently, the mean systolic and diastolic blood pressures and mean pulse rate were calculated.

Aortic stiffness was determined by means of pulse wave velocity. The Sphygmocor® system was used to non-invasively measure stiffness of the aorta (Pulse wave velocity system, PWV medical, Sydney, Australia).[42] After 5 to 10 minutes rest of the subject in

supine position, aortic PWV was measured by sequentially recordings of arterial pressure waveform at the carotid artery and the femoral artery using a hand-held micromanometer-tipped probe on the skin at the site of maximum arterial pulsation. Gating the recordings at those two sites to the electrocardiogram (ECG) allowed PWV to be measured. Recordings were taken when a reproducible signal was obtained with high amplitude excursion, i.e. usually 10 consecutive beats to cover complete respiratory cycle. The system software, using the R wave of a simultaneously recorded ECG as a reference frame, was calculated the wave transit time. A distance from the carotid-sampling site to the suprasternal notch and suprasternal notch to the femoral artery was measured using a compass.[43]

Bone mineral density measurement

Bone mineral density (BMD) was measured with dual energy X-ray absorptiometry (DEXA) using a Lunar prodigy® DEXA instrument. Scanning was performed according to the instructions of the manufacturer. BMD was measured of lumbar vertebrae (L1-L4 individually and together) and proximal femur (femoral neck, trochanter, inter-trochanter, Ward's triangle and total hip, left-or right if left not available). A T-score ≤-2.5 denotes osteoporosis, a T-score between –1 and –2.5 denotes osteopenia.[44] The DEXA scan was also used to measure total and trunk lean body mass (see total body DEXA-scan). Quality assurance, including calibration was performed routinely every morning for DEXA (if that day a measurement is planned), using the standard provided by the manufacturer.[45]

Prostatic measurements

Effects on the treatments on the prostate were examined by digital rectal examination, transrectal ultrasound of the prostate and by monitoring serum prostate-specific antigen (PSA) levels and by IPSS. The IPSS, developed by the American Urological Association (AUA), contains seven items that measure frequency and severity of urological symptoms, together with an additional item measuring the overall impact of these symptoms on quality of life. Each of the seven symptom items has a response scale with six choices, scored from 0 (absence of the symptom) to 5 (symptom always present). Symptoms are considered mild for scores between 0 and 7, moderate for scores between 8 and 19, and severe for scores between 20 and 35.[46]

Digital rectal examination was performed at baseline, 13 weeks and at the end of treatment (26 weeks). Biplanal transrectal ultrasonography of the prostate was performed at baseline and at the end of treatment (26 weeks) with a 7-MHZ transrectal probe (Bruel and Kjaer Model 2110 Falcon). If rectal ultrasound was abnormal, patients were excluded and referred for further evaluation. Serum prostate specific antigen (PSA) levels were measured by an immunnometric assay (IMMULITE® 2000 PSA, Diagnostic Products Corporation, Los Angeles, California, USA) at baseline, week 13 and at the end of the study. An increase of >1.4 μg/L between measurements at any time was cause for concern. Abnormal values required repeat testing; if values remained high, co-morbid illness was ruled out. This was reason to exclude a patient and send to his general practitioner.

Laboratory measurements

Fasting blood samples were obtained between 8.00 and 11.00 AM to minimize diurnal variation. The level of total testosterone and sex hormone binding globulin (SHBG) were measured with a solid-phase, competitive, chemiluminescent enzyme immunoassay (IMMULITE ® 2000, Diagnostic Products Corporation, Los Angeles, California, USA) at baseline and at the end of the study. The intra-assay coefficient of variation of this assay was 7.2% and the inter-assay coefficient of variation was 8.2 % for testosterone and 2.5% and 5.2 % for SHBG, respectively. Hematology (hemoglobin and hematocrit) and routine biochemistry (liver functions and creatinine) were measured by standard autoanalyzer methodologies (Synchron LX®, Beckman Coulter, Fullteron, California, USA) at baseline, after 13 weeks, and at the end of the study. During the study, hemoglobin levels of ≤ 7 mmol/L, hematocrit levels ≥ 0.50, liver function values ≥ three times normal upper normal reference level (ASAT: 15-45 U/L; ALAT: 10-50 U/L; AF: 40-130 U/L ; γ-GT: 15-70 U/L), or creatinine levels of ≥ 180 μmol/l led to an extra blood check after a week. If the values were still too high study participation was discontinued. All laboratory measurements were done at the SHO laboratory, Velp, The Netherlands.

Adverse events

An adverse event (AE) was defined as any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which did not necessarily have a causal relationship with this treatment. An AE could therefore be any

unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporarily associated with the use of a medicinal report, whether or not considered related to the medicinal product. Whether or not an abnormal laboratory/vital sign were entered on the AE form depends on whether or not the finding was clinically relevant in the opinion of the investigator. Information regarding AEs was obtained by questioning or examining the subject. At each visit during the treatment period, all new complaints and symptoms (i.e. those not existing before the treatment period) were recorded (and coded) on the AE Form. Pre-existing complaints or symptoms that increased in intensity or frequency during the treatment period were entered on the AE Form also. All AEs were characterized in terms of their start and stop dates, maximum intensity, action taken on trial medication, relationship to trial medication, and subject outcome. If a subject discontinued the trial because of an AE, this was noted on the AE Form. Serious adverse events (SAE) forms were supplied by Organon. If the AE meets the definition of an SAE, the procedure for reporting SAEs was followed. A serious adverse event (SAE) was defined as any untoward medical occurrence that at any dose: resulted in death, was life-threatening, required in-patient hospitalization or prolongation of existing hospitalization, resulted in persistent or significant disability or incapacity, noted the term life threatening refers to an event in which the patient was at risk of death at the time of the event; it did not refer to an event, which hypothetically might have caused death if it had been more severe. Medical and scientific judgment was exercised in deciding whether expedited reporting was appropriate in other situations, such as important medical events that might not be immediately life-threatening or result in death or hospitalization, but may jeopardized the patient or might required intervention to prevent one of the other outcomes listed in the definition above. These were considered serious. All SAEs were reported to the METC and to Organon NV, Oss, the Netherlands. Every attempt was made to obtain any relevant laboratory or hospital reports that pertain to the SAE.

Compliance

Compliance was monitored by spare capsule counting at each study visit. After finalization of the study serum testosterone concentrations were assessed in the final visit blood samples as an extra check on compliance.

Power calculation

The pre-specified number of subjects was 240 in total, 120 in each intervention arm. This number was based on conventional assumptions of α=0.05 and β=0.20, withdrawal from intervention of 15% and an improvement of 25% on MHAQ and of 18% on the 15 Words test. These improvements were realistic, since they have been previously reported in short-term small studies.

Data analysis

The primary analysis will be done by linear regression analysis with change in outcome parameter between final visit and baseline visit as the dependent and treatment group as the independent variable. All analyses will be based on an intention-to-treat approach (i.e., the intention-to-treat group will consist of all subjects, including those who withdrew from blinded medication, who received at least one dose of study drug and who had at least one post-baseline assessment of the outcome variable). In addition to an intention-to-treat analysis, a per-protocol analysis will be performed. The per-protocol group will consist of all subjects from the intention-to-treat group who did not have any major protocol violations. Furthermore, subgroup analysis will be performed for the following predefined subgroups according to baseline measurements: waist girth (< 100 cm versus ≥ 100 cm); testosterone level (<12 versus ≥ 12 nmol/L), age (< median versus ≥ median), and baseline level of outcome under study (< median versus ≥ median). Differences between final visit and baseline for continuous measures were expressed as means and 95% confidence intervals; unpaired t-tests were used for testing. Level of significance was set at P<0.05. All analyses are performed with SPSS, statistical software package, version 11.

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Chapter 4.1

Low testosterone concentrations and the symptoms of testosterone deficiency according to the ADAM and AMS questionnaire

Abstract

Context Serum testosterone levels decline significantly with aging. This is associated with many symptoms and signs of aging. However, it is difficult to identify the aging males with androgen deficiency who might benefit from testosterone replacement therapy.
Objective To assess the association between the testosterone concentration and the symptoms of testosterone deficiency according to two screening questionnaires, and to investigate the effects of testosterone supplementation during 6 months to elderly men with a low-normal testosterone concentration on symptoms of testosterone deficiency according to the screening questionnaires.
Design, Setting and Participants 587 community-dwelling healthy men between 60 and 80 years of age were eligible for a testosterone measurement and filling in an ADAM- and AMS-questionnaire. Of these 587 men, 237 men with testosterone levels < 13.7 nmol/L were included in a double-blind, randomized, placebo-controlled trial of testosterone supplementation.
Intervention The 237 men were randomly assigned to receive 80 mg of testosterone undecanoate or a matching placebo twice daily for 6 months.
Main Outcome Measures The symptoms of testosterone deficiency were assessed using the ADAM (Androgen Deficiency in Ageing Males) and the AMS (Ageing Males' Symptoms rating scale) questionnaires.
Results There was no significant association between the total testosterone concentration and the scores on the ADAM and the AMS questionnaire. However, age was significantly associated with the scores on the ADAM (OR 1.14, 95% CI 1.05-1.23) and the AMS (OR 1.03, 95% CI 1.01-1.08) questionnaire
After supplementation with testosterone, there were no effects on the scores on the ADAM and the AMS questionnaire in the testosterone group compared with the placebo group.
Conclusion The findings do not support the use of the ADAM or AMS questionnaire in the evaluation of late onset hypogonadism in aging men. Moreover, 6 months of oral testosterone supplementation had no significant effect on scores of the ADAM and AMS questionnaire in this group of elderly men with low-normal testosterone levels.

Introduction

Male aging is associated with a gradual but progressive decline in serum levels of testosterone[1], but the extent of the decline differs considerably between individuals. There is an association between the decline of testosterone and many symptoms and signs of aging, such as decrease in muscle mass and muscle strength, cognitive decline, diminished libido and erectile quality, decrease in bone mass and increase of (abdominal) fat mass. However, despite growing evidence that the decline of testosterone is associated with adverse health effects, it is difficult to identify the aging males with (relative) androgen deficiency who might benefit from testosterone replacement therapy. This is due to the slow rate of decline in testosterone levels, concomitant diseases, changes in dietary and exercise habits, and the multitude of other physiological changes that occur during the same time.

As a result, the definition of late-onset hypogonadism is a topic of lively debate. The International Society for the Study of the Aging Male (ISSAM) defined late-onset hypogonadism (LOH) as a clinical *and* biochemical syndrome associated with advancing age and characterized by typical symptoms and a deficiency in serum testosterone levels.[2] However, there are still no generally accepted lower limits of normal testosterone concentrations and it is unclear whether geographically different thresholds depend on ethnic differences or on the physicians' perception. Moreover, the typical symptoms for LOH may not be specific for testosterone deficiency. In order to identify clinical signs and symptoms of (relative) androgen deficiency, various screening questionnaires have been proposed.

The aims of this study were: 1. to assess the association between the testosterone concentration and the symptoms of testosterone deficiency according to two screening questionnaires, and 2. to assess the effects of testosterone supplementation during 6 months to elderly men with a low-normal testosterone concentration on symptoms of testosterone deficiency according to the screening questionnaires.

Subjects and Methods

This study compromised a baseline assessment and a subsequent randomized, double-blind, placebo-controlled trial. Details of the study design, recruitment, and procedures have been

published previously.[3] The Institutional Review Board of the University Medical Centre Utrecht approved the study protocol and all participants provided written informed consent.

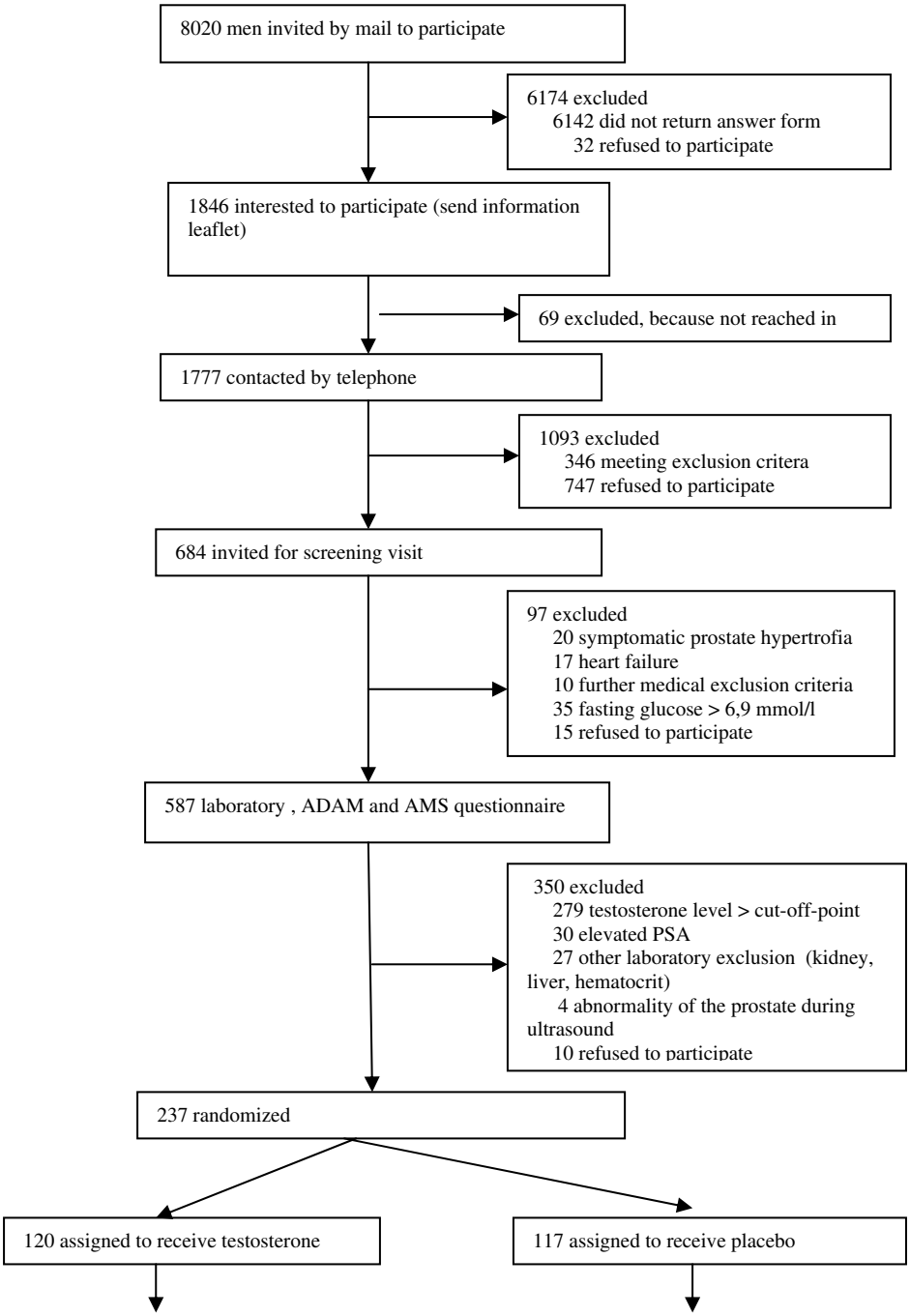
Participants

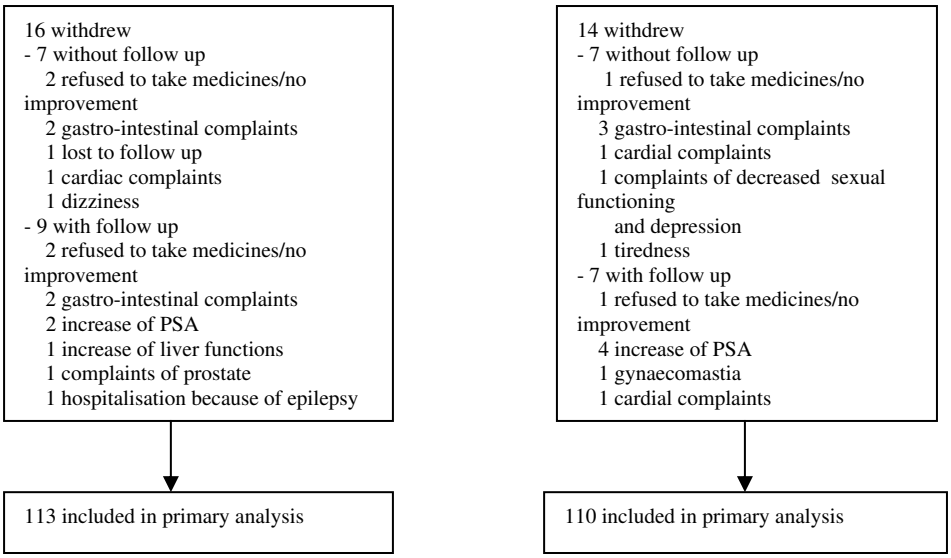
Participants were recruited by direct mailing to 8020 randomly selected men between 60 and 80 years of age whose addresses were obtained from the municipal register of the city of Utrecht, the Netherlands.

Inclusion criteria included: a testosterone level below the 50th percentile of the study population based testosterone distribution and an age between 60 and 80 years. The 50th percentile cut-off level of testosterone was determined to be 13.7 nmol/L after screening of 50 candidates. This was comparable with the 50th percentile of the testosterone level at the end of the study (13.8 nmol/L). Exclusion criteria included: myocardial infarction or cerebrovascular accident within the past 6 months, heart failure unless medically treated and not symptomatic, malignancy within the past 5 years except for non-melanoma skin cancer, any hormone dependent tumor in the history, serious liver- or renal diseases (more than 3 times the upper limit of reference value), hematological abnormalities (hemoglobin $\leq$ 7.0 mmol/l, hematocrit $\geq$ 0.50), epilepsy or the use of anti-epileptic medication, migraine more than once a month, diabetes mellitus, a fasting glucose level $\geq$ 6.9 mmol/l, corticosteroid use ($\geq$ 7.5 mg orally a day within the past 6 months, with the exception of short bouts of prednisone for the period of 7 days, or inhalation of $\geq$ 800 µg a day during the past 6 months), use of testosterone esters and alike substances within the past 60 days, prostate hypertrophy in medical history, and an elevated PSA level (age 60-69 years $\geq$ 4.5 µg/l; 70 years and above $\geq$ 6.5 µg/l).

Following an initial telephone contact 684 men were invited for a screening visit. Of these 684 men, 587 men were eligible for a testosterone measurement, agreed to participate and filled in an ADAM- and AMS-questionnaire. Finally, 237 men with the lowest 50th percentile of testosterone level and no exclusion-criteria were randomly assigned to the intervention or the placebo group. A randomization list without stratification using blocks of 6 was computer generated. Randomization numbers were assigned to the subjects for enrolment into the trial. The flow of study subjects participants recruitment and enrolment is shown in *figure 1*.

FIGURE 1 *Participant Flow Diagram*





Intervention

The intervention consisted of two capsules of 40 mg testosterone undecanoate (Andriol® Testocaps, N.V. Organon, Oss, the Netherlands) twice a day with breakfast and dinner (= a total daily dose of 160 mg testosterone undecanoate), or matching placebo, for a total duration of 6 months. Adherence was monitored by capsule counting at each study visit.

ADAM-questionnaire (Androgen Deficiency in Ageing Males)

This questionnaire contains 10 questions regarding the most common symptoms observed in age-related decline in androgens. All questions should be answered yes or no. A positive questionnaire result, indicating an androgen deficient state, is defined as a 'yes' answer to question 1 or 7 or any 3 other questions.[4] The questionnaire was assessed at baseline and after 6 months of supplementation.

AMS-questionnaire (Ageing Males' Symptoms rating scale)

The AMS scale was designed and standardized as self-administrated scale (a) to assess symptoms of aging (independent for those which are disease-related) between groups of males under different conditions, (b) to evaluate the severity of symptoms over time, and

(c) to measure changes pre- and post-androgen replacement therapy. Although this questionnaire was not developed as a screening instrument of androgen deficiency, the results of screening are comparable with other screening questionnaires for the diagnosis of androgen deficiency.[5,6] It is a 17-question questionnaire investigating age related health complaints divided in three dimensions (psychological, somatovegetative and sexual). The psychological and sexual sub-domains consist of each 5 questions and the somatovegetative sub-domain consists of 7 questions. Each question can be scored from 1 (no symptoms) to 5 (very severe symptoms). Within each dimension, cumulative scores indicate the severity of the complaints on each sub-domain; also the cumulative of all dimensions indicate an overall view of Ageing Males' Symptoms. The cumulative score can range from 5 to 85 points. The severity of the symptoms can be defined as: no/little (17-26 points), mild (27-36 points), moderate (37-49 points) and severe (≥ 50 points). The questionnaire was assessed at baseline and after 6 months of supplementation.

Laboratory

Fasting blood samples were obtained between 8.00 and 11.00 AM to minimize diurnal variation. The serum levels of testosterone was measured with a solid-phase, competitive, chemiluminescent enzyme immunoassay (IMMULITE ® 2000 Total Testosterone, Diagnostic Products Corporation, Los Angeles, California, USA) at baseline and at the end of the study.

Data analysis

For the first research question, to assess the association between the testosterone concentration and the symptoms of testosterone deficiency according to two screening questionnaires, we used the data of the 587 men we screened for in- or exclusion in the trial. Logistic regression analysis was used to calculate odds ratios (OR) and 95% confidence intervals (95% CI) for the relation between the scores on the androgen deficiency questionnaires and testosterone concentration or age. The diagnostic accuracy of the ADAM- and AMS-questionnaires was evaluated using receiver operating characteristic (ROC) curve analysis, reporting the area under the curve (AUC) and its 95% confidence interval (95% CI). We studied the diagnostic accuracy for 3 different cut-off

points for testosterone deficiency, , i.e. testosterone levels of 8 nmol/l, 12 nmol/l, and 13.7 nmol/l (lowest third of study population), respectively.

For the second research question, to assess the effects of testosterone supplementation during 6 months to 237 elderly men with a low-normal testosterone concentration on symptoms of testosterone deficiency according to the screening questionnaires, changes between final visit and baseline for continuous measures were expressed as means and 95% confidence intervals; unpaired t-tests were used for testing the difference in change between treatment groups. Logistic regressions was used to calculate odds ratios (OR) and 95% confidence intervals (95% CI) for the effect of treatment at the final visit for a state of androgen deficiency or not; models were adjusted for baseline visit score. The level of significance was set at $P < 0.05$. Because the percentage missing data was very small (< 3.6%), we did not use any specific strategies to handle this and the missing data were treated as missing values in the analysis.

All analyses were performed with the SPSS statistical software package, version 11.0 (SPSS Inc, Chicago, Ill)

Results

The baseline characteristics of the large group of 587 men are shown in *table 1*. The mean age of the participants was 67 ± 5 years and the mean serum testosterone concentration was 13.8 ± 1.9 nmol/L. The prevalence of symptomatic androgen deficiency according to the ISSAM (positive score on ADAM symptoms and low total testosterone concentration) was 30.8% when a cut-off point of 12 nmol/l was used and 5.3% when a cut-off point of 8 nmnol/l was used. More than 83% of the participants had a positive score on ADAM symptoms and 19.3% of the participants had moderate of severe symptoms on the AMS questionnaire.

TABLE 1. *Subject characteristics at baseline*

	Participants N=587 Mean (SD)
Age	67.3 (5.0) (range 60 - 80)
Total Testosterone (nmol/l)	13.8 (1.9) (range 0.5 – 31.0)
Score AMS – total	29.9 (8.6) (range 17 – 66)
Score AMS – psychological	7.4 (3.1) (range 5 – 21)
Score AMS – somatovegetative	12.3 (3.9) (range 7 – 27)
Score AMS – sexual	10.1 (3.4) (range 5 – 20)
Score ADAM - total	3.9 (2.3) (range 0 – 10)
	Number (%)
Score ADAM – positive score	488 (83.1)

Androgen deficiency questionnaires and T-levels

There was no relation between the androgen deficiency questionnaires and the testosterone concentration in the group of 587 men, whichever cut-off point of testosterone we studied (*table 2*). However, a higher age was associated with a 3% increased score at the AMS-questionnaire (OR 1.03, 95% CI 1.01-1.08), a 15% increase of the score on the sexual subscale of the AMS-questionnaire (OR 1.15, 95% CI 1.09-1.21) and a 14% increased score at the ADAM questionnaire (OR 1.14, 95% CI 1.05-1.23).

TABLE 2. *Relation between the androgen deficiency questionnaires and the testosterone concentration for different cut off points of testosterone or age (n=587)*

	Testosterone < 13.7 mmol/l OR (95% CI)	P-value	Testosterone < 12 mmol/l OR (95% CI)	P-value	Testosterone < 8 mmol/l OR (95% CI)	P-value	Age > 70 years OR (95% CI)	P-value
Score AMS – total	1.01 (0.99–1.03)	0.42	1.01 (0.99–1.03)	0.31	1.02 (0.98–1.06)	0.35	1.03 (1.01–1.08)	0.008
Score AMS – psychological	1.02 (0.97–1.07)	0.53	1.03 (0.97–1.08)	0.32	1.04 (0.94–1.16)	0.42	1.02 (0.96–1.08)	0.58
Score AMS – somato vegetative	1.01 (0.97–1.06)	0.59	1.02 (0.97–1.06)	0.51	1.03 (0.94–1.12)	0.54	1.02 (0.98–1.07)	0.37
Score AMS – sexual	1.02 (0.97–1.07)	0.39	1.02 (0.97–1.08)	0.37	1.05 (0.95–1.16)	0.34	1.15 (1.09–1.21)	< 0.001
Score ADAM - total	1.04 (0.97–1.12)	0.23	1.05 (0.98–1.13)	0.15	1.07 (0.92–1.24)	0.37	1.14 (1.05–1.23)	0.001

Abbreviations: OR, Odds Ratio; 95% CI, 95 % confidence interval

The areas under the curve in the ROC curve for age, the ADAM questionnaire and the AMS questionnaire for different cut off criteria for testosterone deficiency and for higher age (cut off point > 70 yrs) are shown in *table 3*. There were no significant differences for any of the predictors age, ADAM or AMS questionnaire for any of the cut off points for testosterone deficiency.

Table 3 *ROC curve analysis of the ADAM-questionnaire and the AMS-questionnaire*

	Area under the curve	95% CI	P-value
Cut off point testosterone 13.7 nmol/l			
Age	0.53	0.48-0.58	0.23
ADAM	0.53	0.48–0.57	0.28
AMS	0.52	0.47–0.56	0.50
Cut off point testosterone 12.0 nmol/l			
age	0.51	0.46-0.56	0.66
ADAM	0.54	0.49–0.58	0.16
AMS	0.53	0.48–0.58	0.24
Cut off point testosterone 8.0 nmol/l			
age	0.55	0.45-0.66	0.30
ADAM	0.55	0.45–0.65	0.32
AMS	0.55	0.46–0.65	0.31

Abbreviations: 95% CI, 95 % confidence interval

Effect of T-supplementation

During the 6 months of testosterone supplementation there were 30 early withdrawals, 16 in the testosterone group and 14 in the placebo group. Of the subjects completing the study, more than 90% used at least 80% of their medication. The effects of the treatment on safety parameters and testosterone and SHBG levels have been reported previously.[7]

The changes from baseline to month 6 on the ADAM-questionnaire and the AMS-questionnaire for the testosterone group and the placebo group are shown in *table 4*.

There were no significant differences in these questionnaires between both groups after 6 months of intervention. Also, the frequency of positive ADAM symptoms was similar between the testosterone and placebo groups.

Table 4 *Effects of testosterone supplementation for the ADAM-questionnaire and the AMS- questionnaire*

	Baseline Testosterone (n=113) Mean (SD)	Placebo (n=110) Mean (SD)	6 months Testosterone (n=113) Mean (SD)	Placebo (n=110) Mean (SD)	Δ difference (95% CI)	P-value
Score AMS – total	29.6 (7.8)	29.2 (8.1)	28.8 (8.0)	29.5 (9.2)	-1.09 (-2.6; 0.4)	0.15
Score AMS – psychological	7.3 (3.0)	7.4 (3.3)	7.3 (3.0)	7.6 (3.6)	-0.32 (-0.9; 0.2)	0.27
Score AMS – somato vegetative	12.1 (3.6)	12.0 (3.3)	11.9 (3.8)	12.0 (4.1)	-0.26 (-1.0; 0.5)	0.51
Score AMS – sexual	10.3 (3.3)	9.9 (3.5)	9.7 (3.1)	9.9 (3.5)	-0.51 (-1.2; 0.2)	0.13
Score ADAM - total	4.1 (2.2)	3.7 (2.4)	3.4 (2.4)	3.3 (2.4)	-0.39 (-0.9; 0.1)	0.15
	N (%)	N (%)	N (%)	N (%)	Odds Ratio (SD)	P-value
ADAM - positive	100 (88.5)	85 (77.3)	87 (77.0)	80 (74.1)	0.79 (0.4; 1.6)	0.52

Abbreviations: SD, standard deviation; 95% CI, confidence interval

In subanalyses, according to the testosterone level at baseline (testosterone < 12.0 nmol/l versus testosterone ≥ 12.0 nmol/l, and testosterone < 8.0 nmol/l versus testosterone ≥ 8.0 nmol/l) there were also no differences in the scores of the two questionnaires between both groups after 6 months of testosterone supplementation (data not shown).

Discussion

In this combined cross-sectional study and randomized trial, we found that there was no significant association between the total testosterone concentration and the scores on the ADAM and the AMS questionnaire or age in 587 community-dwelling healthy men between 60 and 80 years of age. Age was stronger associated with questionnaire outcomes than testosterone levels.

In the trial, we found that 160 mg testosterone supplementation daily during six months to 237 elderly men with moderately low circulating testosterone levels had no effect on the scores on the ADAM and the AMS questionnaire.

The lack of association between testosterone concentration and scores on the ADAM and the AMS questionnaires is in line with results of other studies.[8-11] Most study have found a

high sensitivity (larger than 80%) to identify aging males with low testosterone levels, but a low specificity (less than 40%). One of the reasons is that the symptoms associated with late onset hypogonadism are nonspecific and seem to be very common in the elderly population in general. The scores on the questionnaires increase with age but do not correlate with low total testosterone concentrations, which again agrees with findings in other studies.[12,13] Also several highly common pathologies in the elderly, like depression[14,15], may produce similar symptoms. Furthermore, it is recognized that the endocrinological changes associated with male ageing are not exclusively limited to testosterone.[16] Moreover, age associated changes in lifestyle may be important as indicated by the impact of the decrease in physical activity and the effects on muscle strength, bone mineral density and lack of energy. And last but not least, androgen sensitivity is variable, so the threshold below which each symptom starts is still not clear.[17] Because of all these factors, these tests cannot be used for the identification of androgen deficiency in elderly, community-dwelling males. We cannot exclude the possibility that in a younger age group symptomatology may be more specific and the scores on the AMS and ADAM questionnaires may better predict testosterone levels. In a previous validation study of the ADAM questionnaire in men between the age of 40 and 62 years a sensitivity of 88% and a specificity of 60% was found.[4] In our study, there was also no relation with any of the individual questions (except the item decrease in beard growth in the AMS questionnaire; difference -0.1, 95% CI -0.1; -0,0 p=0.01) (data not shown), indicating that different combinations of questions are unlikely to result in a different outcome. Also, there was no association between age and the testosterone concentration. So, higher age only is not a good predictor of low testosterone levels

Because most symptoms associated with hypogonadism are multi-factorial in origin, it is not surprising that we did not find an effect of testosterone supplementation on the symptoms of late onset hypogonadism. This lack of effect is comparable with other studies.[18] Although there are studies that show an improvement in ADAM and AMS scores after testosterone supplementation, most of these were uncontrolled observational or single-blind studies.[4,19-22] In our study and other studies, there was a trend for some of the items of the ADAM questionnaire to improve on placebo alone, highlighting the importance of appropriately placebo-controlled trials.[18]

To fully appreciate these results, some issues need to be addressed. First, the testosterone levels in this study population were low to low-normal. Seventy-one percent of the men had a testosterone level below 12.0 nmol/L, and are considered possibly testosterone deficient according to conventional standards.[2] The testosterone levels were comparable with other studies that found beneficial short-term effects of testosterone supplementation.[23,24]

The men in this trial were selected on the basis of their androgen status and not on the basis of symptoms that might indicate reduced testosterone levels. However, more than 80% of the participant had a positive score on the ADAM questionnaire indicating a possible testosterone deficiency and 58.7 % of the participants met the criteria of hypogonadism according to the ISSAM with a total testosterone concentration below 12.0 nmol/l and a positive score on the ADAM questionnaire.

Six months is a relatively short period for supplementation, but this time period is considered long enough to find positive effects on the symptoms of testosterone deficiency.

In conclusion, our data do not support the use of the ADAM or AMS questionnaire in the evaluation of late onset hypogonadism in ageing men. Moreover, 6 months of oral testosterone supplementation had no significant effect on ADAM and AMS scores in this group elderly men with low-normal testosterone levels.

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Chapter 4.2

Effect of testosterone supplementation on functional mobility, cognition and other parameters in older men: a 6-month randomized controlled trial.

Abstract

Context Serum testosterone levels decline significantly with aging. Testosterone supplementation to older men might beneficially affect the aging processes.
Objective To investigate the effect of testosterone supplementation on functional mobility, cognitive function, bone mineral density, body composition, plasma lipids, quality of life, and safety parameters in aging men with low normal testosterone levels.
Design, Setting and Participants Double-blind, randomized, placebo-controlled trial of 237 healthy men between 60 and 80 years of age with a testosterone level < 13.7 nmol/L.
Intervention Participants were randomly assigned to receive 80 mg testosterone undecanoate or a matching placebo twice daily for 6 months.
Main Outcome Measures Functional mobility was assessed using the Stanford health assessment questionnaire, the timed "get up and go" test, the isometric handgrip strength and the isometric leg extensor strength. Cognitive function was assessed using 8 different cognitive instruments. Bone mineral density of the hip and lumbar spine was assessed using dual-energy X-ray absorptiometry (DEXA) scanning. Body composition was assessed using a total body DEXA and an abdominal ultrasound of the fat mass. Metabolic risk factors included levels of fasting plasma lipids, glucose and insulin. Quality of life was measured with the Short-Form Health Survey and the Questions on Life Satisfaction Modules. Safety parameters included serum PSA level, ultrasonographic prostate volume, International Prostate Symptom Score, serum levels of creatinin, AST, ALT, γGT, hemoglobin and hematocrit.
Results A total of 207 men completed the study. During the study, there was an increase of lean body mass and a decrease of fat mass in the testosterone group compared to the placebo group. The increase in lean body mass was not accompanied by an increase of functional mobility or muscle strength. There were no beneficial effects on cognitive function or bone mineral density. Treatment resulted in a net decrease of the total cholesterol concentration, but also a decrease of the HDL cholesterol. There was an increase of the hormone related quality of life in the testosterone group. There were no negative effects on prostate safety.

Conclusion The results of this trial shows that testosterone supplementation during 6 months to elderly men with a low normal testosterone concentration had a positive effect on body composition and quality of life, but no other relevant beneficial effects were seen.

Introduction

Male aging is associated with a gradual but progressive decline in serum levels of testosterone.[1] This occurs to a greater extent in some men than others. There is an association between the decline of testosterone and many symptoms and signs of aging such as decrease in muscle mass and muscle strength, cognitive decline, decrease in bone mass and increase of (abdominal) fat mass. Despite the rapid increase of people aged 60 years and older, there is hardly research on how to prevent or delay these age-related disabilities. In recent years, the potential anti-aging effects of sex hormones, including testosterone, have become a focus of interest.

Clinical trials examining the effects of testosterone supplementation on aging have provided mixed findings.[2-5] These different findings likely reflect differences in study design, including age, gonadal and health status of the study population, the type and duration of treatment and the chosen instruments to study aging. Importantly, most studies had only limited power to detect effects due to small sample sizes. Moreover, most trials have studied only 1 or 2 aspects of aging, in stead of the whole spectrum of aging. Additional data are needed to elucidate whether older men receiving testosterone supplementation experience benefits. Also the safety of testosterone supplementation remains controversial. For these reasons the US Institute of Medicine of the National Academies (IOM) Committee On Assessing The Need For Clinical Trial Of Testosterone Replacement Therapy recommended in 2004 that first short-term, randomized, placebo-controlled studies to examine the efficacy and safety of testosterone therapy in aging men should be conducted, before embarking on long-term studies.[6]

We conducted a randomized, double-blind, placebo-controlled study to assess the effects of testosterone supplementation on functional mobility, cognition, bone mineral density, body composition, lipids, quality of life, and safety parameters in elderly men with low normal testosterone levels during a period of six months.

Subjects and Methods

This study had a randomized, double-blind, placebo-controlled design. Details of the study design, recruitment, and procedures have been published previously.[7] The Institutional

Review Board of the University Medical Centre Utrecht approved the study protocol and all participants provided written informed consent.

Participants

Participants were recruited by direct mailing to 8020 randomly selected men between 60 and 80 years of age whose addresses were obtained from the municipal register of the city of Utrecht, the Netherlands. We did not pay volunteers for participation, but only reimbursed travel expenses.

Inclusion criteria included: a testosterone level below the 50th percentile of the study population based testosterone distribution and an age between 60 and 80 years. The 50th percentile cut-off level of testosterone was determined to be 13.7 nmol/L after screening of 50 candidates. This was comparable with the 50th percentile of the testosterone level at the end of the study (13.8 nmol/L). Exclusion criteria included: myocardial infarction or cerebrovascular accident within the past 6 months, heart failure unless medically treated and not symptomatic, malignancy within the past 5 years except for non-melanoma skin cancer, any hormone dependent tumor in the history, serious liver- or renal diseases (more than 3 times the upper limit of reference value), hematological abnormalities (hemoglobin $\leq$ 7.0 mmol/l, hematocrit $\geq$ 0.50), epilepsy or the use of anti-epileptic medication, migraine more than once a month, diabetes mellitus, a fasting glucose level $\geq$ 6.9 mmol/l, corticosteroid use ($\geq$ 7.5 mg orally a day within the past 6 months, with the exception of short bouts of prednisone for the period of 7 days, or inhalation of $\geq$ 800 µg a day during the past 6 months), use of testosterone esters and alike substances within the past 60 days, prostate hypertrophy in medical history, and an elevated PSA level (age 60-69 years $\geq$ 4.5 µg/l; 70 years and above $\geq$ 6,5 µg/l).

Following an initial telephone contact 684 men were screened with medical history, laboratory testing and digital rectal examination. Finally, 237 men were eligible for entry into the study and agreed to participate.

Randomization and blinding

After completing the baseline tests, participants were randomly assigned to the intervention or the placebo group. A randomization list without stratification using blocks of 6 was computer generated using the Almedica Drug Labeling System (Almedica Technology

Group Inc., Allendale, NJ, USA) by Organon NV, Oss, The Netherlands. One box with active medication and one box with placebo medication were delivered at the University Medical Centre Utrecht Pharmacy with the randomization list. Pharmacy personnel labeled the jars for the participants and provided the study medication upon prescription of the trial physician. Randomization numbers were assigned to the subjects in order of enrolment into the trial.

The key to the randomization numbers i.e. treatment codes was available 24 hours a day at the Pharmacy department of the University Medical Centre Utrecht. Deblinding in cases of emergency was possible at individual base, so no other treatment codes were visible to the research physicians during emergency deblinding. Deblinding was not necessary during the trial.

To assess the efficacy of blinding, at the end of intervention the participants were asked whether they thought they had been assigned to the placebo or the testosterone group.

Intervention

The intervention consisted of two capsules of 40 mg testosterone undecanoate (Andriol® Testocaps, N.V. Organon, Oss, the Netherlands) twice a day with breakfast and dinner (= a total daily dose of 160 mg testosterone undecanoate), or matching placebo, for a total duration of 6 months. Adherence was monitored by capsule counting at each study visit.

Functional mobility

Timed "Get Up and Go" test

During the timed "Get Up & Go" test, the time taken by a subject to rise from a standard chair, walk three meters, turn around, return and sit down again, was measured. The subject was requested to sit with his back against the chair and arms resting on the chair and perform the test three times. The fastest time was recorded in seconds.[8]

Stanford Health Assessment Questionnaire (HAQ)

The Standford Health Assessment Questionnaire (HAQ) is a self-administered questionnaire to measure physical ability. We used a Dutch version of the HAQ, which consists of 24 questions about ordinary activities in eight categories.[9] The categories are dressing, arising, eating, walking, hygiene, reach, grip and common activities. All questions have four alternatives to choose from, ranging from "without difficulty" (assigned a score

of zero) to “unable to perform” (assigned a score of three). Moreover, subjects can indicate whether they need aid or need an assistance device. When a subject indicates the need for aid or a device on a certain question, the corresponding category score is increased to two when that score was zero or one.

The total score on the HAQ is calculated by taking the highest score within each category and subsequently, calculating the mean of the eight category scores. This can range from zero (no disability) to three (completely disabled).

Isometric grip strength testing (IGS)

Isometric handgrip strength (IGS) was measured using an adjustable hand dynamometer (JAMAR® dynamometer).[10] The size of the grip was set so that the participants felt comfortable. The subjects were in standing position and were instructed to keep their shoulders adducted and neutrally rotated, the arm was vertical and the wrist was in a neutral position. The subjects squeezed the grip with maximal strength, alternating the left and right hand. Each test was repeated at least five times until no further improvements were seen. The best measure at each side, recorded in kilograms, was used for analysis.

Isometric leg extensor strength (ILES)

Maximal voluntary isometric knee leg strength was measured using the Hoggan MicroFET hand-held dynamometer.[11] The subjects were in a seated position at a mat-table with the hip flexed to 90 degrees, the knee stretched to 180 degrees and the legs dependent. The dynamometer was applied perpendicularly to each lower extremity just proximal to the malleoli. Participants were instructed to take a second or two to come to maximum effort and to then push as hard as possible during another three seconds, while the investigator was giving counterforce. Five maximal voluntary contractions were made at each side, and if the examiner was not confident that a maximal effort was reached two more efforts were made. The best measure for each side, recorded in Newton, was used for analysis.

Cognitive function

Subjects were tested in a silenced room during the morning. The tests were administered by trained doctors. Equal versions of the test were administered during baseline and at the end of the study, except for the Shepart Mental Rotation test for which alternate versions were used.

15-Words test

The Dutch version of the Rey Auditory Verbal Learning Test is a test for verbal episodic memory. In this test, the participants are asked to recall 15 words immediately (immediate recall) for five times consecutively (maximum score is 75) and after 15 minutes (delayed recall, maximum score is 15).[12]

Digit Symbol Substitution Test

The digit symbol substitution test, a subtest of the Wechsler Adult Intelligence Scale (WAIS), measures cognitive and perceptual speed. The participant is given a code that pairs symbols with digits. The test consists of matching as many digits to their corresponding symbols as possible in 90 seconds.[13] Participants were scored a point for each correct response.

Trail Making Test

The trail making test is a complex attention and mental flexibility task. In this test, pseudo-randomly placed circles with numbers (Trail Making A1), with letters (Trail Making A2), and with both numbers and letters (Trail Making B) have to be connected with a line as fast as possible in a fixed order. In the event of error, the subjects were immediately informed and asked to restart from the point of error: this was done with the timer left running. The time taken to complete the trail without error was recorded.[14]

Benton Judgment of Line Orientation

This test measures basic perceptual processes contributing to extra-personal spatial perception. The test requires the subject to identify which 2 of 11 lines presented in a semicircular array have the same orientation in two-dimensional space as two-target lines.[15] There are 30 items and participants get a point for each correct answer.

Shepard rotation task

Visuospatial performance was assessed by the Vandenberg and Kuse adaptation of Shepard and Metzler's three-dimensional mental rotations test.[16] The test consists of 20 items in which the subject is presented with a three-dimensional geometric target line drawing and four test drawings, and is required to indicate which two of four test drawings depict the target drawing in rotated positions. Two parallel test versions are made by taking the odd and even items on time 1 (baseline) and time 2 (after intervention) respectively (10 items for each test). These parallel versions have been shown to correlate strongly with each other and to have a high reliability.[17] The test is scored by adding one point for each correct

answer and subtracting a point for each incorrect answer. So, the score can change between –10 (no correct answer) and +10 (all of them correct). Subjects are instructed to "work as quickly as possible, but do not sacrifice accuracy for speed". They were allowed 10 minutes to complete the test.

Bone Mineral Density

Bone mineral density (BMD) was measured with dual energy X-ray absorptiometry (DEXA) using a Lunar prodigy® DEXA instrument at baseline and at the end of the 6-month intervention. Scanning was performed according to the instructions of the manufacturer. BMD was measured of lumbar vertebrae (L1-L4 individually and together) and proximal femur (femoral neck, trochanter, inter-trochanter, Ward's triangle and total hip, left or right if left is not available). Quality assurance, including calibration was performed routinely every morning for DEXA, using the standard provided by the manufacturer.

Anthropometry

Weight was measured, after taking of coat, sweaters and shoes, to the nearest 0.5 kg and height was measured to the nearest 0.5 cm. Body mass index (BMI) was calculated as the weight in kilograms divided by the square of the height in meters.

All circumference measurements were done with a standard household centimetre. Waist circumference was measured at the level of midway the distance between the lower rib and the iliac crest, after normal expiration without pressure of the centimetre at the skin. All measurements were performed in duplicate, and the average of the readings was taken as the value for each circumference with results rounded to the nearest 0.1 cm.

Body composition

Total body DEXA

Total body composition measurements were performed with dual energy X-ray absorptiometry (DEXA) using a Lunar prodigy® DEXA instrument. Scanning was performed according to the instructions of the manufacturer. After placement of the subject on the table, there was scanning of the whole subject from dorsal to ventral. Both legs and

feet were endorotated and fixed to on another. Calculations were made regarding fat-mass, fat-free mass and lean body mass. Quality assurance, including calibration was performed routinely every morning for DEXA, using the standard provided by the manufacturer.

Abdominal ultrasound of the fat mass

Ultrasonography of the fat mass was performed in all subjects with an Ultramark 9®. The distances between the posterior edge of the abdominal muscles and the lumbar spine or psoas muscles were measured using electronical callipers. For all images the transducer was placed on a straight line drawn between the left and right midpoint of lower rib and iliac crest. A mark was made in the middle, 10 cm from the left and right side. Distances were measured from three different angles: medial, left and right for intra-abdominal fat mass and medial for subcutaneous fat mass. Measurements were made at the end of quiet expiration, applying minimal pressure without displacement of intra-abdominal contents as observed by ultrasound image.

Laboratory

Fasting blood samples were obtained between 8.00 and 11.00 AM to minimize diurnal variation. The serum levels of testosterone and sex hormone binding globulin (SHBG) were measured with a solid-phase, competitive, chemiluminescent enzyme immunoassay (IMMULITE ® 2000 Total Testosterone, Diagnostic Products Corporation, Los Angeles, California, USA) at baseline and at the end of the study. The levels of free testosterone (FT) and bioavailable testosterone (BT) were calculated from total testosterone, SHBG and albumin concentrations.[18]

Fasting glucose levels were assessed using a GlucoTouch reflectometer (LifeScan, Inc., Benelux), a reagent-strip glucose oxidase method. Venous whole blood was immediately applied to the test strip.

Fasting plasma insulin levels, total cholesterol, high-density lipoprotein (HDL) cholesterol and triglycerides were measured using commercially available assays at baseline and at the final visit. Low-density lipoprotein (LDL) cholesterol was calculated with the Friedewald equation.[19]

To assess insulin sensitivity in the subjects, we calculated the homeostasis model assessment of insulin resistance (HOMA-IR) and the quantitative insulin sensitivity check index (QUICKI). HOMA-IR and QUICKI were calculated using:

HOMA-IR = [fasting insulin (mU/l) * fasting glucose (mmol/l)]/22.5,
QUICKI = 1/[log (fasting insulin (mU/l)) + log (fasting glucose (mg/dl))].
We used HOMA-IR and QUICKI to measure insulin resistance and sensitivity instead of the hyperinsulinemic euglycemic clamp, which is the gold standard for measuring insulin resistance. However, all these measurements have been validated and proved to be strongly correlated with insulin resistance measured by clamp (correlation coefficients of -0.82 and 0.81, respectively).[20-22]

Blood pressure

Systolic and diastolic blood pressures were measured in duplicate at the left arm with the subjects in sitting position after 5 minutes of rest with an automated and calibrated oscillometric device (Omron Healthcare Europe, Hoofddorp, the Netherlands). Subsequently, the mean systolic and diastolic blood pressures were calculated.

Metabolic syndrome

The metabolic syndrome according to the National Cholesterol Education Program (NCEP) Adult Treatment Panel III (2001) was defined as present when 3 or more of the following criteria were met: fasting plasma glucose of at least 6.1 mmol/L (110 mg/dL), serum triglycerides of at least 1.7 mmol/L (150 mg/dL), serum HDL cholesterol less than 1.0 mmol/l (40 mg/dL), blood pressure of least 130/85 mmHg or waist girth of more than 102 cm.[23]

Quality of Life

Quality of Life was measured with the Short Form-36 Health Survey as a generic Quality of Life questionnaire (SF-36) and the Questions on Life Satisfaction Modules as a hormone specific questionnaire.

Short Form-36 Health Survey

The SF-36 includes nine measures of functioning relating to 1) physical functioning; 2) social functioning; 3) role limitations because of health problems (physical role); 4) role limitations due to emotional problems (emotional role); 5) mental health; 6) vitality; 7) bodily pain; 8) general health perception; and 9) reported health transition from the last

month. Raw scores were transformed to a standardized scale ranging from 0 to 100, with the higher score representing better status.[24]

Questions on Life Satisfaction Modules

The Questions on Life Satisfaction Modules is a questionnaire translated from the questionnaire "Fragen zur Lebenszufriedenheit"(FLZ) according to the method described by Henrich and Herschbach.[25] The questionnaire is extended with a module on hypopituarism[26] and divided in a "general", a "health" and a "hormone" section, the first two sections include eight items and the last section includes nine items. All items were recorded on a 5-point scale according to their individual importance (I) and degree of satisfaction (S). As effect measure, a combination of importance and satisfaction (I-1)*(S*2-5) was calculated. In addition the sum of the combination values was calculated for each section. The scores of the "general" and "health" section can change between –96 and + 160, while the score of the "hormone" section can change between –108 and +180. The higher the scores the better the quality of life status.

Safety

The safety of testosterone supplementation was assessed with measuring of the prostate, liver and kidney function, and haematological parameters.

Prostate

Effects on the prostate were studied using serum prostate-specific antigen levels, rectal ultrasound of the prostate, and by the International Prostate Symptom Score.

Serum prostate specific antigen (PSA) levels were measured by an immunnometric assay (IMMULITE® 2000 PSA, Diagnostic Products Corporation, Los Angeles, California, USA) at baseline, week 13 and at the end of the study. The intra- and inter-assay coefficient of variation was 3.5 and 5.0% respectively. Increases of $\geq$ 1.4 μg/L above baseline level on two consecutive measurements during 1-2 weeks prompted treatment discontinuation.

Biplanal transrectal ultrasonography of the prostate, using a 7-MHz transrectal probe (Bruel and Kjaer Model 2101 Falcon), were performed at entry and at the end of the study by an experienced urologist. For each subject the volume of the total prostate was determined with a calliper-based method: height x width x length x $\pi/6$. [27] Furthermore attention was placed on the presence of hypoechogenic lesions in the prostate. The sonographic criteria

for prostate cancer described by Lee and colleagues were used. [28] If abnormalities were found, patients were sent to the urology outpatient clinic for further evaluation.
The International Prostate Symptom Score (IPSS) is developed by the American Urological Association (AUA) and comprises 7 questions regarding urological symptoms. [29] The questions can be scored from 0 (no complaints at all) to 5 (almost always). The cumulative scores of all seven questions are an indication of the severity of lower urinary tract symptoms. The maximum score is 35. The subjects filled in the IPSS at baseline, after 6 and 13 weeks, and at the end of the study.

Liver and kidney function and haematological parameters

Liver function (aspartate aminotransferase (AST, alanine aminotransferase (ALT), alkaline phosphatase (AF) and γ-glutamyl transferase (GGT)), kidney function (albumin and creatinin) and hematological parameters (hemoglobin and hematocrit) were measured in serum by standard autoanalyzer methodologies (Synchron LX®, Beckman Coulter, Fullteron, California, USA) at baseline, after 13 weeks, and at the end of the study. During the study, hemoglobin levels of ≤ 7 mmol/l, hematocrit levels ≥ 0.50, liver function values $\geq$ three times normal upper normal reference level (ASAT: 15-45 U/L; ALAT: 10-50 U/L; AF: 40-130 U/L ; γ-GT: 15-70 U/L), or creatinin levels of ≥ 180 µmol/l led to an extra blood check after a week. If the values were still too high study participation was discontinued.
All laboratory measurements were done in the SHO laboratory, Velp, the Netherlands.

Adverse Events

An adverse event was defined as any untoward medical occurrence in a participant, which does not necessarily have to have a causal relationship with the treatment. An AE could therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal report, whether or not considered related to the medicinal product.
Information regarding adverse events was obtained by questioning or examining the subject. At each visit during the treatment period, all new complaints and symptoms (i.e. those not existing before the treatment period) were recorded on the adverse event form. Pre-existing complaints or symptoms that increased in intensity or frequency during the treatment period were entered on the adverse event form also.

A serious adverse event was defined as any medical occurrence that resulted in death, was life-threatening, required in-patient hospitalisation or resulted in persistent or significant disability or incapacity. All serious adverse events were reported to the Institutional Review Board and to Organon NV, Oss, The Netherlands.

Data analysis

We performed power calculations for the primary end point: the 15 Words test for cognitive function. The planned number of subjects was 240 in total, 120 in each intervention arm. This number was based on conventional assumptions of α=0.05 and β=0.20, withdrawal from intervention of 15% and an improvement of 18% on the 15 Words test. This means an improvement of 6 words on the 15 words test.

Data were analyzed according to a modified intention-to-treat principle, including all those who had 2 measurements, including baseline, in the groups to which they were randomized. According to the protocol a second visit was performed after 3 months and a final visit was performed after 6 months. When a participant remained in the study for less then 3 months, no second visit or close out visit was performed, because this time is too short to find any beneficial effects of the treatment. When a participant dropped out between 3 and 6 months, a close-out visit was performed at the time of drop out. 12 participants (5.1%) (6 in the testosterone group and 6 in the placebo group) did not complete 3 months of treatment and 2 participants (0.8%) (1 in the testosterone group and 1 in the placebo group) were unable of unwilling to participate in a second and final visit. In total, 207 participants completed 6 months of intervention (104 in the testosterone group and 103 in the placebo group) and 16 participants did not complete 6 months, but received a close-out visit (9 in the testosterone group and 7 in the placebo group). These 223 participants (94.1%) were included in the primary, modified intention-to-treat analysis.

Changes between final visit and baseline for continuous measures were expressed as means and 95% confidence intervals; unpaired t-tests were used for testing the difference in change between treatment groups. All comparisons were two-tailed and the level of significance was set at $P < 0.05$. Because the percentage missing data was very small (< 3.6%), we did not use any specific strategies to handle this and the missing data were treated as missing values in the analysis. Repeated-measures ANOVA was used to test the

statistical significance of the effects of testosterone versus placebo for the safety parameters.

All analyses were performed with the SPSS statistical software package, version 11.0 (SPSS Inc, Chicago, Ill)

Results

The flow of study subjects participants recruitment and enrolment is shown in *figure 1*. Between January 2004 and October 2004, we randomly enrolled 237 men in the study, 120 were assigned to testosterone and 117 to placebo. There were 30 early withdrawals, 16 in the testosterone group and 14 in the placebo group. From the withdrawals, there were 7 without follow-up in both groups. So, the primary analysis included 113 in the testosterone group and 110 in the placebo group. The baseline characteristics of the participants are shown in *table 1*.

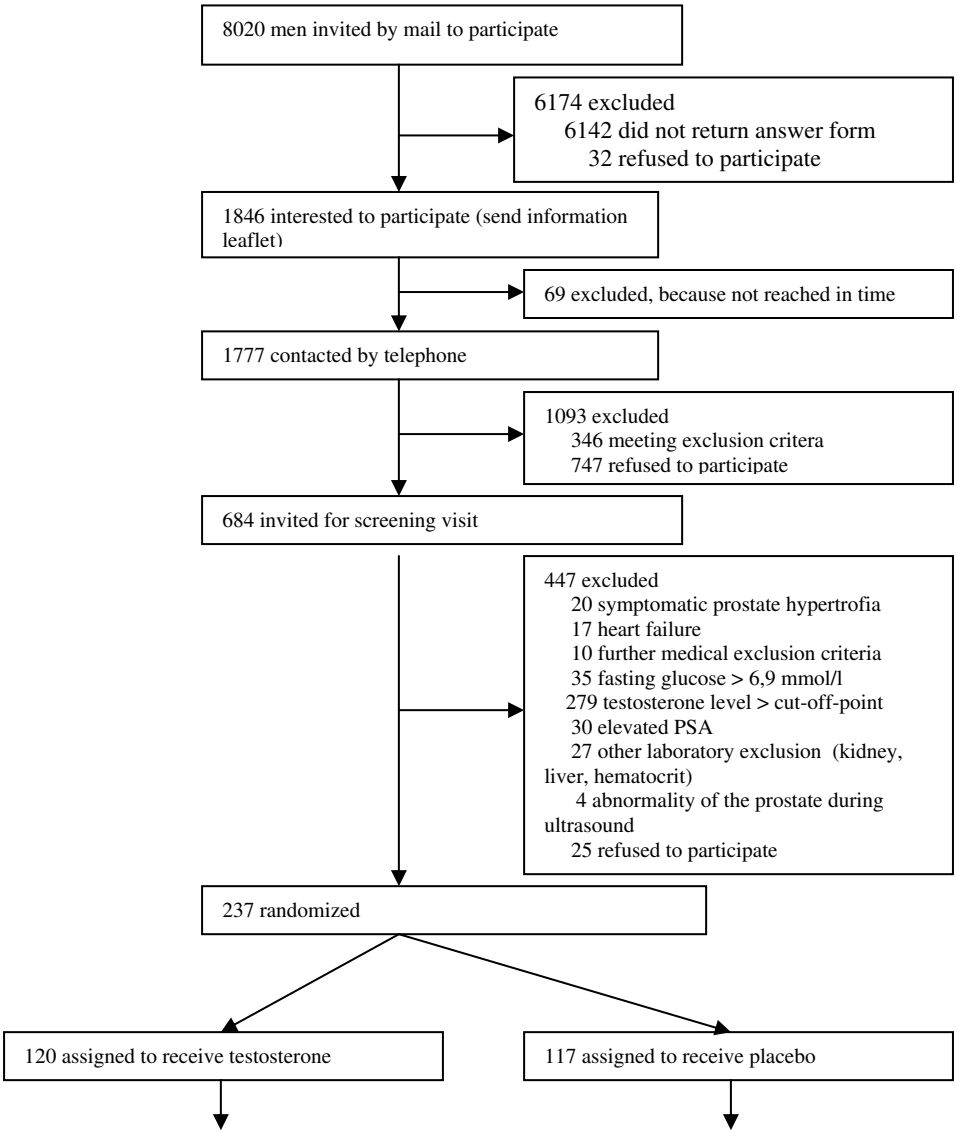
***TABLE 1**. Subject characteristics at baseline according to randomization group*

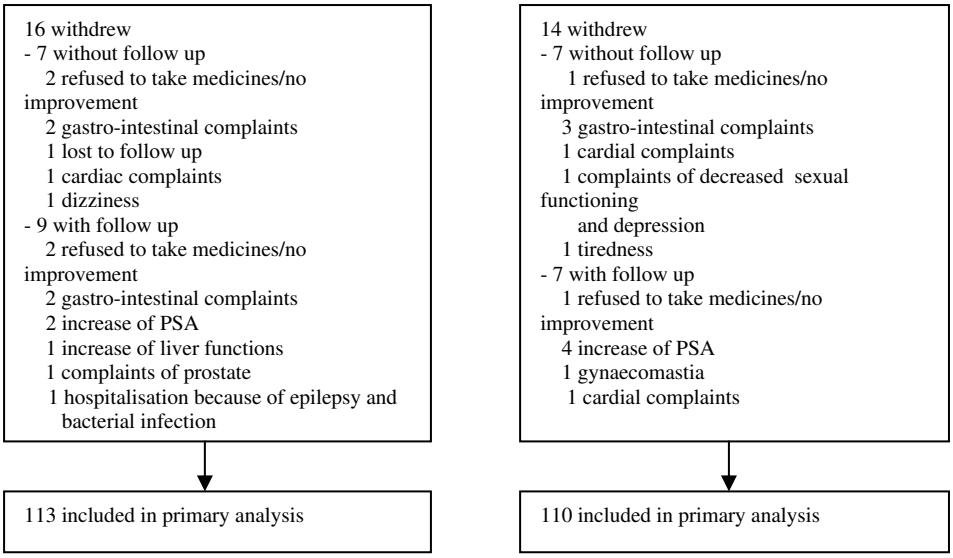
	Testosterone n=113 Mean (SD)	Placebo n=110 Mean (SD)
Age (yr)	67.1 (5.0)	67.4 (4.9)
Testosterone (nmol/L)	11.0 (1.9)	10.4 (1.9)
SHBG (nmol/L)	33.0 (10.7)	32.8 (10.0)
Albumin (g/L)	43.9 (2.3)	43.8 (2.4)
FT (nmol/L)	0.22 (0.02)	0.21 (0.0)
BT (nmol/L)	5.2 (1.1)	5.0 (1.2)
Body Mass Index (kg/m²)	27.4 (3.8)	27.3 (3.9)
Systolic blood pressure (mmHg)	155 (23.3)	151.4 (22.7)
Diastolic blood-pressure (mmHg)	89.2 (12.0)	86.8 (11.7)
	Number (%)	Number (%)
Smokers	21 (17.5)	15 (12.8)
Alcohol users	99 (82.5)	90 (76.9)
Prior cardiovascular disease ‡	35 (48.6)	37 (51.4)

‡ Includes myocardial infarction, angina, hypertension or stroke
Abbreviations: SD, standard deviation; SHBG, Serum Hormone Binding Globulin, FT, Free Testosterone; BT, Bioavailable Testosterone
Conversions: To convert testosterone from nmol/L to ng/dL divide by 0.0347.

The mean age of the participants was 67 ± 5 years and the serum testosterone concentration was 10.7 ± 1.9 nmol/L. There were no major differences between the 2 groups at baseline.

FIGURE 1 *Participant Flow Diagram*





Total testosterone was unchanged from baseline in the testosterone group and increased slightly in the placebo group; the difference between the testosterone and placebo group at month 6 was -3.2 nmol/l (95% confidence interval [CI] -4,2; -2.2, p<0.001). SHBG levels declined from baseline in the testosterone group and did not in the placebo group (difference -10.1 nmol/l, 95% CI -11.7; -8.5, p<0.001). Also the between-group difference for FT and BT was statistically significant at month 6 (FT difference -0.03, 95% CI -0.05; -0.00, p=0.04, and BT difference=-0.69, 95% CI -1.24; -0.13, p=0.02, respectively. The compliance, assessed by counting returned capsules, was good in both groups. Over 90% of participants used at least 80% of their medication. Blinding had been effective, since there was no difference in distribution of the answers on the treatment group the participants were thought to be randomised to between men in the testosterone group and men in the placebo group (p-value chi-square test 0.976).

Functional Mobility (table 2)

There were no significant differences between functional mobility among the two groups at baseline. Subjects in the testosterone and placebo groups had no significant change in score

on the Stanford Hamilton Assessment Questionnaire. Also measures of isometric grip strength, isometric leg extensor strength and timed get up and go test were not significantly altered by treatment with testosterone compared to placebo.

TABLE 2. *Results for Functional Mobility*

	Baseline Testosterone (n=113)*	Placebo (n=110)*	Final Visit Testosterone (n=113)*	Placebo (n=110)*	Δ difference (95% CI)	P-value
HAQ (score 0-3)	0.02 (0.1)	0.06 (0.2)	0.05 (0.1)	0.07 (0.2)	0.0 (-0.0/0.0)	0.61
IGS left (kg)	43.0 (9.7)	44.4 (11.6)	42.3 (8.8)	42.7 (8.2)	-1.1 (-3.5/1.3)	0.54
IGS right (kg)	44.6 (8.7)	46.5 (9.5)	43.0 (7.5)	43.4 (7.7)	-1.7 (-3.6/0.2)	0.16
ILES left (N)	78.8 (29.4)	84.5 (36.3)	73.3 (25.0)	75.2 (24.8)	0.2 (-9.8/10.2)	0.83
ILES right (N)	79.8 (29.0)	84.3 (35.9)	73.2 (24.3)	77.0 (26.0)	-2.0 (-12.1/8.0)	0.48
Timed Get Up and Go Test (sec)	4.24 (0.9)	4.24 (1.0)	4.27 (0.7)	4.34 (1.0)	0.1 (-0.1/0.2)	0.70

Results are the mean (SD)
Abbreviations: CI, Confidence Interval; HAQ, Health Assessment Questionnaire; IGS, Isometric Grip Strength; ILES, Isometric Leg Extension Strength.
* Occasionally data were missing for some participants, but the percentage missing values never exceeded 3.6%

Cognitive function (table 3)

Baseline performance on the cognitive function tests was similar for the 2 groups. At the end of the study both groups had higher scores on most of the tests, but the differences were small and there were no significant differences in change of cognition between the testosterone group and the placebo group.

Bone Mineral Density (table 4)

The mean scores at baseline were 1.03 (SD 0.14) and 1.22 (SD 0.19) for the placebo group and 1.03 (SD 0.15) and 1.21 (SD 0.18) for the testosterone group for hip and lumbar spine respectively. Neither subjects in the testosterone group nor in the placebo group had significant increases in BMD at any of the sites.

TABLE 3. *Results for Cognitive function*

	Baseline Testosterone (n=113)*	Placebo (n=110)*	Final Visit Testosterone (n=113)*	Placebo (n=110)*	Δ difference (95% CI)	P-value
Benton Judgment of line orientation (maximum score 30)	25.6 (3.7)	25.8 (3.7)	25.9 (3.2)	26.1 (2.9)	0.0 (-0.7/0.7)	0.86
Digit Symbol substitution (score number of symbols)	44.8 (10.9)	46.0 (10.4)	47.0 (11.0)	47.9 (10.5)	0.4 (-0.9/1.7)	0.57
Shepard Mental rotation (maximum score 10)	4.8 (7.1)	5.9 (6.4)	6.3 (6.2)	7.5 (6.7)	-0.1 (-1.6/1.5)	0.93
Rey Auditory Verbal Learning Test – immediate recall (maximum score 75)	35.5 (9.5)	34.9 (9.6)	37.8 (10.2)	36.6 (8.3)	0.5 (-1.3/2.3)	0.57
Rey Auditory Verbal Learning Test - delayed recall (maximum score 15)	7.1 (2.6)	6.9 (2.8)	7.8 (2.8)	7.5 (2.5)	0.1 (-0.5/0.7)	0.69
Trail making A1 (sec)	47 (18)	48 (16)	44 (16)	43 (13)	1.3 (-2.3/4.9)	0.50
Trail making A2 (sec)	53 (33)	55 (34)	49 (28)	47 (22)	2.5 (-3.0/8.0)	0.45
Trail making B (sec)	108 (51)	101 (43)	107 (66)	95 (43)	5.4 (-7.2/17.9)	0.40

Results are the mean (SD)
Abbreviations: CI, Confidence Interval
* Occasionally data were missing for some participants, but the percentage missing values never exceeded 3.6%

TABLE 4. *Results for the Bone Mineral Density*

	Baseline Testosterone (n=113)*	Placebo (n=110)*	Final Visit Testosterone (n=113)*	Placebo (n=110)*	Δ difference (95% CI)	P-value
Total hip (g/cm^2)	1.03 (0.1)	1.03 (0.2)	1.02 (0.2)	1.03 (0.2)	0.0 (-0.0/0.0)	0.77
Lumbar Spine (g/cm^2)	1.22 (0.2)	1.21 (0.2)	1.23 (0.2)	1.23 (0.2)	-0.0 (-0.0/0.0)	0.47

Results are the mean (SD)
Abbreviations: CI, Confidence Interval
* Occasionally data were missing for some participants, but the percentage missing values never exceeded 3.6%

Body composition (table 5)

The mean BMI at baseline was 27.4 (SD 3.8) for the testosterone group and 27.5 (SD 3.8) for the testosterone group. Total body fat mass and the fat percentage of the body decreased significantly in the testosterone group, while the placebo group remained stable after treatment; the fat percentage decreased from 27.7 (SD 6.0) to 26.4 (SD 6.2.) ($p<0.001$) and the total body fat mass decreased from 23.2 (SD 7.9) to 22.7 (SD 8.1) in the testosterone group ($p<0.001$). There was a significant increase of the total body lean body mass in the

testosterone group from 58.9 (SD 6.8) to 60.0 (SD 6.0), and a decrease in the placebo group from 58.3 (SD 7.6) to 58.0 (SD 75) (p=0.000). There were no significant differences in the change of baseline in body mass index and intra-abdominal fat mass measured by ultrasound.

TABLE 5. *Results for Body Composition*

	Baseline Testosterone (n=113)*	Placebo (n=110)*	Final Visit Testosterone (n=113)*	Placebo (n=110)*	Δ difference (95% CI)	P-value
BMI (kg/m²)	27.4 (3.8)	27.3 (3.9)	27.5 (3.8)	27.4 (3.9)	0.0 (-0.2/0.3)	0.76
Total fat mass (kg)	23.2 (7.9)	22.9 (7.2)	22.2 (8.1)	22.8 (7.1)	-1.3 (-1.8/-0.8)	0.000
Total lean mass (kg)	58.9 (6.8)	58.3 (7.6)	60.0 (6.6)	58.0 (7.5)	1.2 (0.7/1.7)	0.000
Fat mass percentage (%)	27.7 (6.0)	27.8 (5.4)	26.4 (6.2)	27.8 (5.4)	-1.7 (-2.1/-1.1)	0.000
Intra-abdominal fat - Ultrasound (cm)	8.3 (2.3)	8.2 (2.0)	8.6 (2.5)	8.5 (2.1)	-0.0 (-0.4/0.4)	0.98
Subcutaneous fat - Ultrasound (cm)	2.6 (0.8)	3.5 (0.8)	2.5 (0.8)	2.7 (0.8)	0.7 (-0.8/2.4)	0.34

Results are the mean (SD)
Abbreviations: CI, Confidence Interval; BMI, Body Mass Index.
* Occasionally data were missing for some participants, but the percentage missing values never exceeded 3.6%

Lipids, glucose and metabolic syndrome (table 6 and 7)

At the end of the study both total cholesterol and HDL cholesterol decreased significantly in the testosterone group; total cholesterol decreased from 5.6 (SD 1.0) to 5.4 (SD 1.0) in the testosterone group and from 5.5 (SD 1.0) to 5.4 (SD 1.0) in the placebo group (p=0.03). HDL Cholesterol decreased from 1.2 (SD 0.3) to 1.0 (SD 0.3) in the testosterone group and remained stable in the placebo group (1.1 (SD 0.3) before and after treatment (p<0.001). This resulted in a significantly increase of the ratio total cholesterol versus HDL cholesterol in the testosterone group from 5.1 (SD 1.3) to 5.5 (SD 1.4)) compared with the placebo group 5.0 (SD 1.2) before and after treatment (p<0.001). There were no significant changes in triglycerides and LDL cholesterol. The glucose concentration and the insulin concentration increased significantly in the placebo group compared with the testosterone group; glucose increased from 5.5 (SD 0.6) to 5.7 (SD 0.8) in the placebo group and remained stable in the testosterone group (5.6 (SD 0.7) (p=0.007) and insulin increased from 8.9 (SD 5.5) to 11.5 (SD 11.8) in the placebo group and remained stable in the testosterone group (10.1 (SD 9.8) and 10.2 (SD 7.6) (p=0.04). Also, the QUICKI index

(insulin sensitivity) decreased and the insulin HOMA index (insulin resistance) increased significantly in the placebo group.

TABLE 6. *Results for Plasma Lipids and Glucose*

	Baseline Testosterone (n=113)*	Placebo (n=110)*	Final Visit Testosterone (n=113)*	Placebo (n=110)*	Δ difference (95% CI)	P-value
Glucose (mmol/L)	5.6 (0.6)	5.5 (0.6)	5.6 (0.7)	5.7 (0.8)	-0.2 (-0.4/-0.1)	0.01
Insulin (µIU/L)	10.1 (9.8)	8.9 (5.5)	10.2 (7.6)	11.5 (11.8)	-3.2 (-6.2/-0.1)	0.04
Insulin sensitivity – Quicky	0.3 (0.0)	0.4 (0.0)	0.3 (0.0)	0.3 (0.0)	0.0 (0.0/0.0)	0.03
Insulin resistence – HOMA-IR	2.6 (2.6)	2.3 (1.5)	2.6 (2.0)	2.9 (2.9)	-0.9 (-1.7/-0.1)	0.02
Total Cholesterol (mmol/L)	5.6 (1.0)	5.5 (1.0)	5.4 (1.0)	5.4 (1.0)	-0.2 (-0.4/0.0)	0.03
HDL Cholesterol (mmol/L)	1.2 (0.3)	1.1 (0.3)	1.0 (0.3)	1.1 (0.3)	-0.1 (-0.2/-0.1)	0.000
Triglycerides (mmol/L)	1.6 (1.0)	1.5 (1.1)	1.5 (0.8)	1.6 (0.9)	-0.1 (-0.2/0.1)	0.33
LDL Cholesterol (mmol/L)	3.9 (0.9)	3.8 (0.9)	3.8 (1.0)	3.7 (0.9)	-0.0 (-0.2/0.1)	0.83
Ratio total cholesterol / HDL cholesterol	5.1 (1.3)	5.0 (1.2)	5.5 (1.4)	5.0 (1.2)	0.4 (0.2/0.6)	0.000

Results are the mean (SD)
Abbreviations: CI, Confidence Interval; HDL, high-density lipoprotein; LDL, low-density lipoprotein.
Conversions: To convert glucose from mmol/L to mg/dL divide by 0.0555; total cholesterol, HDL-C, and LDL-C from mmol/L to mg/dL divide by 0.0259; triglycerides from mmol/L to mg/dL divide by 0.0113.
* Occasionally data were missing for some participants, but the percentage missing values never exceeded 3.6%

The effects of testosterone supplementation on blood pressure have been reported previously (Nakhai Pour, submitted). No significant changes were seen in either systolic or diastolic blood pressure following testosterone treatment.

At the end of the study, there was a trend to an increase in the percentage men with a metabolic syndrome in the testosterone group, from 34.5% at baseline to 47.8% after 6 months of supplementation with testosterone (p=0.07). (table 7). This increase was specifically due to the decrease in HDL cholesterol level in the testosterone group.

***TABLE 7**. Results for the Metabolic Syndrome*

	Baseline Testosterone (n=113)*	Placebo (n=110)*	Final visit Testosterone (n=113)*	Placebo (n=110)*	Odds Ratio (SD)	P-value
Waist circumference	32.7	28.2	31.9	26.6	1.3 (0.7/2.3)	0.74
Glucose	24.8	19.1	22.3	27.4	0.8 (0.4/1.4)	0.10
Blood pressure	88.5	83.6	92.9	84.4	2.4 (1.00/5.9)	0.08
HDL cholesterol	36.6	39.1	56.3	42.2	1.8 (1.03/3.0)	0.003
Triglycerides	33.9	29.1	402.	33.0	1.4 (0.8/2.4)	0.46
Metabolic syndrome	34.5	30.9	47.8	35.5	1.7 (0.97/2.8)	0.07

Data are presented as percentage subjects that meet the criteria of the metabolic syndrome
Abbreviations: CI, Confidence Interval; HDL, high-density lipoprotein
* Occasionally data were missing for some participants, but the percentage missing values never exceeded 3.6%

Quality of Life (table 8)

The SF-36 scores were not significantly changed in the testosterone group compared to the placebo group for any of the nine sections of functioning. The Questions on Life Satisfaction Modules was also similar in the two groups for the general and health related quality of life. Only the hormone related quality of life increased significantly in the testosterone group from 59.4 (SD 34.1) to 62.3 (SD 32.9), while there was a decrease in the placebo group from 62.1 (SD 36.5) to 58.5 (SD 33.6) (p=0.03). This was mainly caused through the item “resilience/ability to tolerate stress”. (data not shown)

TABLE 8. *Results for Quality of Life*

	Baseline Testosterone (n=113)*	Placebo (n=110)*	Final Visit Testosterone (n=113)*	Placebo (n=110)*	Δ difference (95% CI)	P-value
SF36 physical functioning	89.8 (12.2)	86.8 (16.2)	89.6 (10.9)	86.1 (17.0)	0.7 (-2.0/3.5)	0.61
SF36 social functioning	92.0 (13.2)	91.0 (15.3)	91.9 (13.1)	89.8 (16.0)	1.5 (-2.2/5.2)	0.42
SF36 physical role	91.9 (21.4)	86.8 (29.1)	86.3 (28.7)	86.1 (29.5)	-4.5 (-12.0/3.0)	0.24
SF-36 emotional role	90.6 (24.8)	89.4 (29.0)	93.8 (20.5)	89.5 (27.3)	3.3 (-4.4/11.0)	0.41
SF36 mental health	81.7 (13.0)	81.5 (15.9)	81.7 (12.8)	81.6 (14.8)	0.2 (-2.3/2.8)	0.86
SF36 vitality	75.0 (14.6)	73.7 (16.5)	75.8 (14.8)	72.5 (16.1)	2.4 (-0.4/5.3)	0.10
SF36 bodily pain	87.7 (16.3)	84.9 (17.6)	87.1 (15.2)	85.5 (17.9)	-1.5 (-5.5/2.5)	0.47
SF36 general health perception	70.7 (16.2)	70.8 (16.0)	70.2 (16.0)	72.6 (16.2)	-2.3 (-5.8/1.2)	0.19
SF36 health transition	50.9 (12.6)	52.5 (13.9)	50.56 (12.1)	51.2 (11.0)	0.9 (-3.1/5.0)	0.65
Herschbach - general	73.9 (25.4)	79.8 (25.2)	74.4 (26.5)	79.5 (26.8)	1.3 (-3.7/6.3)	0.62
Herschbach - health	80.9 (28.2)	78.3 (34.2)	75.9 (28.7)	77.7 (30.7)	-3.8 (-9.9/2.3)	0.22
Herschbach –hormones	107.6 (56.8)	113.6 (63.1)	112.4 (56.9)	106.8 (58.2)	13.6 (-3.6/23.5)	0.01

Results are the mean (SD)
Abbreviations: CI, Confidence Interval; SF36, Short Form-36 Health Survey
* Occasionally data were missing for some participants, but the percentage missing values never exceeded 3.6%

Safety (table 9) and Adverse Events (table 10)

Prostate volume was not significantly changed in the testosterone group compared to the placebo group. There were no significant differences in the prostate-specific antigen concentrations between both groups. Also, the lower urinary tract symptoms measured by the IPSS were similar in the two groups. During the study, there were eight participants with a rise of the PSA with 1.4 µg/L or more (3 testosterone, 5 placebo; six during the first three months and two at the end of the study), who had to discontinue the study. Four participants showed a hypoechogenic lesion at the end of study prostate ultrasonography (2 testosterone, 2 placebo). Also, there was one participant with a possible abnormality of the bladder in the placebo group at the end of the study. Further evaluation of these abnormalities revealed two carcinomas of the prostate in the placebo group.

TABLE 9. *Results for Safety*

	Testosterone (n=113)*			Placebo (n=110)*			Δ difference (95% CI)	P-value
	Baseline	13 weeks	Final Visit	Baseline	13 weeks	Final Visit		
PSA (µg/l)	1.6 (1.1)	1.6 (1.1)	1.6 (1.1)	1.7 (1.1)	1.8 (1.6)	1.7 (1.3)		0.58
Prostate Volume (cc)	28.3 (12.6)		30.7 (13.1)	28.0 (9.9)		29.2 (10.4)	1.0 (-1.6/3.7)	0.43
IPSS	6.3 (5.1)	6.4 (4.8)	6.6 (4.8)	6.7 (4.9)	6.1 (4.3)	6.8 (4.6)		0.94
Creatinin (µmol/L)	93.7 (18.2)	99.8 (21.6)	101.5 (18.3)	93.1 (15.2)	94.0 (16.3)	94.9 (15.9)		0.051
ASAT (U/L)	22.8 (7.3)	22.5 (6.4)	23.0 (7.0)	24.2 (12.5)	23.2 (8.5)	24.0 (8.7)		0.31
ALAT (U/L)	26.1 (11.1)	24.9 (11.9)	25.3 (11.1)	27.0 (13.9)	27.2 (12.6)	27.9 (12.2)		0.17
AF (U/L)	71.8 (19.5)	68.2 (20.7)	70.3 (19.5)	70.1 (18.2)	70.8 (17.7)	73.0 (19.5)		0.74
GGT (U/L)	29.3 (15.8)	32.1 (26.4)	31.5 (14.7)	30.3 (19.8)	30.0 (18.2)	31.5 (18.0)		0.82
Hemoglobin (mmol/l)	9.2 (0.5)	9.35 (0.6)	9.5 (0.6)	9.1 (0.6)	9.1 (0.6)	9.2 (0.6)		0.02
Hematocrit (%)	0.45 (0.0)	0.46 (0.0)	0.46 (0.0)	0.45 (0.0)	0.45 (0.0)	0.45 (0.0)		0.01

Results are the mean (SD)
Abbreviations: CI, Confidence Interval; PSA, Prostate Specific Antigen; IPSS, International Prostate Symptom Score; ASAT, Aspartate Aminotransferase.; ALAT, Alanine Aminotransferase; AF, Alkaline Phosphatase; GGT, γ-Glutamyl Transferase
* Occasionally data were missing for some participants, but the percentage missing values never exceeded 3.6%

There were no significant differences in liver or kidney function. There was one participant in the testosterone group who discontinued the study medication because of liver function values of more than three times upper normal reference level. After discontinuation of the medication, the liver functions normalised. Both hemoglobin levels and hematocrit increased significantly in the testosterone group compared to the placebo group (p is respectively 0.02 and 0.009). This increase occurred during the first three months of supplementation levels remained stable after that period (data not shown). Two of the study subjects developed red cell parameters just above the normal range at the end of the study. The other subjects did not reach predetermined hemoglobin and hematocrit levels for discontinuation of study medication.

129 participants (54.3%) experienced one or more adverse events. The mean number of adverse events per participant was 0.87 in the testosterone group and 0.90 in the placebo

group. The most frequent adverse events were gastro-intestinal complaints, cardiovascular complaints and urologic complaints. There were no statistical significant differences in types of adverse events between both groups.

During the study there were 15 serious adverse events, 5 in the testosterone group and 10 in the placebo group, 13 of them because of hospitalisation during the study period. Five of the hospitalisations were already planned before the study started. The hospitalisations were not related to the study medication. The other two serious adverse events were the two prostate carcinomas in the placebo group we described above.

TABLE 10. *Adverse Events*

Adverse event	Testosterone n=113	Placebo n=110	P-value
Gastro-intestinal complaints	10	9	0,86
Cardiovascular complaints	7	3	0,21
Gynaecomastia/tender mammae	1	2	0,55
Urologic complaints	11	10	0,98
Skin problems	7	7	0,17
Musculoskeletal complaints	11	9	0,35
Lung problems	12	5	0,26
Neoplasms (benign/malignant)	2	3	0,63
Oedema	1	0	0,32
Neurologic complaints	4	1	0,18
Other complaints	38	56	0.19
Total No of Adverse Events	**104**	**105**	

Discussion

In this large double-blind, placebo-controlled, randomized trial, we found that 80 mg testosterone undecanoate supplementation twice daily administered orally during six months to elderly men with low normal circulating testosterone levels had beneficial effects on body composition with an increase of the lean body mass and a decrease of the fat mass. The increase of the lean body mass, however, was not accompanied by an increase of functional mobility or muscle strength. There were no beneficial effects on cognition or bone mineral density. The decrease of the fat mass was accompanied by a decrease of the total cholesterol concentration, but also a decrease of the HDL cholesterol. This resulted in an increase of the ratio total cholesterol versus HDL cholesterol. The decrease of the fat mass was also accompanied with a decrease of the glucose level together with an increase

of the insulin sensitivity . There was an increase of the hormone related quality of life in the testosterone group. Testosterone supplementation was safe and well tolerated.

To fully appreciate these results, some issues need to be addressed. First, the testosterone levels in this study population were low to low-normal. Seventy-one percent of the men had a testosterone level below 12.0 nmol/L, and are considered possibly testosterone deficient according to conventional standards.[30] The testosterone levels were comparable with other studies that found beneficial short-term effects of testosterone supplementation.[31,32]

The men in this trial were selected on the basis of their androgen status and not on the basis of their health status or symptoms that might indicate reduced testosterone levels. So, most of the participants were healthy and had no important pre-existing health problems.

Six months is a relatively short period for supplementation. However, other studies with even a shorter intervention period have shown treatment effects.[31,33,34] Moreover, for the endpoints chosen, effects, if any, should have been reached within six months, with the exception of bone mineral density, for which treatment duration may have been on the short side.

The total daily dose of 160 mg testosterone undecanoate orally as used in this study is the dose that is used in clinical practice and in other studies.[35,36] The fact that the serum testosterone levels were not increased at the end of the study in the testosterone treatment group is a known effect of oral supplementation of testosterone undecanoate capsules, and agrees with other studies. [35] [36] Due to the pharmacokinetic profile of oral testosterone undecanoate, the testosterone level as measured in a single blood sample is highly dependent on the time of sampling in reaction to the time of intake of the capsules. Although the final testosterone level was not increased, we know from various studies that the pharmacological profile of testosterone undecanoate yields increased testosterone levels most of the 24 hours[37,38], so the circulating hormone level is not unchanged and we can expect significant physiological alterations. Unfortunately we were not able to measure a post-dose level, which would undoubtedly have been higher. We attribute the increase in testosterone in the placebo group largely to regression to the mean, as a result of the fact that we measured testosterone levels only once. Of course we cannot exclude more or larger effects when giving higher doses, but as the risks involved with giving higher doses are

unknown, there was no other option than to supply the dosis that is used in clinical practice. Moreover, this study did show the same statistically significant biochemical (increased hematocrit) and physical effects (decreased fat mass and increased lean body mass) as studies reporting an increase of the serum testosterone concentration with the use of intra-muscular or transdermal testosterone. Moreover, at the moment this study was designed patches and gels that provide more steady testosterone levels were not available in the Netherlands.

Compliance is always a concern. However, based on count of returned study medication, more than 90% of the subjects completing the study used at least 80% of their medication, and these numbers did not differ between treatment groups.

We adjusted the analysis of the outcome values for variables that might be different at baseline (testosterone level, age, smoking, alcohol, blood pressure (systolic and diastolic) and BMI). This did not lead to any different conclusion about the efficacy of testosterone.

To address effects that dropouts might have had, we imputed the outcome values for the persons for whom we did not have these available with a regression-based imputation method. With these imputed outcomes included in the dataset, we repeated all analyses. This did not lead to any different conclusion about the efficacy of testosterone.

The levels of free testosterone (FT) and bioavailable testosterone (BT) were calculated from total testosterone, SHBG and albumin concentrations. This appears to be a rapid, affordable, simple, and reliable method, and suitable for clinical routine, although not ideal compared to equilibrium dialysis.

The increase in lean body mass and the decrease in fat mass in this study are comparable to those reported in most other testosterone supplementation studies in hypogonadal men.[39] There were no effects of testosterone on body mass index, waist circumference and subcutaneous and intra-abdominal fat mass measured with ultrasound, probably because these measurements are not sensitive enough to detect small changes. In this study, the increase in lean body mass was not accompanied by an increase in muscle strength or functional mobility. Muscle strength is a key factor in maintaining independence in elderly people. Decreased muscle strength is a risk factor for falls, frailty and disability.[40,41] Observational epidemiological studies have not only shown an association between testosterone levels and muscle mass and strength, but also between testosterone levels and physical performance and fall risk.[42,43] Still, in other studies with testosterone

supplementation the effects of the increase in lean body mass on muscle strength are inconsistent. The majority of studies show a discrepancy between changes in lean body mass and muscle performance.[2-5,39] A recent meta-analysis suggest that testosterone supplementation in healthy elderly man might produce a moderate increase in muscle strength, but the mean effect size was strongly influenced by one study.[44] There are hardly any previous studies about the effects of testosterone supplementation on functional mobility.

The decrease in fat mass was accompanied by a decrease in plasma glucose concentration and an increase in insulin sensitivity. This is important, because type 2 diabetes mellitus is a growing public health problem. Other studies with testosterone supplementation have also shown a decrease in blood glucose concentrations, plasma insulin levels and mean glycated hemoglobin (HbA1C) and an increase of insulin sensitivity, but these were mainly based on subjects with type 2 diabetes or abdominal obesity.[45,46] There are almost no well-designed studies about the effects of testosterone supplementation on insulin resistance in healthy elderly men, like the participants in this study.

The decrease in fat mass was also accompanied by a decrease in total cholesterol, mainly because of a decrease in HDL cholesterol. Exogenous testosterone increases the activity of hepatic lipoprotein lipase (LPL), an enzyme involved in HDL catabolism.[47] This should reduce HDL levels but available data are controversial. Two recent meta-analyses have shown different results. According to the first meta-analysis intramuscular administration of testosterone to hypogonadal men is associated with a small, dosage-dependent decrease in HDL-cholesterol and concomitant declines in total cholesterol and LDL cholesterol.[48] The other meta-analysis revealed also a significant decrease in total cholesterol after supplementation of testosterone (oral, intramuscular or transdermal) to eugonadal or hypogonadal men that was more pronounced in the group with lower baseline testosterone levels, but the reduction in HDL-cholesterol was only detectable in studies with higher pre-treatment testosterone concentrations and there were no effects on the LDL cholesterol.[39] In the latter, the effects on HDL cholesterol were smaller in studies using intramuscular testosterone esters than in studies using oral and transdermal testosterone. This agrees with our study and could reflect higher serum levels of oestradiol achieved with intramuscular testosterone injections, that are important in maintaining HDL-cholesterol concentration in men to counteract the effects of testosterone on LPL activity.[47]

The Metabolic Syndrome represents a complex clustering of several interrelated physiological and metabolic alterations, including insulin resistance, dyslipidemia, abdominal fat and hypertension.[23] The presence of the syndrome is a strong risk factor for cardiovascular disease and type 2 diabetes mellitus.[49,50] Epidemiological studies have shown an association between low androgen levels and the metabolic syndrome.[51-53] However, there are no studies on the effects of testosterone supplementation on the metabolic syndrome. We found a non-significant increase in the percentage men who met the criteria of the metabolic syndrome. This was mainly caused by the decrease in HDL cholesterol levels. The effects of these changes on risk of cardiovascular disease and type 2 diabetes are still unknown.

With advancing age, men lose bone mineral density, which leads to increased risk for fractures. Osteoporotic fractures cause significant morbidity and excess mortality and prevention of these fractures is important. Up to 20% of men with vertebral fractures[54] and 50% of men with hip fractures[55] have biochemical evidence of hypogonadism, suggesting a potential role of testosterone supplementation for prevention. There were no effects of testosterone supplementation on bone mineral density in this study, probably because the intervention period was too short. Two meta-analyses of the effects on testosterone supplementation on bone health have shown that testosterone supplementation moderately increased lumbar bone density in men after a minimum of 12-36 months of treatment, but the results on femoral neck bone are inconclusive.[39,56] Intra-muscular testosterone yielded a larger effect on bone mineral density than oral or transdermal preparations. However, none of these studies showed a decreased rate of fractures with testosterone therapy.

The prevalence of age-associated cognitive decline in the general older population is estimated to be between 20-35%.[57] Cognitive decline can precede dementia and subsequent institutionalization. Epidemiological studies have reported a positive association between testosterone level and cognition [58-61] and between testosterone level and the incidence of Alzheimer disease.[59,62,63] Furthermore, on the basis of basic research and animal studies, testosterone is suggested to exert a protective effect on cognitive function.[64-66] However, there were no effects of testosterone supplementation on cognitive function in this study. The results of other studies are similarly unconvincing.[2,31,33,67] Several lines of evidence suggest that testosterone has a selective effect on (visuo)spatial abilities. In this study different domains of cognitive function were tested, including a sensitive and widely used

visuospatial test (mental rotation performance), without any effect. The 95% confidence interval of the difference in Shepard Mental Rotation that we found for testosterone treatment excludes an effect larger than approximately 0.2 SD of the baseline distribution of the Shepard Mental Rotation. If even smaller effects are considered clinically relevant, obviously even larger studies than this one are necessary.

Most of the participants in this study had no pre-existing cognitive abnormalities. The participants have scored between the 50th and 70th percentile for all "cognitive tests, suggesting that they scored perhaps slightly better than average for their age group, but still with plenty of room for improvement. Moreover, even studies with testosterone supplementation in men who have mild cognitive impairment or Alzheimer's disease have shown mixed findings.[34,68,69]

It has been suggested that the age-related decline in testosterone levels in men, because of the negative effects mentioned above, also might adversely affect quality of life. However, there is little evidence about the effects of testosterone supplementation on quality of live. Most studies that assessed health related perception of quality of life using the SF-36 did not show any benefit.[70,71] That is in agreement with our findings, and might be due to the fact that this questionnaire is too general and not sensitive enough. In our study, we have also used a questionnaire, developed to measure hormone deficiency dependent quality of life, and here we have found modest beneficial results, especially on the item "resilience/ability to tolerate stress".

There is serious concern that men receiving hormone replacement may be vulnerable to increased health risks. Known side effects of androgen supplementation are gynecomastia, edema and an increase of hematocrit. However, the most important concern of androgen supplementation in old age is the risk of the development and/or progression of prostate disease such as benign prostate hyperplasia and prostate carcinoma. Both conditions are highly dependent on androgen action, nevertheless, there are only limited clinical data available on the effects of testosterone replacement on the prostate in hypogonadal males. A recent compilation of published prospective studies revealed only five cases of prostate cancer among 461 men (1.1 percent) followed for six to 36 months, an incidence rate similar to that in the unexposed general population. [72] Also a recent study, where prostate biopsies were taken before and after 6 months of testosterone supplementation have shown no treatment-related changes in prostate histology, tissue biomarkers, gene expression or

cancer incidence or severity. [73] However, several case-reports have suggested that testosterone therapy may convert occult prostate cancer into a clinically symptomatic lesion. [74,75] Occult prostate cancer is common in elderly men. Autopsy and biopsy studies have confirmed that approximately 15-35% of elderly men have occult prostate cancer, despite a normal PSA and a normal digital rectal examination .[76,77] Normally, only a small percentage of the occult prostate cancers will evolve into a clinical carcinoma during lifetime, but it is possible that testosterone substitution stimulates this process. If this hypothesis is true, the clinical carcinoma will develop during the first months of testosterone substitution. In this study, there were no indications that this hypothesis is true. None of the participants with testosterone supplementation developed prostate carcinoma, whereas two in the placebo group did. Multiple studies have examined the effects of exogenous testosterone replacement on serum PSA, prostate volume and voiding symptoms with varying results. In a systemic review of published studies of testosterone replacement therapy in men with hypogonadism the mean increase of PSA levels was between 0.30 ng/ml in young men and 0.43 ng/ml in elderly men. [78] We found no overall effect on serum PSA, prostate volume and voiding symptoms in this trial. A stimulatory effect of testosterone on erythropoiesis has been documented in several studies.[72] This effect was confirmed in our study, but the elevation of the hematocrit and the hemoglobin was without any clinical consequences. There were no significant testosterone effects on liver and kidney functions in our study. There was no difference in (serious) adverse events between both groups.

This study is, as far as we know, the largest study of testosterone supplementation ever, with the most endpoints and a solid design. The compliance was high and the drop-out rate was low. We found a change in body composition which was accompanied by different effects on metabolic risk factors. There were no beneficial effects of testosterone supplementation on functional mobility, bone mineral density and cognitive function. There was an increase of the hormone related quality of life in the testosterone group. Testosterone supplementation was safe and well tolerated. The findings in this study do not support a net benefit of 6 months of modest testosterone supplementation in healthy men with circulating testosterone levels in the lower range on several indicators of health and functional and cognitive performance.

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Chapter 4.3

Effect of testosterone supplementation on sexual functioning in ageing men: a 6-month randomized controlled trial.

Abstract

Serum testosterone levels decline significantly with aging and this has been associated with reduced sexual function. We have conducted a double-blind, randomized, placebo-controlled trial to investigate the effect of testosterone supplementation on sexual function in 237 elderly men with a testosterone level < 13.7 nmol/L. Participants were randomly assigned to receive oral testosterone undecanoate or a placebo for 6 months. A total of 207 men completed the study. After treatment, there were no differences in scores on sexual function between the groups. Subanalysis showed that although a baseline testosterone level in the lowest tertile was associated with significantly lower scores for sexual fantasies, desire of sexual contact and frequency of sexual contact, supplementation of testosterone did not result in improvement on any of these tests in this group. In conclusion, the findings do not support the view that testosterone undecanoate supplementation during 6 months to elderly men with low normal testosterone concentrations favorably affects sexual function.

Introduction

Many older men remain sexually active, but activity declines with age from 83.7% sexually active among men between 57-64 years of age, 67% among men between 65 to 74 years of age, and 38.5% among men between 75 to 85 years of age.[1] According to a recent survey, half of men who are sexually active report at least one bothersome sexual problem, of which the most common are erectile dysfunction (37%). Other frequent sexual problems are lack of interest in sex (28%), inability to climax (20%) and climaxing too quickly (28%).[1] Impairment of sexual function has a major impact on quality of life.[2]

In aging men, sexual problems are believed to be related partly to hormonal status. Up to 35% of men with erectile dysfunction may have hypogonadism.[3, 4] Serum testosterone levels decline gradually with aging.[5] This decline coincides with increasing signs and symptoms of aging, including tiredness and lack of energy, diminished cognition, reduced muscle mass and strength, reduced bone density, depression and diminished well-being. However, the association between sexual functioning (diminished libido, erectile dysfunction) and testosterone levels is not consistent across studies. Although some investigators have reported a significant relation between testosterone levels and sexual function[6, 7], others have found no association[8, 9], possibly because of the relative impact of other chronic conditions common in elderly men, notably diabetes mellitus, depression and cardiovascular diseases, that may also affect sexual function.

Clinical trials examining the effect of testosterone supplementation on sexual function in aging men have produced conflicting findings.[10, 11] These divergent findings likely reflect differences in study design. In particular, most of the studies were probably too small in size and duration. Additional data are needed to determine whether older men receiving testosterone supplementation experience benefits on sexual functioning.

We conducted a randomized, double-blind placebo-controlled study to assess the effect of testosterone supplementation on sexual functioning in ageing men with moderately low testosterone levels for a period of six months.

Subjects and Methods

This study had a randomized, double-blind, placebo-controlled design. Details of the study design, recruitment, and procedures have been published previously.[12] The Institutional Review Board of the University Medical Centre Utrecht approved the study protocol and all

participants provided written informed consent. This study is a secondary analysis of a larger, previously published study.[13]

Participants

Participants were recruited by direct mailing to 8020 randomly selected men between 60 and 80 years of age whose addresses were obtained from the municipal register of the city of Utrecht, the Netherlands.

Inclusion criteria included: a testosterone level below the 50th percentile of the study population based testosterone distribution and an age between 60 and 80 years. The 50th percentile cut-off level of testosterone was determined to be 13.7 nmol/L after screening of 50 candidates. This was similar to the 50th percentile of the testosterone level at the end of the study (13.8 nmol/L). Exclusion criteria included: myocardial infarction or cerebrovascular accident within the past 6 months, heart failure unless medically treated and not symptomatic, malignancy within the past 5 years except for non-melanoma skin cancer, any hormone dependent tumor in the history, serious liver- or renal diseases (more than 3 times the upper limit of reference value), hematological abnormalities (hemoglobin ≤ 7.0 mmol/l, hematocrit ≥ 0.50), epilepsy or the use of anti-epileptic medication, migraine more than once a month, diabetes mellitus, a fasting glucose level ≥ 6.9 mmol/l, corticosteroid use (≥ 7.5 mg orally a day within the past 6 months, with the exception of short bouts of prednisone for the period of 7 days, or inhalation of ≥ 800 µg a day during the past 6 months), use of testosterone esters and alike substances within the past 60 days, prostate hypertrophy in medical history, and an elevated PSA level (age 60-69 years ≥ 4.5 µg/l; 70 years and above ≥ 6,5 µg/l).

Following an initial telephone contact 684 men were screened with medical history, laboratory testing and digital rectal examination. Finally, 237 men were eligible for entry into the study and agreed to participate. Data collection took place between January 2004 and October 2004, and of the 237 men enrolled 120 were assigned to testosterone and 117 to placebo.

Intervention

After entry into the study, subjects were randomly assigned to treatment with either two capsules of 40 mg testosterone undecanoate (Andriol® Testocaps, N.V. Organon, Oss, the

Netherlands) twice a day with breakfast and dinner, or matching placebo, for a total duration of 26 weeks. Adherence was monitored by capsule counting at 3 and 6 months.

Laboratory

Fasting blood samples were obtained between 8.00 and 11.00 AM to minimize diurnal variation. The serum levels of testosterone and sex hormone binding globulin (SHBG) were measured with a solid-phase, competitive, chemiluminescent enzyme immunoassay (IMMULITE ® 2000 Total Testosterone, Diagnostic Products Corporation, Los Angeles, California, USA) at baseline and at the end of the study. Normal ranges for testosterone and SHBG are respectively 6.3-26.8 nmol/L and 13-71 nmol/L. The levels of free testosterone (FT) and bioavailable testosterone (BT) were calculated from total testosterone, SHBG and albumin concentrations.[14]

Sexual functioning

The 'Eleven questions on Sexual Functioning' (ESF) questionnaire was used to assess sexual well being at baseline and at the final visit.[15] The questionnaire consists of 11 multiple-choice questions of each 5 to 7 answer categories, for some questions relating to frequency of the item in the question and for others relating to appreciation of the item in the question. Subsequently, each answer was categorised in two categories as follows. For the questions about sexual fantasies, masturbation, desire of sexual contact and frequency of sexual contact the answers were grouped as less than once a week, or once a week or more. For the questions about difficulty with achieving and maintaining erection, achieving a climax, climaxing too quickly or climaxing too slowly, and pain with sexual contact the answers were grouped as not once, or sometimes, regularly, often or every time. For the question about quality of sexual functioning the answers were categorised into dissatisfied and neutral/satisfied. Sexual function domains were categorized in a way that the number of participants in the smallest group was still high enough to have enough power for statistical analysis.

Possible sexual dysfunction was defined when the participant reported one or more of the following in the last month: masturbation several times a day, not once desire for sexual or several times a day desire for sexual contact, the penis was regularly less hard or hard for a shorter time than wanted with sexual contact, the participant regularly did not reach an

orgasm or the orgasm was less or more quickly than wanted, regular pain in the genitals or dissatisfaction about the present sex life, according to the questionnaire's manual.

Data analysis

Logistic regressions was used to calculate odds ratios (OR) and 95% confidence intervals (95% CI) for the effect of treatment at the final visit; models were adjusted for baseline visit score. The level of statistical significance was set at $P<0.05$.

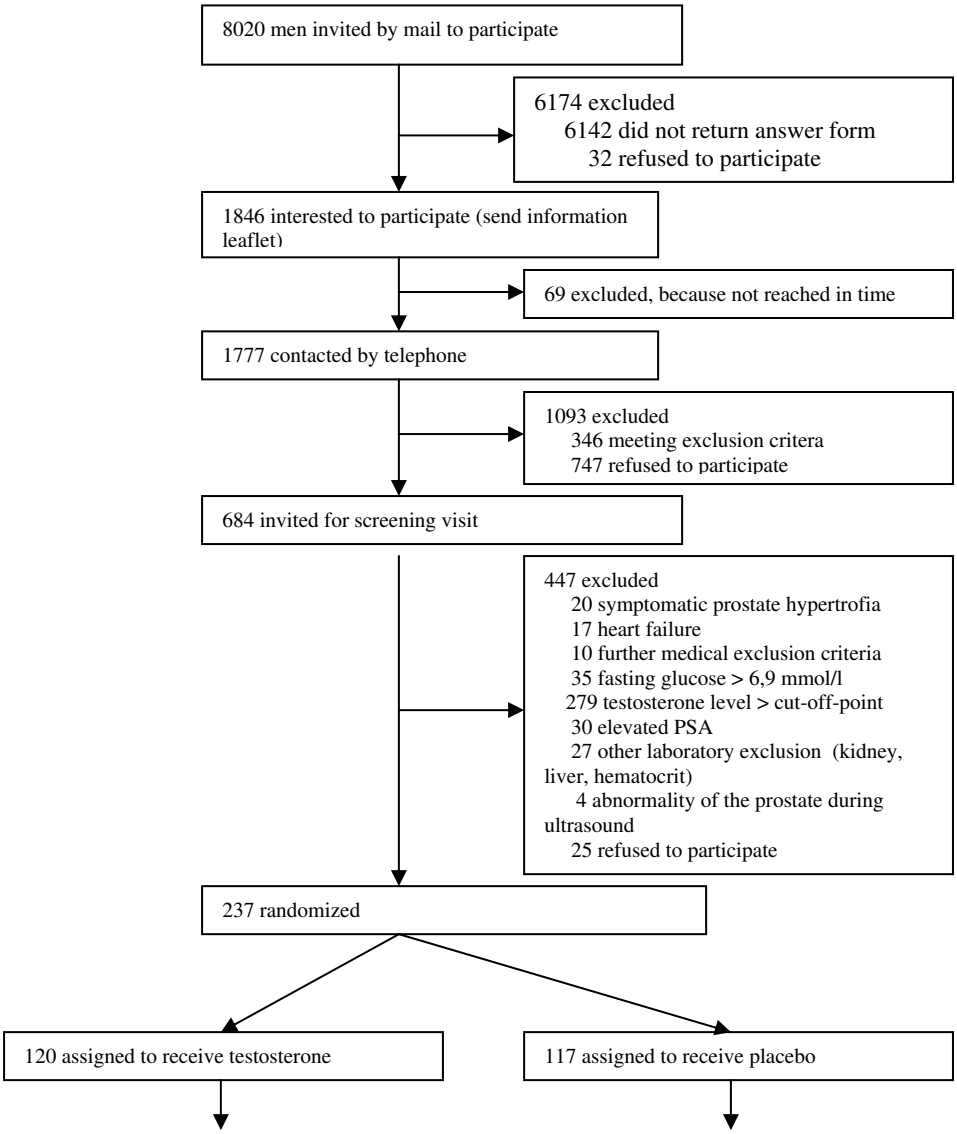
Furthermore, we studied whether the effect of testosterone supplementation differed across predetermined subgroups of testosterone-level (lowest tertile (<9.65) versus highest two tertiles (≥ 9.65 nmol/l)) and age (below median (60-66.5years) versus over median (66.5-80 years)).

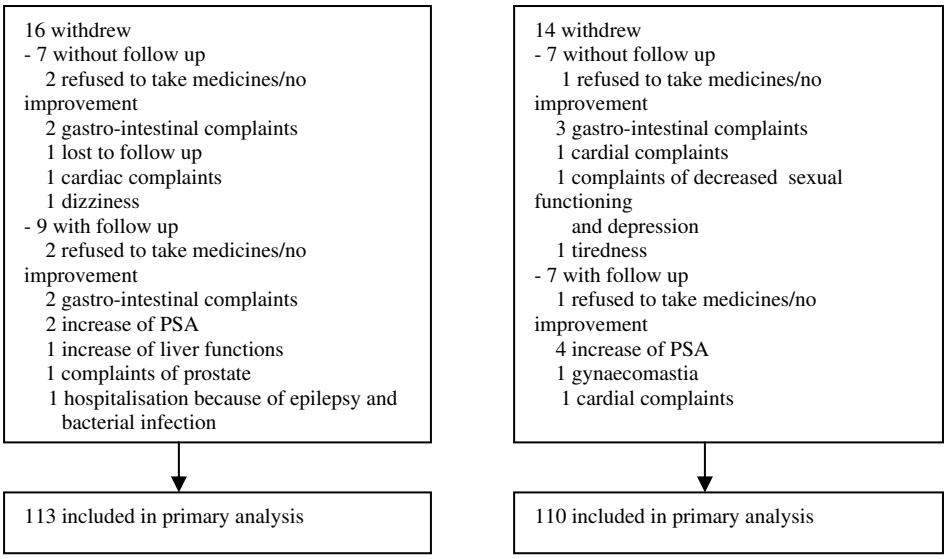
Because the percentage missing data was very small (<6.7%), we did not use any specific strategies to handle this and the missing data were treated as missing values in the analysis. All analyses were performed with SPSS, statistical software package, version 11.0 (SPSS Inc, Chicago, Ill)

Results

The baseline characteristics of the participants are shown in *table 1*. The mean age of the participants was 67 ± 5 years and the mean serum testosterone concentration was 10.7 ± 1.9 nmol/L. The following problems with sexual functioning were reported: 11.9% had a total lack of interest in sexual contact, and 24.7% of the participants had no sexual contact in the last month , while 66.7% of these participants were married or unmarried living together, 30.1% of the participants had an inability to climax and 10.5% had major problems with erectile function every time they had sexual contact. In total, 78.9 % of the participants reported any sexual dysfunction. The percentage men using PDE5 inhibitors was 1.7%. There were no major differences between the two groups at baseline.

FIGURE 1 *Participant Flow Diagram*





There were 30 early withdrawals, 16 in the testosterone group and 14 in the placebo group. Of the subjects completing the study, more than 90% used at least 80% of their medication. The effects of the treatment on safety parameters and testosterone and SHBG levels have been reported previously.[13] Briefly, there were no statistically significant differences in frequencies and types of adverse events between testosterone and placebo groups from baseline to month 6. Total testosterone was unchanged from baseline in the testosterone group and increased slightly in the placebo group; the difference between the testosterone and placebo group at month 6 was -3.2 nmol/l (95% confidence interval [CI] -4,2; -2.2, $p<0.001$). SHBG levels declined from baseline in the testosterone group and did not in the placebo group (difference -10.1 nmol/l, 95% CI -11.7; -8.5, $p<0.001$). Also the between-group difference for FT and BT was statistically significant at month 6 (FT difference -0.03, 95% CI -0.05; -0.00, $p=0.04$, and BT difference -0.69, 95% CI -1.24; -0.13, $p=0.02$, respectively).

TABLE 1. *Subject characteristics at baseline according to randomization group*

	Testosterone (n=113) Mean (SD)	Placebo (n=110) Mean (SD)
Age (yr)	67.1 (5.0)	67.4 (4.9)
Testosterone (nmol/L)	11.0 (1.9)	10.4 (1.9)
SHBG (nmol/L)	33.0 ± 10.7	32.8 ± 10.0
Free Testosterone (nmol/L)	0.22 ± 0.02	0.21 ± 0.0
Bioavailable Testosterone (nmol/L)	5.2 ± 1.1	5.0 ± 1.2
Body Mass Index (kg/m²)	27.4 (3.8)	27.3 (3.9)
Systolic blood pressure (mmHg)	155 (23.3)	151.4 (22.7)
Diastolic blood-pressure (mmHg)	89.2 (12.0)	86.8 (11.7)
	Number (%)	Number (%)
Smokers	21 (17.5)	15 (12.8)
Alcohol users	99 (82.5)	90 (76.9)
Prior cardiovascular disease‡	35 (48.6)	37 (51.4)
Married or unmarried living together	95 (84.1)	87 (79.1)
No sexual contact last month	32 (29.1)	22 (20.2)
Total lack of interest in sex	14 (12.8)	12 (11.0)
Erectile dysfunction with every sexual contact	11 (10.0)	12 (11.0)
Inability to climax	41 (37.3)	28 (25.7)
Any sexual dysfunction	91 (82.7)	85 (77.3)

‡ Includes myocardial infarction, angina, hypertension or stroke
Abbreviations: SHBG, Serum Hormone Binding Globulin.
Conversions: To convert testosterone from nmol/L to ng/dL divide by 0.0347.

The changes from baseline to month 6 on sexual functioning for the testosterone group and the placebo group are shown in *table 2*. There were no significant differences in sexual functioning between both groups after 6 months of intervention.

TABLE 2. *Effects of testosterone undecanoate treatment versus placebo on sexual functioning.*

ESF questionnaire item	Baseline Testosterone (n=113)* number (%)	Placebo (n=110)* number (%)	Final Visit Testosterone (n=113)* number (%)	Placebo (n=110)* number (%)	OR (95% CI)	P-value
Sexual fantasies†	71 (64.0)	73 (67.0)	70 (61.9)	74 (69,8)	0.70 (0.37-1.32)	0.27
Frequency of masturbation†	87 (78.4)	92 (84.4)	93 (82.3)	94 (87.9)	0.83 (0.34-2.03)	0.68
Desire of sexual contact†	64 (58.7)	69 (63.3)	73 (65.2)	69 (64.5)	1.17 (0.64-2.13)	0.61
Frequency of sexual activity †	78 (70.9)	77 (72.0)	85 (75.2)	77 (72.0)	1.19 (0.60-2.38)	0.62
Difficulty in achieving erection‡	57 (51.8)	68 (62.4)	58 (51.8)	60 (56.1)	1.20 (0.62-2.33)	0.58
Difficulty in maintaining erection‡	61 (55.5)	68 (62.4)	60 (53.1)	62 (57.9)	1.01 (0.51-2.00)	0.98
Difficulty in reaching climax‡	69 (62.7)	81 (74.3)	75 (66.4)	75 (70.1)	1.44 (0.66-3.13)	0.36
Climaxing too slowly‡	40 (38.8)	46 (43.8)	38 (35.8)	48 (47.1)	0.77 (0.40-1.49)	0.44
Climaxing too quickly‡	39 (37.9)	35 (33.3)	32 (30.2)	26 (25.5)	1.32 (0.63-2.77)	0.46
Pain and discomfort‡	13 (11.9)	8 (7.3)	6 (5.3)	6 (5.6)	0.59 (0.16-2.23)	0.43
Quality of sexual functioning #	46 (41.4)	32 (29.4)	35 (31.0)	36 (33.6)	0.55 (0.27-1.13)	0.11

Effects are expressed as odds ratios for the treatment effect on the probability of being in the less favourable category of the individual items of the Eleven Questions on Sexual Functioning questionnaire
Abbreviations: CI, Confidence Interval; OR, Odds Ratio
† < once a week vs once a week or more as reference
‡ never vs. ever as reference
# dissatisfied vs neutral/satisfied as reference

In subgroup analysis, participants were divided into two groups according to their testosterone concentration at baseline (testosterone < 9.96 nmol/l versus testosterone ≥ 9.96 nmol/l). A baseline testosterone level in the lowest tertile was associated with significantly lower scores for sexual fantasies, desire of sexual contact and frequency of sexual contact, (OR (95% CI) 2.3 (1.2-4.4), 2.1 (1.1- 4.0) and 2.6 (1.3- 5.3) respectively). After adjustments for body mass index, blood pressure, smoking, use of alcohol, age and prior cardiovascular disease (myocardial infarction, angina, hypertension or stroke) this difference was still statistically significant for sexual fantasies, desire of sexual contact and frequency of sexual contact (*table 3*).

TABLE 3. *Subject characteristics at baseline according to baseline testosterone level*

ESF questionnaire item	Testosterone ≥ 9.96 nmol/L (n=149) Number (%)	Testosterone < 9.96 nmol/L (n=74) Number (%)	OR (95% CI)	P-value	After correction* OR (95% CI)	P-value
Sexual fantasies †	89 (60.5)	55 (75.3)	2.0 (1.1-3.7)	0.03	2.3 (1.2-4.4)	0.02
Frequency of masturbation †	114 (77.6)	65 (89.0)	2.4 (1.0-5.4)	0.04	2.1 (0.9-5.0)	0.09
Desire of sexual contact †	81 (55.5)	52 (72.2)	2.1 (1.1-3.8)	0.02	2.1 (1.1-4.0)	0.03
Frequency of sexual activity †	96 (65.3)	59 (81.9)	2.4 (1.2-4.8)	0.01	2.6 (1.3-5.3)	0.01
Difficulty in achieving erection ‡	83 (56.5)	42 (58.3)	1.1 (0.6-1.9)	0.79	1.0 (0.5-1.8)	0.92
Difficulty in maintaining erection ‡	88 (59.9)	41 (56.9)	0.9 (0.5-1.6)	0.68	0.9 (0.5-1.5)	0.60
Frequency of climax ‡	104 (70.7)	46 (63.9)	0.7 (0.4-1.3)	0.31	0.7 (0.4-1.3)	0.27
Climaxing too slowly ‡	59 (41.8)	27 (40.3)	0.9 (0.5-1.7)	0.83	0.9 (0.5-1.6)	0.68
Climaxing too quickly ‡	49 (34.8)	25 (37.3)	1.1 (0.6-2.0)	0.72	1.2 (0.6-2.2)	0.67
Pain and discomfort ‡	12 (8.2)	9 (12.7)	1.6 (0.7-4.1)	0.29	2.3 (0.9-6.3)	0.10
Quality of sexual functioning #	54 (36.7)	24 (32.9)	0.8 (0.5-1.5)	0.57	0.8 (0.4-1.5)	0.53

Effects are expressed as odds ratios for the treatment effect on the probability of being in the less favourable category of the individual items of the Eleven Questions on Sexual Functioning questionnaire
Abbreviations: CI, Confidence Interval; OR, Odds Ratio
* correction for body mass index, blood pressure, smoking, use of alcohol, age and prior cardiovascular disease (myocardial infarction, angina, hypertension or stroke)
† < once a week vs once a week or more as reference
‡ never vs. ever as reference
# dissatisfied vs neutral/satisfied as reference

However, after 6 months of supplementation with testosterone, there were also no significant differences in sexual functioning in the group with the lowest testosterone concentration (*table 4*).

TABLE 4. *Effects of testosterone undecanoate treatment versus placebo on sexual functioning according to baseline testosterone levels.*

Testosterone < 9.65 mol/L (n=74) ESF questionnaire item	Baseline Testosterone (n=35)* number (%)	Placebo (n=39)* number (%)	Final Visit Testosterone (n=35)* number (%)	Placebo (n=39)* number (%)	OR (95% CI)	P-value
Sexual fantasies †	25 (73.5)	30 (76.9)	26 (74.3)	30 (78.9)	0.75 (0.22-2.51)	0.64
Frequency of masturbation †	29 (85.3)	36 (92.3)	31(88,6)	36 (94,7)	0.54 (0.05-5.84)	0.61
Desire of sexual contact †	22 (66.7)	30 (76.9)	24 (68.6)	28 (73.7)	0.91 (0.31-2.66)	0.87
Frequency of sexual activity †	26 (78.8)	33 (84.6)	24 (68.6)	29 (76.3)	0.65 (0.19-2.18)	0.49
Difficulty in achieving erection ‡	18 (54.5)	24 (61.5)	18 (52.9)	17 (44.7)	2.11 (0.68-6.49)	0.20
Difficulty in maintaining erection ‡	17 (51.5)	24 (61.5)	19 (54.3)	18 (47.4)	1.77 (0.61-5.17)	0.30
Difficulty in reaching climax ‡	18 (54.5)	28 (71.8)	22 (62.9)	25 (65.8)	2.12 (0.50-8.97)	0.31
Climaxing too slowly ‡	11 (36.7)	16 (43.2)	11 (33.2)	15 (41.7)	0.99 (0.31-3.14)	0.99
Climaxing too quickly ‡	12 (40.0)	13 (35.1)	13 (39.4)	8 (22.2)	3.32 (0.74-14.96)	0.12
Pain and discomfort ‡	4 (11.4)	5 (12.8)	2 (5.7)	2 (5.1)	0.56 (0.04-7.51)	0.66
Quality of sexual functioning #	17 (50.0)	7 (17.9)	10 (28.6)	9 (23.7)	0.56 (0.15-2.13)	0.40

Testosterone ≥ 9.65 nmol/L (n=149) ESF questionnaire item	Baseline Testosterone (n=78)* number (%)	Placebo (n=71)* number (%)	Final Visit Testosterone (n=78)* number (%)	Placebo (n=70)* number (%)	OR (95% CI)	P-value
Sexual fantasies †	46 (59.7)	43 (61.4)	44 (56.4)	44 (64.7)	0.70 (0.33-1.49)	0.36
Frequency of masturbation †	58 (75.3)	56 (80.0)	62 (79.5)	58 (84.1)	0.92 (0.34-2.46)	0.87
Desire of sexual contact †	42 (55.3)	39 (55.7)	49 (62.8)	41 (59.4)	1.31 (0.64-2.70)	0.47
Frequency of sexual activity †	52 (67.5)	44 (62.9)	61 (78.2)	48 (69.0)	1.58 (0.67-3.71)	0.30
Difficulty in achieving erection ‡	39 (50.6)	44 (62.9)	40 (51.3)	43 (62.3)	0.86 (0.37-1.96)	0.71
Difficulty in maintaining erection ‡	44 (57.1)	44 (62.9)	41 (52.6)	44 (63.8)	0.62 (0.24-1.58)	0.32
Difficulty in reaching climax ‡	51 (66.2)	53 (75.7)	53 (6.9)	50 (72.5)	1.20 (0.47-3.07)	0.71
Climaxing too slowly ‡	29 (39.7)	30 (44.1)	27 (37.0)	33 (50.0)	0.67 (0.30-1.51)	0.33
Climaxing too quickly ‡	27 (37.0)	22 (32.4)	19 (26.0)	18 (27.3)	0.96 (0.40-2.31)	0.92
Pain and discomfort ‡	9 (11.5)	3 (4.3)	4 (5.1)	4 (5.6)	0.49 (0.10-2.58)	0.40
Quality of sexual functioning #	29 (37.7)	25 (35.7)	25 (32.1)	27 (39.1)	0.59 (0.24-1.41)	0.23

Effects are expressed as odds ratios for the treatment effect on the probability of being in the less favourable category of the individual items of the Eleven Questions on Sexual Functioning questionnaire
Abbreviations: CI, Confidence Interval; OR, Odds Ratio
† < once a week vs once a week or more as reference
‡ never vs. ever as reference
# dissatisfied vs neutral/satisfied as reference

Next, participants were divided according to their age at baseline (age < 69.57 years versus age ≥ 69.57 years). A baseline age ≥ 69.57 years was associated with significantly lower scores for sexual fantasies and desire of sexual contact (OR (95% CI) 2.0 (1.0–3.9) and 2.6 (1.4–5.1) respectively). After adjustments for body mass index, blood pressure, smoking, use of alcohol, baseline testosterone concentration and prior cardiovascular disease these differences were still statistically significant (*table 5*).

TABLE 5. *Subject characteristics at baseline according to baseline age.*

ESF questionnaire item	Age ≥ 69.57 years (n=75) Number (%)	Age < 69.57 years (n=148) Number (%)	OR (95% CI)	P-value	After correction* OR (95% CI)	P-value
Sexual fantasies †	57 (77.0)	87 (59.6)	2.3 (1.2-4.3)	0.01	2.0 (1.0-3.9)	0.047
Frequency of masturbation †	64 (86.5)	115 (78.8)	1.7 (0.8-3.7)	0.17	1.6 (0.7-3.7)	0.23
Desire of sexual contact †	55 (75.3)	78 (53.8)	2.6 (1.4-4.9)	0.002	2.6 (1.4-5.1)	0.004
Frequency of sexual activity †	57 (77.0)	98 (67.6)	1.6 (0.8-3.1)	0.15	1.9 (0.9-3.8)	0.08
Difficulty in achieving erection ‡	44 (59.5)	81 (55.9)	1.2 (0.7-2.0)	0.61	1.2 (0.7-2.2)	0.51
Difficulty in maintaining erection ‡	44 (59.5)	85 (58.6)	1.0 (0.6-1.8)	0.91	1.2 (0.5-1.8)	0.96
Frequency of climax ‡	46 (62.6)	104 (71.7)	0.6 (0.4-1.2)	0.15	0.6 (0.3-1.1)	0.12
Climaxing too slowly ‡	32 (45.7)	54 (39.1)	1.3 (0.7-2.3)	0.36	1.2 (0.7-2.3)	0.52
Climaxing too quickly‡	19 (27.1)	55 (39.9)	0.6 (0.3-1.1)	0.07	0.5 (0.3-1.1)	0.07
Pain and discomfort ‡	4 (5.4)	17 (11.8)	0.4 (0.1-1.3)	0.14	0.4 (0.1-1.3)	0.14
Quality of sexual functioning #	27 (36.0)	51 (34.5)	1.1 (0.6-1.9)	0.82	1.2 (0.7-2.3)	0.52

Effects are expressed as odds ratios for the treatment effect on the probability of being in the less favourable category of the individual items of the Eleven Questions on Sexual Functioning questionnaire
Abbreviations: CI, Confidence Interval; OR, Odds Ratio
* correction for body mass index, blood pressure, smoking, use of alcohol, testosterone concentration at baseline and prior cardiovascular disease (myocardial infarction, angina, hypertension or stroke)
† < once a week vs once a week or more as reference
‡ never vs. ever as reference
# dissatisfied vs neutral/satisfied as reference

After 6 months of supplementation with testosterone, there were no significant differences in sexual functioning in the group with the oldest participants (*table 6*).

TABLE 6. *Effects of testosterone undecanoate treatment versus placebo on sexual functioning according to baseline age.*

Age ≥ 69.57 years (n=75) ESF questionnaire item	Baseline Testosterone (n=37)* number (%)	Placebo (n=38)* number (%)	Final Visit Testosterone (n=37)* number (%)	Placebo (n=38)* number (%)	OR (95% CI)	P-value
Sexual fantasies †	27 (75.0)	30 (78.9)	27 (73.0)	28 (73.7)	1.18 (0.39–3.59)	0.78
Frequency of masturbation †	32 (88.9)	32 (84.2)	33 (89.2)	34 (89.5)	0.89 (0.10–7.73)	0.91
Desire of sexual contact †	26 (74.3)	29 (76.3)	29 (78.4)	26 (68.4)	2.61 (0.78–8.73)	0.12
Frequency of sexual activity †	29 (80.6)	28 (73.7)	31 (83.8)	30 (78.9)	1.03 (0.23–4.75)	0.97
Difficulty in achieving erection ‡	17 (47.2)	27 (71.1)	19 (51.4)	24 (63.2)	1.75 (0.42–7.24)	0.44
Difficulty in maintaining erection ‡	18 (50.0)	26 (68.4)	19 (51.4)	25 (65.8)	1.15 (0.25–5.24)	0.86
Difficulty in reaching climax ‡	19 (52.8)	27 (71.1)	20 (54.1)	17 (48.6)	1.70 (0.40–7.18)	0.47
Climaxing too slowly ‡	14 (41.2)	18 (50.0)	14 (40.0)	17 (48.6)	1.12 (0.31–3.97)	0.86
Climaxing too quickly‡	10 (29.4)	9 (25.0)	7 (20.0)	5 (14.3)	1.74 (0.40–7.61)	0.46
Pain and discomfort ‡	2 (5.6)	2 (5.3)	1 (2.7)	2 (5.3)	0.36 (0.02–7.27)	0.50
Quality of sexual functioning #	14 (38.9)	13 (34.2)	12 (32.4)	15 (39.5)	0.54 (0.16-1.84)	0.33

Age < 69.57 years (n=148) ESF questionnaire item	Baseline Testosterone (n=76)* number (%)	Placebo (n=72)* number (%)	Final Visit Testosterone (n=76)* number (%)	Placebo (n=72)* number (%)	OR (95% CI)	P-value
Sexual fantasies †	44 (58.7)	43 (60.6)	43 (56.6)	46 (67.6)	0.54 (0.25–1.20)	0.13
Frequency of masturbation †	55 (73.3)	60 (84.5)	60 (78.9)	60 (87.0)	0.77 (0.28–2.12)	0.61
Desire of sexual contact †	38 (51.4)	40 (56.3)	44 (58.7)	43 (62.3)	0.89 (0.44–1.79)	0.73
Frequency of sexual activity †	49 (66.2)	49 (69.0)	54 (71.1)	47 (68.1)	1.22 (0.56–2.68)	0.62
Difficulty in achieving erection ‡	40 (54.1)	41 (57.7)	39 (52.0)	36 (52.2)	1.17 (0.54–2.51)	0.69
Difficulty in maintaining erection ‡	43 (58.1)	42 (59.2)	41 (53.9)	37 (53.6)	1.07 (0.49–2.32)	0.87
Difficulty in reaching climax ‡	50 (67.6)	54 (76.1)	55 (72.4)	51 (73.9)	1.34 (0.52–3.46)	0.54
Climaxing too slowly	26 (37.7)	28 (40.6)	24 (33.8)	31 (46.3)	0.68 (0.31–1.49)	0.33
Climaxing too quickly‡	29 (42.0)	26 (37.7)	25 (35.2)	21 (31.3)	1.20 (0.50–2.85)	0.68
Pain and discomfort ‡	11 (15.1)	6 (8.5)	5 (6.6)	4 (5.8)	0.71 (0.16–3.24)	0.66
Quality of sexual functioning #	32 (42.7)	19 (26.8)	23 (30.3)	21 (30.4)	0.57 (0.23–1.40)	0.22

Effects are expressed as odds ratios for the treatment effect on the probability of being in the less favourable category of the individual items of the Eleven Questions on Sexual Functioning questionnaire
Abbreviations: CI, Confidence Interval; OR, Odds Ratio
† < once a week vs once a week or more as reference
‡ never vs. ever as reference
# dissatisfied vs neutral/satisfied as reference

Discussion

In this large double-blind, placebo-controlled, randomized trial, we found that orally administered 80 mg testosterone undecanoate supplementation twice daily during six months to elderly men with low normal circulating testosterone levels had no beneficial effects on sexual function.

To fully appreciate these results, some issues need to be addressed. First, although the trial was of long duration relative to several previous studies, six months still is a relatively short period for supplementation. However, other studies have suggested that 6 months is long enough to find beneficial effects of testosterone supplementation on sexual function.[16-18] Moreover, this is the time period that the US Institute of Medicine of the National Academies (IOM) Committee On Assessing The Need For Clinical Trial Of Testosterone Replacement Therapy recommended to be more closely examined in a randomized trial.[19] Compliance is a common problem, especially in hormone substitution trials. However, in this study the participants were highly motivated and compliance was good. The drop-out rate was low at 13% and 207 participants for analyses make this one of the largest double-blind, placebo-controlled, randomized trials of testosterone supplementation conducted so far.

After supplementation with testosterone, there was a decrease of the SHBG concentration without an increase of the total, free and bio-available testosterone concentration. This is conform other studies using this medication.[20, 21] This finding relates to the fact that the testosterone concentrations were pre-dose levels, measured just before the intake of the following testosterone capsule. Since the pharmacokinetical profile of Andriol Testocaps, shows a maximum rise of testosterone after 5 to 6 hours, this is the moment with the lowest testosterone concentrations. Because of logistic and financial reasons, post-dose testosterone measurements were not included in the protocol of our study. Second, it is known that androgen supplementation results in a decline of SHBG concentrations, which affects the free testosterone concentration, because most of the testosterone is bound to SHBG. The free testosterone fraction is metabolised fast, so the total testosterone concentration decreases, especially when measured pre-dose. Third, exogenous testosterone supplementation induces a suppression of LH, resulting in a decrease in endogenous testosterone production. When measuring testosterone at a moment when levels through exogenous testosterone are low, i.e. pre-dose, a net decrease of testosterone can be found.

Although the final testosterone level was not increased, we know from various studies that the pharmacological profile of testosterone undecanoate yields increased testosterone levels most of the 24 hours.[22, 23] Moreover, at the moment this study was designed patches and gels that provide more steady testosterone levels were not available in the Netherlands. The total daily dose of 160 mg testosterone undecanoate orally as used in this study is the dose that is used in clinical practice and in other studies.[17] In our study we have found the same significant biochemical an physical effects (decreased fat mass and increased lean body mass, improvement in hormone-related quality of life)[13] as expected with the use of testosterone supplementation. Although some small studies have found positive results with this type of hormone supplementation on sexual function[17, 24-26], we could not confirm this effect, in agreement with other studies.[27, 28]

The men in this trial were selected on the basis of their androgen status and not on the basis of hypogonadal symptoms or their sexual function. More than 83% of the participants had a positive score on the ADAM questionnaire (Androgen Deficiency in Ageing Males), indicating symptomatic hypogonadism. Moreover, when we compare the men in our study with other population based studies, they were similar with regard to frequency of sexual activity, but the frequency of sexual problems reported by the men in our study (80%) was materially than the 33-50% reported in other studies.[1, 29] This is possibly the result of the low-normal testosterone levels of our study participants on the basis of which they were selected.

The testosterone levels in this study population were low to low-normal. Seventy-one percent of the men had a testosterone level below 12.0 nmol/L, and are considered possibly testosterone deficient according to conventional standards.[30] Critical testosterone levels to maintain sexual functions have been suggested to lie around 10.5 nmol/L, although they vary widely between subjects[31], and other studies did not confirm this cut-off point.[11] Symptoms of testosterone deficiency accumulate gradually with decreasing testosterone levels, with the levels of testosterone differing between individuals and within a subject not all symptoms of testosterone deficiency will manifest themselves at the same blood testosterone levels. [32, 33] The testosterone levels in our study were comparable with other studies that reported beneficial effects of testosterone supplementation on sexual functioning.[16, 17] Moreover, the current available testosterone assays are sub-optimal for the investigation of male reproductive disorders, because of imprecision and inaccuracy of

these assays. Also reference ranges will vary because of the differences that exist between methods and this makes the suggestion of a fixed concentration less helpful.

In this study the Eleven questions on Sexual functioning was used to measure sexual function. This questionnaire is derived from the Questionnaire for screening Sexual Dysfunctions (QSD).[15] This is a validated questionnaire, that is used for clinical and scientific purposes in the Netherlands.[34, 35] The most often used questionnaire internationally is the International Index of Erectile Function (IIEF).[36] This 15-item, self assessed questionnaire is developed for the assessment of erectile function and it is not intended for use as a measure for overall sexual function. In this questionnaire 5 factors or response domains are identified: erectile function, orgasmic function, sexual desire, intercourse satisfaction and overall satisfaction. The ESF used in our study is comparable with the IEFF: 7 of the 11 questions are similar to questions of the IEFF and the questions of the ESF cover 4 of the 5 domains described in the IEFF. Moreover the ESF is intended for use as a measure of overall sexual function and that's the reason why we have chosen for this questionnaire.

The results of our study are consistent with some studies[37, 38], but other studies have shown positive results of supplementation of testosterone on sexual function.[16, 17] A meta-analysis has shown that testosterone use may be associated with small improvements in erectile function and moderate improvement in libido in elderly men.[10, 11] However, unexplained inconsistent results across trials, wide confidence intervals and possible reporting bias weaken these inferences. This meta-analysis have also shown that sexual function is more likely to improve with testosterone supplementation in men with severe degrees of hypogonadism.[10, 11] In our study, we found a negative association between baseline testosterone concentration and sexual fantasies, desire of sexual contact and frequency of sexual contact, but no association between baseline testosterone concentration and erectile functioning as in previous studies.[6, 39] Despite this difference, after 6 months of supplementation with testosterone there was no improvement of these items of sexual function in the testosterone group compared with the placebo group in the lowest testosterone concentration in this study. Also, we found an inverse association between higher age and sexual fantasies and desire of sexual contact. We know from previous studies that aging is accompanied by an increase of sexual problems.[1] Nevertheless, there

was no effect of testosterone supplementation on sexual functioning in the testosterone group compared with the placebo group in the oldest participants in this study.
Some studies that did provide evidence for a beneficial effect of testosterone supplementation on sexual functioning were conducted in younger men, and this might be explained by the fact that they do have less co-morbid illness that interfere with sexual functioning.[24, 40] Because of the relatively high age of the participants in our study, it is likely that there were more causes for erectile dysfunction than the low normal testosterone concentrations alone, like neurological causes, cardiovascular diseases (arterial insufficiency, venous leak) and depression. Testosterone replacement therapy alone will not correct these problems.

In conclusion, the findings of this large randomised trial do not support the presence of an effect of testosterone supplementation on sexual function in men with low-normal testosterone levels during a period of 6 months.

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Chapter 5
General discussion

The concept of frailty

Frailty is a geriatric syndrome describes as: "*A state of increased vulnerability to stressors that results from decreased physiological reserves and multi-system dysregulation, limited capacity to maintain homeostasis and to respond to internal and external stresses. Frailty is an aggregate expression of risk resulting from age- or disease-associated physiologic accumulation of subthreshold decrements affecting multiple physiologic systems resulting in adverse health outcomes, like falls and fractures, disabilities, hospitalization, institutionalization and mortality*".[1] Although frailty is associated with advanced age, chronic diseases and increased disability (defined as the inability to perform activities of daily living (ADL), instrumental activities of daily living (IADL), or difficulty with mobility), evidence suggests that neither old age, chronic diseases or disability alone identify those at highest risk of adverse outcomes. Not all elderly people are frail. Three to seven percent of persons aged more than 65 years are frail; this percentage increases to 26% for persons older than 80 years and to 32% for those older than 90 years.[1-3] Among frail elderly persons 60% have difficulty completing IADL, and 27% cannot complete ADL; furthermore, only 28% of disabled persons are frail.[2,3] In fact, frailty predicts adverse outcomes independent of these factors, suggesting an independent etiology.[4]

There is no consensus how frailty should be definitely diagnosed or graded. The most widely accepted definition of frailty has been developed by Fried and coworkers (2001).[2] This definition concentrates on the physical aspects of frailty. The Fried definition proposes five items: weight loss, exhaustion, weakness, slow walking speed and low levels of physical activity. Frailty is diagnosed when at least three criteria are met. An individual is said to be pre-frail when one or two of these criteria are present (table 1). Pre-frailty can predict frailty. The same factors that predict frailty, in general, also predict pre-frailty. This provides support for the hypothesis that pre-frailty and frailty represent degrees of severity within the same chronic, progressive process.[5]

Table 1 Criteria for the phenotypic definition of frailty developed by Fried et al. (2001)[2]

Weight loss	> 10 pounds or 5% of weight loss in the last year
Exhaustion	Center for Epidemiologic Studies Depression Scale (2 points)*
Weakness	Grip strength (lowest 20%)
Gait speed	15-foot walk test (lowest 20%)
Low physical activity	Minnesota Leisure Time Activity Questionnaire (lowest 20%)
Diagnosis of frailty	3 or more criteria met
Diagnosis of pre-frailty	1-2 criteria met

*In the last week, how often did you feel this way? I felt that everything was an effort; I could not get going – a moderate amount of time or most of the time

In recent years, there is increasing evidence that not only weight loss, but also obesity may promote frailty.[6-8]

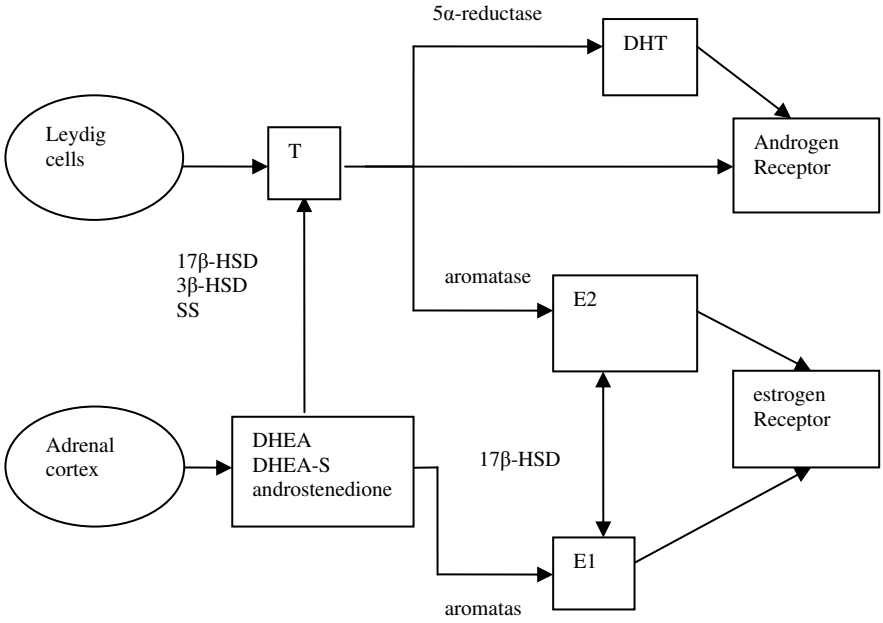
Testosterone and aging in men

In the last decades the scientific interest has grown in the potential importance of androgens in etiology, prevention and treatment of frailty.

With aging, there is a gradual decline in testosterone concentrations in men.[9] Testosterone is mainly produced by testiculair Leydig cells. With aging, the number of Leydig cells decreases, as does their secretory capacity.[10] Moreover there is a decreased sensitivity to sex hormone feedback of the hypothalamic-pituitary-gondal axis.[11] In the circulation testosterone is bound to albumin (binds 38% of testosterone) and sex hormone binding globulin (SHBG) (binds 60% of testosterone); this is the inactive testosterone. Only the free, unbound fraction of testosterone (2% of testosterone) is hormonally active. In addition, some of the albumin-bound testosterone can easily dissociate from the protein and enter target tissue; thus, the amount of bio-available testosterone is larger than just the amount of the free testosterone. Due to rising SHBG levels with aging, the decrease of free,

active testosterone concentrations is even more pronounced.[10,12] Testosterone can be irreversibly converted in peripheral tissue, like fat mass, by the enzyme 5α-reductase to the more potent 5α-dihydrotestosterone (DHT). Both testosterone and DHT can activate the androgen receptor (AR) to exert its androgenic effects. Testosterone can also be converted in peripheral tissue to estradiol (E2) by the aromatase enzyme, followed by activation of the estrogen receptors (ER-α and ER-β). The adrenal cortex secretes large amounts of androgens, like dehydroepiandrosterone (DHEA), DHEA-sulfate (DHEA-s) en androstenedione. These adrenal androgens can be metabolized either directly or indirectly in a rather complex pathway to estrone (E1) by the aromatase enzyme or to testosterone by the enzymes steroid sulfatase, 17β-hydroxysteroid dehydrogenase (17β-HSD) and/or 3β-HSD.[13] (see figure 1). More than 85% of the circulating estrogen levels in men are derived from peripheral aromatization of testosterone. However, the Leydig cells can also secrete small amounts of estrogens. Estrogen levels remain relative stable with aging as a result of increasing aromatase activity with age and the age-associated increase in fat mass.[14] However, because estrogens are also bound to SHBG in circulation, there is a decrease of the free, unbound estrogen concentration.[15]

Figure 1 Testosterone metabolism

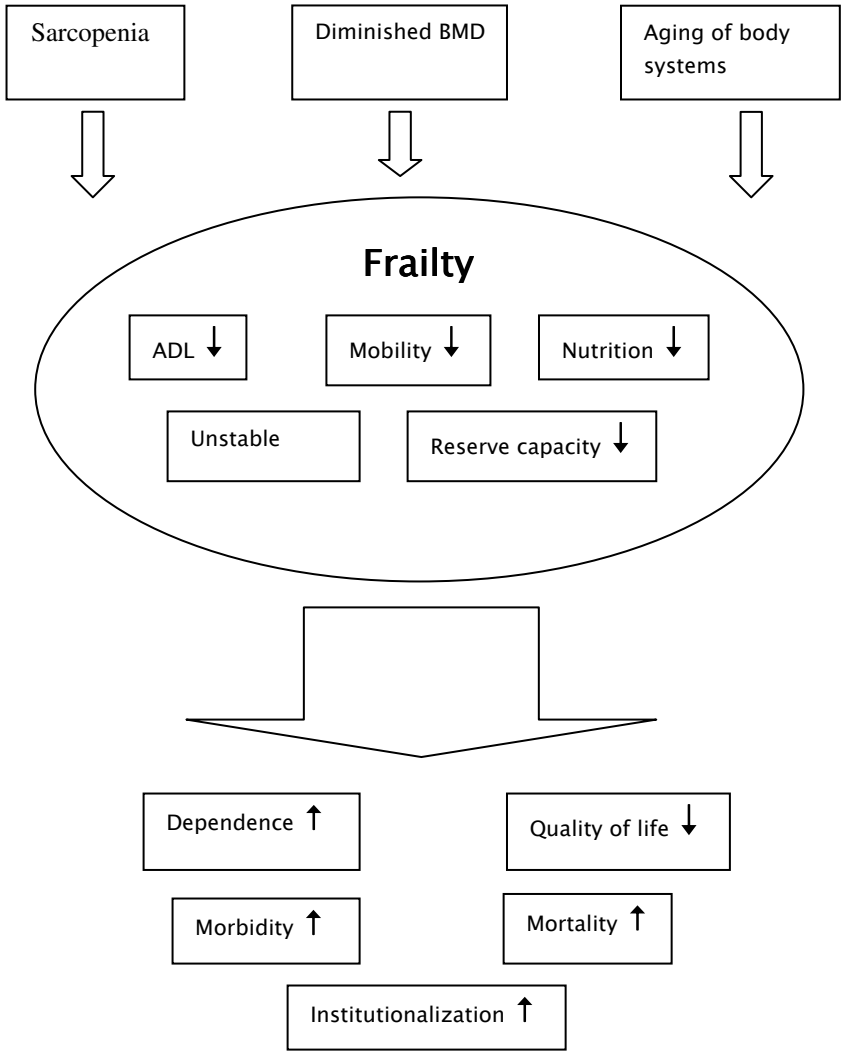


Abbreviations: T, Testosterone; DHT, 5α-dihydrotestosterone; DHEA, dihydroepiandrosterone; DHEA-S, dihydroepiandrosterone sulfate; E2, estradiol; E1, estron; 17β-HSD, 17β-hydroxysteroid dehydrogenase; 3β-HSD, 3β-hydroxysteroid dehydrogenase; SS, steroid sulfatase

The pathophysiologic mechanisms of frailty

Like we have discussed already in the general background, two physical changes associated with aging appear to be the major drivers of frailty, namely, loss of muscle mass (sarcopenia) and bone mineral density (BMD) (osteopenia), as shown in figure 2.[2,16,17]

Figure 2 Pathological pathways of frailty



Abbreviations: BMD, bone mineral density; ADL, activities of daily living

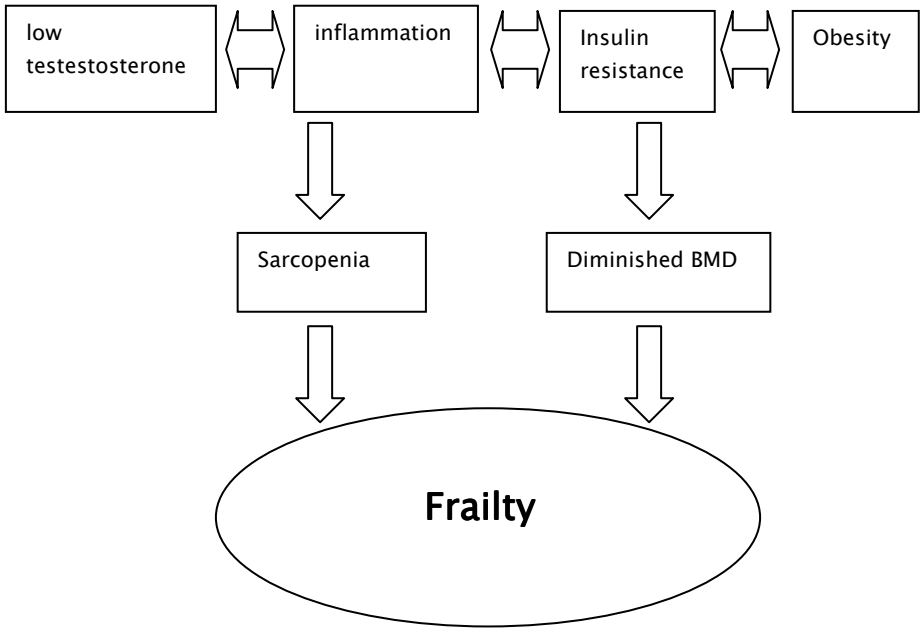
Sarcopenia is defined as losses of muscle mass greater than 2 standard deviations below the mean for young healthy controls. The prevalence of sarcopenia rangs from 13-24% in

persons aged 65 to 70 years and amounts to over 50% for those older than 80 years.[18] Sarcopenia results in a loss of muscle strength and function. This increases the risk of falls and, together with a decrease of bone mineral density, fractures. Consequently, this can lead to loss of independence and disability.

The pathophysiologic mechanisms of the frailty process are not yet fully understood. Frailty is a complex syndrome that is believed to result from multiple contributing factors. One of the factors that is clearly associated with frailty is a low testosterone level. Low testosterone levels are associated with several characteristics of frailty, like muscle strength and bone mineral density (*chapter 2.2* of this thesis). But there is still no convincing evidence that testosterone is associated with frailty itself. Also other factors, like inflammation, insulin resistance and obesity, are associated with sarcopenia (*chapter 2.1* of this thesis) and osteoporosis, and finally with frailty.

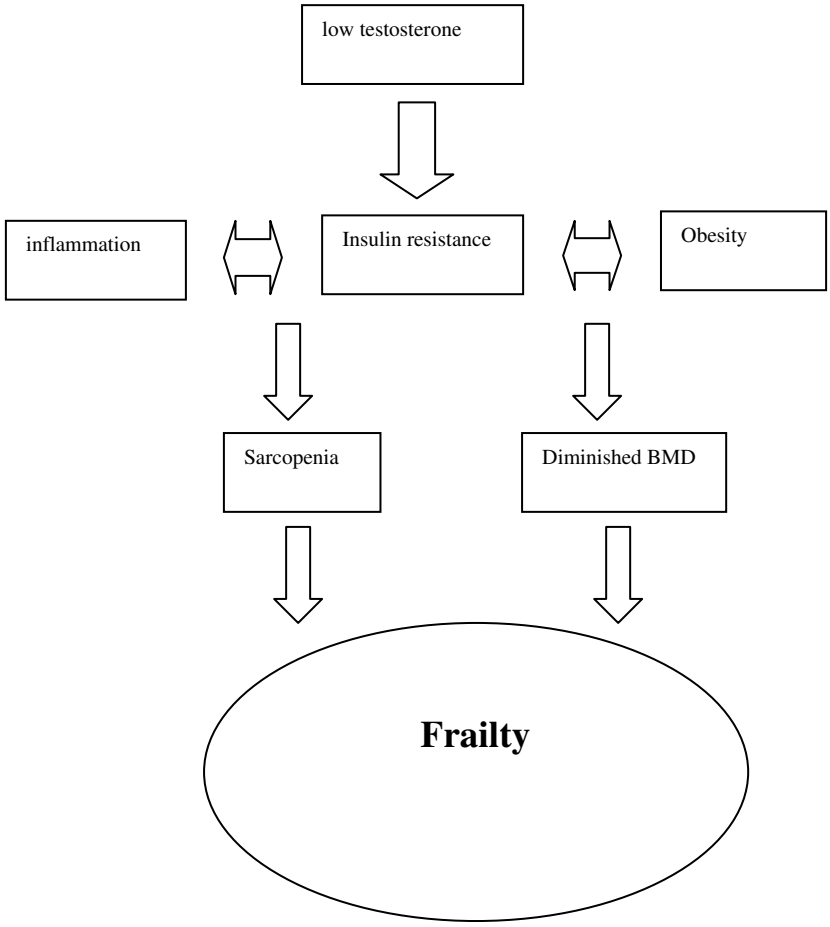
There are two possible hypotheses how these factors can lead to frailty. The first hypothesis is that all four factors are independently related to loss of muscle strength and bone mineral density, but the factors are also related to each other. (figure 3a)

Figure 3A Relation between low testosterone, inflammation, glucose metabolism and obesity, and frailty – hypothesis A



The second hypothesis is that a low testosterone concentration is the main driving factor. A low testosterone levels promotes inflammation, disturbances of the glucose metabolism and changes of body composition, with frailty as the eventual consequence. (figure 3b)

Figure 3B Relation between low testosterone, inflammation, glucose metabolism and obesity, and frailty – hypothesis B



We will briefly discuss the available evidence for these two hypotheses.

Inflammation

In *chapter 2.1* of this thesis we have shown that a higher level of the inflammatory marker hs-CRP is associated with lower muscle strength. Our results are comparable with other studies.[19-24] Inflammatory biomarkers may contribute to loss of function via indirect and direct effects on muscle catabolism.[25] Inflammation affects muscle strength by its effect on body composition by accelerating changes that are typical of the aging process.[26] Higher levels of CRP have been associated with obesity and insulin resistance.[21,27-29] These findings could be explained through the inverse association between physical activity and markers of inflammation.[30] Evidence for a direct effect of cytokines on muscle mass comes from animal studies. Experimental studies have shown that administration of IL-6 or TNF-α in rats increases muscle protein breakdown, decreases the rate of protein synthesis, reduces the total skeletal muscle amino acid concentration, and causes muscle wasting.[31-34]

Higher inflammatory markers are also associated with a lower bone mineral density.[35-39], although not in all studies.[40-42] However, it is well demonstrated by animal and in vitro studies that IL-6 and TNF-α are responsible for osteoclastogenesis and increased trabecular bone resorption, while simultaneously inhibiting osteoblast function.[19,43-48]

In humans increases in inflammatory markers have also been associated with frailty[5,19,49,50], even in the absence of significant clinical morbidity.[50] Higher circulating levels of IL-6 can predict disability onset in older persons.[26] This may be attributable to a direct effect of IL-6 on muscle atrophy and/or to the pathophysiologic role played by IL-6 in specific diseases. In combination with the mechanistic evidence from animal studies, this strongly suggests that there is a disease-independent inflammatory mechanism involved in the development of frailty and disability.

Low testosterone levels are inversely related with inflammatory markers.[51,52] The mechanism remains unclear, but it has been hypothesized that testosterone can inhibit the production of cytokines or that it down-regulate the production of the IL-6 receptor.[19,53-55], although data are conflicting regarding the effects of inflammatory markers on testosterone as well. There is evidence from in vitro studies that IL-6 and TNF-α inhibit testosterone secretion by their influence on the central (hypothalamic-pituitary) and peripheral (testicular) components of the gonadal axis.[56] However, induction of hypogonadism in older men is followed by a significant increase in IL-6 and TNF-α .[57] In addition, several studies have shown decreased levels of inflammatory markers after substitution of

testosterone.[58-62] However, we were not able to replicate this effect of testosterone supplementation with Andriol on hs-CRP in our own intervention studie.[63]

Insulin resistance

As shown in *chapter 2.1* of this thesis, higher glucose and insulin levels and lower insulin sensitivity are associated with lower muscle strength. Other studies have found similar results in people with and without diabetes mellitus.[64-66]Also longitudinal studies have shown that type 2 diabetes mellitus is associated with accelerated loss of muscle strength.[67] Insulin has a powerful anabolic effect on proteins, because it stimulates protein synthesis.[68,69] This effect is possible modulated by insulin-induced changes in muscle blood flow and amino acid availability.[69] Therefore, insulin resistance may promote muscle breakdown. A disturbed glucose metabolism can also have indirect effects on muscle mass and strength, since insulin resistance is associated with obesity, a decline in physical activity and increased inflammatory cytokines.[19,70]

Insulin resistance and hyperglycemia are also associated with a lower bone mineral density.[71,72] Insulin has a direct bone anabolic effect and hyperglycemia may have several adverse effects on bone metabolism.[71] First, glucose is the principal energy source for osteocloasts. In addition, hyperglycemia leads to non-enzymatic glycosylation of various bone proteins, including type 1 collagen, which may impair bone quality. Last, hyperglycemia caused hypercalciuria, which has indirect influences on the skelet.

Accumulating evidence suggest that frailty is not only associated with prevalent diabetes mellitus[7], but also with glucose intolerance in older adults without diabetes mellitus.[5,50] On the other hand, people who develop frailty are more likely to develop DM.[5] This suggests that the two disorders may have common pathogenic mechanisms, but it is still unclear what the direction of the causal relationship is.

Evidence has indicated that low testosterone levels are associated with increased risk of type 2 diabetes mellitus in cross-sectional and prospective studies (reviewed in[73]). Moreover, induction of hypogonadism with gonadotropin-releasing hormone analogs is associated with hyperinsulinemia in men.[74-76] The mechanism by which endogenous testosterone affects glucose metabolism is still not fully understood, but may be secondary to the effect of testosterone on body fat distribution.[77] In our Andriol intervention study (*chapter 4.1)* testosterone supplementation resulted in a decrease in plasma glucose

concentration and an increase in insulin resistance. Other studies with testosterone supplementation have also shown a decrease in blood glucose concentrations, plasma insulin levels and mean glycated hemoglobin (HbA1C) and an increase of insulin sensitivity, although these studies were mainly based on subjects with type 2 diabetes mellitus or abdominal obesity.[78,79]

Obesity

Lower muscle strength is associated with a higher fat mass and obesity (sarcopenic obesity), as shown in *chapter 2.1* of this thesis and in other studies.[80-83] One of the explanations for this association is that obesity can lead to increases in the amount of fat within and around muscles. Fat infiltration into muscle is associated with lower muscle strength and leg performance capacity.[84,85] Obesity can also negatively affect muscle strength through the upregulation of proinflammatory cytokine production.[86] A third explanation is that obese persons tend to be less physically active and this may contribute to decreased muscle strength.[87] However, the reverse is also possible. Roubenoff suggested that the loss of muscle mass results in lower physical activity, which, in turn, results in reduced energy expenditure, fat gain, and obesity.[88] Finally, obesity can have an indirect effect on muscle mass through its association with insulin resistance.

Several studies have shown that obesity protects against bone loss.[89-91] We have shown in *chapter 2.2.* of this thesis that estradiol is the main hormone in bone metabolism. Since estradiol levels are higher in obese persons through an increase of peripheral aromatization of testosterone, this might be an important mechanism through which obesity protects against bone loss.

Despite the various effects of obesity on muscle tissue and bone metabolism, obesity is still associated with frailty.[7] An increased fat mass, as shown in *chapter 2.1* of this thesis, and decreased lean body mass can predict poor physical performance.[83] Moreover, frail elderly have a significant higher BMI than non- frail elderly, despite the weight loss variable to identify frailty.[50]

Low testosterone concentrations are associated with a higher fat mass and a lower muscle mass (*chapter 2.1* of this thesis). Body fat distribution also changes with lower testosterone concentrations, with visceral abdominal fat increase and subcutaneous abdominal fat decrease. These changes occur even when there are no significant changes in body mass

index (BMI).[92] Moreover, induction of hypogonadism in men with gonadotropin-releasing hormone analogs is associated with onset of central obesity.[76] Androgen receptors are present to a greater degree in visceral adipocytes than in subcutaneous adipocytes and it has been recently shown that androgens, via androgen receptors, inhibit the differentiation of preadipocytes into mature adipocytes.[93] Androgens are also known to increase lean body mass and decrease visceral fat mass by inhibiting lipoprotein lipase activity, therefore inhibiting triglyceride uptake and accelerating triglyceride release from abdominal adipose tissue.[94]

Testosterone supplementation results in an increase of lean body mass and a decrease of fat mass (this thesis *chapter 4.1*). The increase in lean body mass and the decrease in fat mass in this study are comparable to those reported in most other testosterone supplementation studies.[78,95]

Relation between obesity, inflammation and disturbed glucose metabolism

It is now evident that adipose tissue is an active metabolic tissue that secretes hormones and proteins. In adipose tissue, either adipocytes directly or infiltrating macrophages produce pro-inflammatory cytokines, such as IL-6 and TNF-a, and adipokines, such as leptin and adiponectin, that up-regulate the inflammatory response[96-98], which, in return, may contribute to a decline of muscle mass and strength[21] and a decrease of bone mineral density. So, increases in fat mass, particularly visceral fat, may lead to increased secretion of a number of pro-inflammatory cytokines[99,100] that are positively associated with central obesity.[21,101] The degree of obesity directly affects inflammation which in turn contributs to the development and progression of sarcopenia.[86] Some studies have shown that sarcopenic obese participants were more likely to be disabled than participants who were just obese or sarcopenic.[102,103] The opposite is also possible, since the use of cytokine inhibitors is associated with weight gain.[104]

A growing body of evidence has shown that an increase in circulating cytokines contributes to insulin resistance.[100,105-109] The mechanism by which cytokines influences insulin resistance is probably multifaceted. IL-6 inhibits nonoxidative glucose metabolism[110,111], as well as lipoprotein lipase with a secondary increase in plasma triglyceride concentrations.[111] In addition, IL-6 activates the suppression of cytokine signaling proteins.[112,113] As has been well established, obesity is also associated with insulin

resistance. Some studies have found that inflammatory molecules mediate obesity-related insulin resistance through a cross-talk between cytokine receptors and insulin receptor signaling pathways.[114,115]

Insulin resistance and obesity are both components of the metabolic syndrome, and chronic inflammation is strongly related to the metabolic syndrome. In this thesis, we choose to look for the individual components of the metabolic syndrome instead of the syndrome as a whole, because we know that in elderly people the single components of the metabolic syndrome are equally good or even better predicters of negative outcomes, like cardiovascular diseases and mortality than the metabolic syndrome itself.[116-118]

Conclusions and implications

It is now evident that inflammation, insulin resistance and obesity, all have an important role in the pathogenesis of sarcopenia, low bone mineral density and frailty. All three factors have direct effects on muscle and bone mass, as well as indirect effects by influencing each other. However, the exact role of testosterone in the pathogenesis is still unclear. Based on the currently available evidence we have presented, I still believe that the second hypothesis is the most likely. Testosterone is direct or indirect associated with sarcopenia and bone mineral density by its effects on body composition, glucose metabolism and inflammation. Induction of hypogonadism is followed by an increase of inflammation, insulin resistance and obesity. Moreover, testosterone supplementation may have positive effects on all these aspects, although we could not fully confirm this in our cross-sectional study and testosterone supplementation study. We found no direct association between testosterone and muscle strength or BMD. However, it is possible that testosterone has an indirect effect on muscle strength through its effects on inflammation, glucose metabolism and obesity, and on BMD through its effects on estrogen levels. Other explanations are that the participants in our cross-sectional study, as well in the testosterone supplementation study, were too healthy and not frail enough to find these associations and/or effects, or that other unknown determinants, also play a role and influence the associations. We found a positive effect of testosterone supplementation on body composition and glucose metabolism, but there was no effect on muscle strength, physical performance and BMD. Possible explanations are that the study period was not long enough or that the dose and way of supplementation were not optimal. In our view, 6

months supplementation should be long enough to exert effects on body composition, glucose metabolism, sexual functioning and hs-CRP levels, but probably too short to find effect on cognition, arterial stiffness and BMD. Moreover, in our trial the change in serum testosterone levels after six months was lower than anticipated. This could reflect limited reliability of oral bioavailability, a too low dose, short half life and fluctuating serum levels. This might imply that the efficacy of oral testosterone supplementation, at least in the given formulation and dose, is limited. In recent years, a number of newer testosterone preparations are developed that give more steady and higher testosterone levels. It is still an open question whether using these newer, different testosterone preparations, will result in beneficial effects on inflammation, insulin resistance and obesity, and through these risk factors ultimately on frailty.

In summary, although, there is ample evidence that testosterone is a major factor in the pathogenesis of frailty, the evidence is not conclusive and further studies are necessary to elucidate the exact role of testosterone in the pathogenesis, prevention and treatment of frailty. Large-scale prospective studies are needed to establish the biological significance of testosterone in the etiology of frailty. And, even more important, randomized, double-blind, placebo-controlled testosterone supplementation studies performed in subjects with a high risk for frailty (elderly men with obesity, insulin resistance, chronic low-grade inflammation **and** low testosterone levels), of sufficient duration and with other testosterone preparations are necessary to answer the central question whether testosterone replacement therapy can help for the prevention and/or treatment of frailty.

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Chapter 6a
Summary

With aging, there is an increase of the incidence of frailty. Frailty is associated with adverse health outcomes, like falls and fractures, disabilities, hospitalization, institutionalization and mortality. It is generally considered that frailty, unlike the aging process, is in part reversible and amenable to interventions. Therefore, it is important to expand our understanding of the mechanisms that play a role in the pathophysiology of frailty, so we can develop effective measures to prevent frailty in future. Two physical changes associated with aging appear to be the main cause of frailty, namely, loss of muscle mass (sarcopenia) and bone mineral density. The last decades more attention has been raised to the potential importance of androgens in etiology, prevention and treatment of frailty.

In this thesis, we investigated the relations between androgens, sarcopenia, bone mineral density and frailty in men and we assessed the effects of testosterone supplementation on these parameters.

The data described in this thesis have been derived from two studies, a cross-sectional study on endogenous androgen levels in 400 men aged 40-80 years and a randomized, double-blind, placebo-controlled trial with testosterone supplementation in 237 men aged 60-80 years.

In *chapter 2*, the relation of endogenous sex hormones with the 2 most important components of frailty, namely muscle strength and bone mineral density, is explored.

In *chapter 2.1* we studied the association of endogenous sex hormone levels, chronic low grade inflammation, glucose metabolism and obesity with muscle strength and physical performance in men. Chronic low grade inflammation, high glucose and insulin levels and higher fat mass were all associated with lower muscle strength and a decrease of physical performance, while testosterone had no direct effect on muscle strength and physical performance.

The associations between endogenous sex hormone levels, polymorphisms of the aromatase gene and the estrogen receptor genes α and β, and bone mineral density in men are described in *chapter 2.2*. Estradiol levels, but not testosterone levels, were associated with bone mineral density. The main source of estradiol is peripheral conversion of testosterone by the aromatase enzyme and estradiol exerts its effects on bone primarily via the estrogen receptors. Several single nucleotide polymorphisms (SNP) in the aromatase gene and the estrogen receptor genes have been described, and we additionally assessed the association

between these gene polymorphisms and bone mass in men. None of the SNPs were associated with bone mineral density. Genetic variation of the aromatase gene was associated with estradiol levels.

Chapter 3 presents the design and baseline characteristics of a randomized, double-blind, placebo-controlled testosterone supplementation trial in 237 elderly men with low to low-normal testosterone levels to investigate the effects of testosterone supplementation on functional mobility, quality of life, body composition, cognitive function, bone mineral density, vascular function, sexual function and safety parameters.

In *chapter 4* the results of this testosterone supplementation study are described.
Despite the growing evidence that the decline of testosterone is associated with adverse health effects, it is difficult to identify the aging males with (relative) androgen deficiency who might benefit from testosterone replacement therapy. Therefore, we assessed the association between the testosterone concentration and the symptoms of testosterone deficiency according to two screening questionnaires (the ADAM-questionnaire (Androgen Deficiency in Ageing Males) and the AMS-questionnaire (Ageing Males' Symptoms rating scale)), and the effects of testosterone supplementation on these symptoms of testosterone deficiency according to this two screening questionnaires in *chapter 4.1*. We found a significant association between age and the scores on the ADAM and the AMS questionnaire, but the total testosterone concentration was not significantly associated with the scores on these two questionnaires. Testosterone supplementation during 6 months with testosterone undecanoate 160 mg/day had no effect on the scores of the ADAM and the AMS questionnaires.
The effects of testosterone supplementation on parameters associated with aging are presented in *chapter 4.2*. After 6 months of supplementation, there was an increase of lean body mass and a decrease of fat mass in the testosterone group compared to the placebo group. The increase in lean body mass was not accompanied by an increase of functional mobility or muscle strength. The decrease of fat mass was accompanied by a decrease in plasma glucose concentration and a decrease in insulin resistance. There were no beneficial effects on cognitive function or bone mineral density. Treatment resulted in a net decrease of the total cholesterol concentration, but also a decrease of the HDL cholesterol. There was

an increase of the hormone related quality of life in the testosterone group. There were no negative effects on prostate safety.
In *chapter 4.3* we investigate the effect of testosterone supplementation on sexual function. After 6 months treatment, there were no differences in scores on sexual function between the testosterone and placebo group. Subanalyses showed that although a baseline testosterone level in the lowest tertile was associated with significantly lower scores for sexual fantasies, desire of sexual contact and frequency of sexual contact, supplementation of testosterone did also not result in improvement on any of these tests in this subgroup.

The main results of the above studies are reviewed and discussed in *chapter 5* of this thesis. We discussed whether it is possible that testosterone is the main factor in the pathogenesis of frailty. There is increasing evidence that a low testosterone levels leads to inflammation, disturbances of the glucose metabolism and changes of body composition, which results to sarcopenia and a low bone mineral density and finally to frailty. However, many questions in this discussion still need to be addressed and await further study.

Chapter 6b
Samenvatting

Samenvatting

Met het ouder worden is er een toename van de incidentie van frailty. Frailty is geassocieerd met negatieve gezondheidsaspecten, zoals vallen en fracturen, lichamelijke beperkingen, opname in het ziekenhuis, opname in verzorgingshuizen en verpleeghuizen, en een verhoogde mortaliteit. Het is algemeen geaccepteerd dat frailty, in tegenstelling tot het verouderingsproces, gedeeltelijk reversibel is en beïnvloedbaar door interventies. Daarom is het belangrijk om meer over de pathofysiologie van frailty te weten te komen, zodat er in de toekomst effectieve maatregelen genomen kunnen worden om frailty te voorkomen. Twee fysiologische veranderingen, die geassocieerd zijn met veroudering, zijn belangrijke oorzaken van frailty, namelijk verlies van spiermassa (sarcopenie) en verlies van botminerale dichtheid.
De laatste jaren is er in toenemende mate aandacht voor het mogelijke belang van androgenen in de etiologie, preventie en behandeling van frailty. In de onderzoeken beschreven in dit proefschrift bestudeerden wij de relatie tussen androgenen, spiermassa, botminerale dichtheid en frailty in mannen, en we onderzochten de effecten van testosteron suppletie op deze parameters.
De gegevens in dit proefschrift zijn afkomstig van twee onderzoeken; een dwarsdoorsnede onderzoek naar endogene androgeenconcentraties bij 400 mannen in de leeftijd van 40-80 jaar, en een gerandomiseerde, dubbelblinde, placebo-gecontroleerde studie met testosteronsuppletie bij 237 mannen tussen 60-80 jaar oud.

In *hoofdstuk 2* wordt de relatie onderzocht tussen endogene geslachtshormonen en twee belangrijke componenten van frailty; spierkracht en botminerale dichtheid.
In *hoofdstuk 2.1* onderzochten we de associatie tussen endogene geslachtshormoon-concentraties, chronische laaaggradige ontsteking, glucose metabolisme en obesitas met spierkracht en lichamelijk functioneren in mannen. Chronische laaggradige ontsteking, hoge glucose en insuline concentraties, en een hoge vetmassa zijn allen geassocieerd met een lagere spierkracht en een afname van het lichamelijk functioneren. De testosteronconcentratie had geen directe relatie met spierkracht en het lichamelijk functioneren.

De associatie tussen endogene geslachtshormoonconcentraties, polymorphismen van het aromatase gen en de genen van de oestrogeenreceptoren α and β, en de botminerale dichtheid in mannen wordt beschreven in *hoofdstuk 2.2*. Estradiolconcentraties, maar niet testosteronconcentraties, zijn geassocieerd met de botminerale dichtheid. De belangrijkste bron van estradiol is de perifere conversie van testosteron door het aromatase enzym. Estradiol oefent zijn effect op het bot uit via de oestrogeenreceptoren α en β. Verschillende "single nucleotide polymorphismen" (SNP) van de aromatasegen en de genen van de oestrogeenreceptoren zijn beschreven. Aansluitend hebben we daarom gekeken naar de associatie tussen de polymorphismen van deze genen en botmassa in mannen. Geen van de onderzochtte SNPs was geassocieerd met de botminerale dichtheid. Genetische variatie van het aromatase gen was geassocieerd met estradiolspiegels.

Hoofdstuk 3 presenteert de opzet en de basiskarakteristieken van een gerandomiseerde, dubbelblinde, placebo-gecontroleerde testosteron suppletie trial bij 237 oudere mannen met laag tot laagnormale testosteronconcentraties. Het doel van deze studie is om het effect van testosteronsuppletie op functionele mobiliteit, kwaliteit van leven, lichaamssamenstelling, cognitie, botminerale dichtheid, risicofactoren voor vasculaire functie, seksueel functioneren en een aantal veiligheidsaspecten te onderzoeken.

In *hoofdstuk 4* worden de resultaten van deze testosteron suppletie trial beschreven. Ondanks toenemend bewijs dat de afname van de testosteronconcentratie geassocieerd is met negatieve gezondheidsaspecten, is het moeilijk om die oudere mannen te identificeren met een (relatief) androgeen tekort die mogelijk baat zouden kunnen hebben van testosteron suppletie. Daarom onderzochten we de associatie tussen de testosteronconcentratie en de symptomen van testosterondeficiëntie met behulp van twee screeningsvragenlijsten (de ADAM-vragenlijst (Androgen Deficiency in Ageing Males) en de AMS-vragenlijst (Ageing Males' Symptoms rating scale)), en vervolgens bestudeerden wij de effecten van testosteron suppletie op de symptomen van testosterondeficiëntie volgens deze twee screeningsvragenlijsten in *hoofdstuk 4.1*. We vonden een significante associatie tussen leeftijd en de scores van de ADAM en de AMS vragenlijsten, maar de totale testosteronconcentratie was niet significant geassocieerd met de scores van deze twee

vragenlijsten. Testosteron suppletie gedurende 6 maanden had geen effect op de scores van de ADAM en de AMS vragenlijsten

De effecten van testosteron suppletie op parameters die geassocieerd zijn met veroudering worden gepresenteerd in *hoofdstuk 4.2*. Na 6 maanden suppletie was er een toename van de spiermassa en een afname van de vetmassa in de testosterongroep vergeleken met de placebogroep. De toename in spiermassa ging niet gepaard met een toename van de functionele mobiliteit of de spierkracht. De afname van de vetmassa ging gepaard met een afname van de plasma glucoseconcentratie en een afname van insuline resistentie. Er waren geen positieve effecten op de cognitie of de botminerale dichtheid. Behandeling leidde tot een afname van de totale cholesterol concentratie, maar ook een afname van de HDL cholesterol concentratie. Er was een verbetering van de hormoongerelateerde kwaliteit van leven in de testosterongroep. Er waren geen negatieve effecten op de prostaat.

In *hoofdstuk 4.3* onderzochten we de effecten van testosteronsuppletie op het seksueel functioneren. Na 6 maanden behandeling met testosteron waren er geen verschillen in scores van het seksueel functioneren tussen de testosteron en de placebo groep. Subanalyses lieten zien dat, ondanks dat een testosteronconcentratie in het laagste tertiel geassocieerd was met lagere scores voor seksueel fantaseren, zin in seksuele contacten en de frequentie van seksuele contacten aan het begin van de studie, testosteron suppletie ook in deze subgroep geen verbetering gaf van de scores van één van deze testen.

De belangrijkste resultaten van de bovengenoemde studies worden beschouwd en bediscusseerd in *hoofdstuk 5* van dit proefschrift. Het begint er steeds meer op te lijken dat testosteron een belangrijke factor in de pathogenese van frailty is. Er is toenemend bewijs dat lage testosteronconcentraties leiden tot chronische laaggradige ontsteking, verstoringen van het glucose metabolisme en veranderingen in de lichaamssamenstelling, hetgeen resulteert in sarcopenie en een lage botminerale dichtheid, en uiteindelijk kan leiden tot frailty. Er is echter nog veel onduidelijk en verder onderzoek naar de rol van androgenen in de pathofysiologie en preventie van frailty is dan ook noodzakelijk

Chapter 7
Dankwoord, Curriculum Vitae en Publicatielijst

Dankwoord

Het schrijven van dit dankwoord is eigenlijk het allerleukste gedeelte van het hele proefschrift, omdat ik nu eindelijk alle mensen kan en mag bedanken die mij, op welke wijze dan ook, hebben geholpen aan de tot stand koming van dit proefschrift.

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Curriculum Vitae

Mariëlle Henriëtte Emmelot-Vonk werd geboren op 28 februari 1972 te Den Burg – Texel. Zij behaalde in 1990 het VWO diploma aan de Rijksscholengemeenschap Texel te Den Burg, waarna zij geneeskunde heeft gestudeerd aan de Rijksuniversiteit Limburg. Aansluitend aan het behalen van haar artsexamen in 1997 heeft zij 6 maanden als ANIOS (arts niet in opleiding tot specialist) klinische geriatrie gewerkt op de afdeling interne geneeskunde van het Havenziekenhuis te Rotterdam. In oktober 1997 is zij gestart met haar opleiding tot klinisch geriater. Eerst 2 jaar de vooropleiding interne geneeskunde in het Havenziekenhuis te Rotterdam (opleider dr. A.G.C. Bauer), vervolgens 2 jaar klinisch geriatrie in het UMC Utrecht (opleider dr. P.A.F. Jansen) en afsluitend 1 jaar psychogeriatrie in Parnassia te Den Haag (opleider dr. R.C. Sival). Sinds oktober 2002 is zij werkzaam als klinisch geriater in het UMC Utrecht. In het najaar 2003 is zij gestart met de werkzaamheden van dit proefschrift.
Mariëlle is in 1999 getrouwd met Tim Emmelot en zij hebben samen 3 kinderen: Lotte (2001), Bram (2004) en Sophie (2005).

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