The decline of serum testosterone levels in community-dwelling men over 70 years of age: descriptive data and predictors of longitudinal changes

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Abstract

Objective: This study was designed to assess longitudinal changes in serum testosterone levels, explore relationships with aging, genetic-, health-, and lifestyle-related factors, and investigate predictors of changes in healthy elderly men.

Design: Population-based, longitudinal, 4-year observational study in 221 community-dwelling men aged 71–86 years at baseline.

Methods: Hormone levels assessed by immunoassay, anthropometry, questionnaires on general health, and genetic polymorphisms. Predictors of changes in testosterone levels explored using linear mixed-effects modeling for longitudinal analyses.

Results: Total testosterone (TT), free testosterone, and bioavailable testosterone (BioT) levels decreased with aging, decreases in BioT being most marked. No changes in sex hormone-binding globulin (SHBG) or estradiol (E2), while LH and FSH levels increased during follow-up. Subjects who gained weight displayed a greater decline in TT levels, mainly due to decreasing SHBG levels. However, baseline body composition was not predictive of subsequent changes in testosterone levels. Baseline E2 (P = 0.023 to 0.004), LH (P = 0.046 to 0.005), and FSH (P < 0.002) levels were independently positively associated with a faster decline in testosterone fractions, although only FSH remained significant when adjusting for baseline testosterone (P = 0.041–0.035). Carriers of a ‘TA’ haplotype of the estrogen receptor α gene (ERα) PvuII and XbaI polymorphisms displayed a slower decline of TT and BioT (P = 0.041–0.007).

Conclusions: In elderly men with already low serum testosterone levels, a further decline was observed, independent of baseline age. The identification of FSH levels as a predictor of this decline appears to reflect the testicular mechanisms of aging-related changes in testosterone production, whereas associations with E2 and ERα polymorphisms are suggestive of estrogen-related processes, possibly related to changes in the neuroendocrine regulation of testosterone production.

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Introduction

It is now widely accepted that normal chronological aging in men is associated with a progressive decline in androgen levels. More than 20% of healthy men over 60 years of age present with serum levels below the reference range for young men. In contrast to women, who experience a rather precipitous and profound reduction of endogenous estradiol (E2) production during menopausal transition, the decline of testosterone production in aging men is much more gradual and individually highly variable. This decline results from both testicular changes and altered neuroendocrine regulation of luteinizing hormone (LH) secretion (1). Between the third and eighth decade of life, a 20–30% decrease in population mean serum testosterone levels can be observed. As serum levels of sex hormone-binding globulin (SHBG) increase with age, the decrease in serum-free testosterone (FT) and non-SHBG-bound testosterone levels, often referred to as bioavailable testosterone (BioT), is of even greater magnitude reaching up to 50% over the same age range (2–5).

Understanding the physiology of this decline is important because testosterone has actions in many tissues and low serum testosterone levels have been associated with a variety of adverse conditions, which
Longitudinal changes in androgen levels were explored. Using mixed-effects modeling, predictors of changes of gonadal steroid and gonadotropin concentrations. Indeed, many of these conditions are non-specific, multifactorially determined, and often associated with normal aging per se (1). Up to now, the risk—benefit ratio for testosterone supplementation in elderly men has not been established (10).

While chronological aging per se seems to be associated with declining testosterone production, co-morbid chronic illnesses, and certain medications as well as the aging-related changes in body composition may accentuate and contribute to these changes (1, 4, 11, 12). Furthermore, lifestyle-related factors such as smoking may also influence testosterone levels (2, 7, 11). In addition, the possibility of genetic polymorphisms influencing this decline cannot be excluded (13). Most information about changes in testosterone levels with aging results from cross-sectional studies (2, 3, 5, 7, 14, 15), while information from longitudinal studies remains more limited (4, 16–19). Moreover, it should be noted that most studies report on testosterone levels of middle-aged men and relatively few studies have focused specifically on men older than 70 years, whereas many of the adverse conditions potentially associated with the age-related decline of testosterone levels occur relatively late in life.

In this population-based longitudinal study, we followed a homogenous group of 221 community-dwelling elderly men, aged 71–86 years at baseline, for a period of 4 years. We report on the distribution and changes of gonadal steroid and gonadotropin concentrations. Using mixed-effects modeling, predictors of longitudinal changes in androgen levels were explored.

Subjects and methods

Study subjects

Subjects were recruited from the population registry of the semi-rural community of Merelbeke (Belgium). A sample of 352 community-dwelling men, aged 71–86 years, agreed to participate (initial participation rate was 47.1%). This longitudinal, population-based study was specifically designed to investigate the process of aging, focusing on hormonal changes, bone metabolism, and body composition in elderly men at yearly intervals over a period of 4 years. The study was approved by the ethics committee of the Ghent University Hospital (Belgium). All the participants gave written informed consent for participation in this study and completed questionnaires pertaining to general health (Geriatric Depression Scale, Short Form-36 and Rapid Disability Rating Scale-2 (20)), dietary habits, and physical activity. Baseline characteristics, exclusion and inclusion criteria have been described extensively in previous publications (20–22). Following exclusions because of past or current history of disorders or treatments known to affect androgen status. 221 eligible subjects were included in the longitudinal analyses, for a total of 912 observations. Five serial measurements were available in 125 subjects, for the remaining subjects two to four data points were available. Reasons for loss to follow-up were institutionalization, withdrawal of consent, death, and occurrence of diseases or treatments known to affect androgen levels. All exclusions were censored before hormonal analysis. Longitudinal changes in BioT over 4 years in 214 men of this longitudinal cohort have previously been shown in Fig. 1 of the review article under reference (1).

Hormonal assays

Each consecutive year, venous blood was obtained between 0800 and 1000 h after overnight fasting and serum was stored at −80 °C until batch analysis. Blood collection was completed over a period of 2 months during summer, except for the first year when blood collection was performed from March till June. Commercial immunoassays were used to determine serum levels of total testosterone (TT), follicle-stimulating hormone (FSH), and LH (Medgenix, Fleurus, Belgium), E2 (Clinical Assay, DiaSorin s.r.l., Saluggia, Italy, according to a modified protocol that doubles the serum amount (22)), SHBG (Orion Diagnostica, Espoo, Finland), insulin (Pharmacia & Upjohn Diagnostics AB), insulin-like growth factor-1 (IGF-1), IGF-binding protein 3 (IGFBP3; Diagnostic Laboratory Systems Inc., Webster, TX, USA), and leptin (Linco Research Inc., St Louis, MO, USA). Testosterone and SHBG levels were determined at all visits, whereas E2, LH, and FSH were assessed at the first and last study visits. All samples from the same subject were assayed in a single assay run using duplicate measurements. The intra- and interassay coefficients of variation (CV) were 4.8 and 5.1% for testosterone and 3.6 and 6.6% for SHBG respectively. Intra- and inter-CV for all other measurements were below 10% and 15% respectively. Serum non-SHBG-bound testosterone (BioT), FT, non-SHBG-bound E2 (‘bioavailable E2’; BioE2), and free E2 (FE2) were calculated from serum TT, total E2, SHBG, and albumin concentrations using a previously validated equation derived from mass action law (23, 24).

Determination of gene polymorphisms

Genomic DNA was extracted from EDTA-treated blood using a commercial kit (Qiagen Midi Kit, Qiagen Inc.).
Androgen receptor gene (AR) CAG repeat length
As previously described (21), PCR to amplify exon 1 of the AR gene with primers 5'-AGCCTGTTGAACCTCTCTGAGC-3' (sense) and 5'-CTGCCTTACACAACTCCTTGGC-3' (anti-sense). After ethanol precipitation, the amplified fragment was directly sequenced on a ABI Prism 310 sequencer (ABI Prism, Perkin–Elmer Applied Biosystems). To confirm repeat length, homozygotic representatives of the different observed allele lengths (7–13) were sequenced. The allelic variant consisting of (TTTA)7 repeats was the most frequently observed one in this population of healthy elderly men. Subjects were classified in three genotype groups according to the presence of the shortest allele length.

CYP19 gene (TTTA)n repeat length
As previously described (22), (TTTA)n repeat length was assessed by fragment analysis of the PCR products using primers published by Haiman et al. (25). The forward primers were 5'-labeled with a fluorescent dye for automated fragment analysis on a ABI Prism 310 sequencer (ABI Prism, Perkin–Elmer Applied Biosystems), using BigDye Terminator Cycle Sequencing Reaction Kit (ABI Prism, Perkin–Elmer Applied Biosystems). To confirm repeat length, homozygotic representatives of the different observed allele lengths (7–13) were sequenced. The allelic variant consisting of (TTTA)7 repeats was the most frequently observed one in this population of healthy elderly men. Subjects were classified in three genotype groups according to the presence of the shortest allele length.

Estrogen receptor α gene (ERα) single nucleotide polymorphisms (SNPs) were determined by a single PCR fragment in accordance with a study by Yamada et al. (26). The amplified products were then digested with the XbaI and PvuII restriction enzymes (New England Biolabs Inc., Beverly, MA, USA). Haplotypes were constructed using Phase software (27). Subjects were then defined by the presence of a ‘TA’ haplotype.

Body composition
At each visit study subjects had their body weight measured to the nearest 0.1 kg on a calibrated balance scale in light indoor clothing without shoes. Height was measured to the nearest 0.1 cm after removal of shoes. Body mass index (BMI) was calculated as the weight (kg) divided by the height squared (m²). Bioelectrical impedance analysis (Bodystat 1500, Bodystat Ltd, Isle of Man, UK) was used to estimate fat and lean mass percentage. The CV was 1.3 and 0.5% for fat mass and lean mass percentage, respectively, as calculated from duplicate measurements in 15 study subjects.

Statistical analysis
Cross-sectional analyses
Continuous variables were described in terms of mean ± S.D. if their distribution was normal according to the Kolmogorov–Smirnov test, and in terms of median, first and third quartiles otherwise. Bivariate partial correlations, adjusting for age and BMI or fat mass percentage, were used to show associations between hormonal and various clinical parameters. SPSS 12.0 software package (SPSS Inc., Chicago, IL, USA) was used for the descriptive analyses and bivariate correlations; a P value <0.05 was considered to indicate statistical significance; all P values were two-tailed.

Longitudinal analyses
Linear mixed-effects models with random intercepts and autoregressive residual correlation structure were used for longitudinal analysis. Using likelihood ratio tests, no evidence of random time slopes was found. Parameters for baseline
predictors were estimated via restricted maximum likelihood estimation and model-based confidence intervals (CI) and P values are reported. No adjustments for baseline testosterone levels were performed when it was anticipated that these levels could be affected by the considered exposure (e.g. genetic polymorphism, smoking, etc.) (28). All the analyses were performed in the software package SAS 9.1.3. Service Pack 4 (SAS Institute, Inc., Cary, NC, USA).

Results

Baseline clinical and hormonal characteristics

Baseline clinical characteristics of the study population are described in Table 1 (n = 218; three subjects excluded from baseline data because of temporary use of medication). Mean age at baseline was 74 years (first and third quartiles: 73–78 years); 40 out of 218 (18.3%) were current smokers, 32 (20.1%) consumed more than two alcoholic beverages per day, 36 (16.5%) were obese (BMI ≥ 30 kg/m²), 80 (36.7%) reported the use of at least three prescription medications, and 71 (32.6%) reported to be physically active for > 2 h per week. After 4 years of follow-up, 20 out of 152 (13.2%) were current smokers, 16 (10.5%) consumed more than two alcoholic beverages per day, 23 (15.1%) were obese, and 78 (51.3%) reported the use of at least three prescription medications. More men than at baseline, i.e. 95 (62.5%), reported to be physically active for > 2 h per week.

Median length of the AR CAG repeat polymorphism was 21 (first to third quartiles: 20–24). Sixty (28.8%) subjects had seven (TTTA) repeats of the CYP19 on both alleles, 97 (46.6%) had only one allele with seven (TTTA) repeats, and in 51 (24.5%) subjects the number of (TTTA) repeats exceeded seven on both alleles. Regarding the ERα polymorphisms, 72 (33.0%) subjects were homozygous for the ‘TA’ haplotype, 105 (48.5%) had only one allele with the ‘TA’ haplotype, and 41 (18.8%) had no ‘TA’ haplotype.

Age at baseline was negatively associated with TT, BioT (ρ = −0.20 to −0.22; P < 0.01), and positively with SHBG, LH, and FSH levels (ρ = 0.13–0.27; P < 0.05). In the age range of our study participants (71–86 years), mean cross-sectional changes of −0.66% (95% CI −1.77; 0.44%), 1.15% (95% CI −2.11; −0.41%), and 1.76% (95% CI −2.75; −0.77%) per year were observed for TT, FT, and BioT respectively. After controlling for age, BMI was negatively associated with TT, FT, BioT, and SHBG (ρ = −0.14 to −0.30; P < 0.05), and positively with FE2 and BioE2 levels (ρ = 0.15; P < 0.05). Both absolute (kg) and relative (%) fat mass displayed a negative association with all three testosterone fractions (ρ = −0.23 to −0.33; P < 0.001). Absolute fat mass was negatively associated with SHBG levels (ρ = −0.24; P < 0.001). After controlling for absolute fat mass, insulin levels were negatively associated with SHBG levels (ρ = −0.15; P = 0.016) and positively with BioE2 levels (ρ = 0.15; P = 0.025). Controlling for total body weight, both IGF-1 and IGF-BP3 were negatively associated with SHBG levels (ρ = −0.21 to −0.29; P < 0.01), and IGF-BP3 displayed a positive association with FT and BioT levels (ρ = 0.14–0.16; P < 0.05). As reported previously, serum leptin levels were negatively correlated with all three testosterone fractions after adjusting for absolute fat mass (29).

No significant differences in sex steroid or SHBG levels between current smokers, past smokers, or subjects who never smoked were found (all P > 0.129). Among current or past smokers, the number of pack year smoked was inversely correlated with both FT (ρ = −0.20; P = 0.011) and BioT (ρ = −0.20; P = 0.009) after controlling for age and BMI. As reported previously, no associations between hormone or SHBG levels and AR CAG repeat length (21) or CYP19 TTTA repeat length (22) were observed in this elderly population. Neither for the ERα Xbal and PvuII SNPs nor for the combined haplotypes were differences in hormone levels detected (all P > 0.32; data not shown).

Evolution of hormonal parameters over time

Table 2 presents descriptive statistics for number of participants, age, and BMI at all visits, as well as for testosterone and SHBG levels. During the 4-year follow-up, 69 subjects left the study. Dropout analysis using logistic regression showed that dropout was associated with lower FT levels and a lower score of functional capacity and mental status at their last visit, lower physical activity scores at baseline and the ‘TA’X haplotype of the ERα (data not shown).

The evolution of TT and BioT levels is displayed in Fig. 1. Mean TT levels declined from 444 ± 148 to 414 ± 157 ng/dl over the 4-year period. The greatest decrease was observed between visits 1 and 2, which
was reflected in SHBG levels with generally higher levels at visit 1 compared with visit 2. The evolution of FT and BioT showed a more gradual decline (mean levels at visit 1 were 7.4±2.1 and 173±50 ng/dl vs 6.9±2.2 and 153±50 ng/dl at visit 5 for FT and BioT respectively). Longitudinal data analysis, adjusting for baseline age and BMI, revealed a yearly mean decrease of −9.5 ng/dl (95% CI −12.7; −6.2 ng/dl), −0.16 ng/dl (95% CI −0.21; −0.10 ng/dl), and −6.1 ng/dl (95% CI −7.3; −4.9 ng/dl) for TT, FT, and BioT respectively. This corresponds to yearly mean percentage decreases of −1.26% (95% CI −2.58; −0.01), −1.33% (95% CI −2.61; −0.01), and −2.43% (95% CI −3.78; −1.08) for TT, FT, and BioT respectively. No significant overall changes in SHBG levels were detected (data not shown).

Percentages of participants with testosterone levels below the reference range for young men (320 ng/dl for TT, 6.5 ng/dl for FT, and 140 ng/dl for BioT (1)) are displayed for all visits in Table 2. At baseline, 19.3, 32.1, and 25.7% of participants presented with levels below this reference range for TT, FT, and BioT respectively. At visit 5, this rose to 30.3, 43.4, and 38.8% respectively.

Table 2 Evolution of age, body mass index (BMI), number of participants, and hormonal levels over time.

<table>
<thead>
<tr>
<th>Visit</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
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<tbody>
<tr>
<td>n</td>
<td>284</td>
<td>284</td>
<td>284</td>
<td>284</td>
<td>284</td>
</tr>
<tr>
<td>Age (years)*</td>
<td>74 (73–78)</td>
<td>75 (73–79)</td>
<td>77 (75–80)</td>
<td>77 (75–78)</td>
<td>78 (76–81)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.3±3.4</td>
<td>26.4±3.5</td>
<td>26.4±3.5</td>
<td>26.4±3.5</td>
<td>26.4±3.5</td>
</tr>
<tr>
<td>SHBG (nmol/l)*</td>
<td>43 (34–56)</td>
<td>42 (33–54)</td>
<td>43 (34–56)</td>
<td>43 (33–57)</td>
<td>44 (34–58)</td>
</tr>
<tr>
<td>Testosterone (ng/dl)</td>
<td>444±143</td>
<td>409±135</td>
<td>410±145</td>
<td>414±141</td>
<td>414±148</td>
</tr>
<tr>
<td>Free T (ng/dl)</td>
<td>7.4±2.0</td>
<td>6.9±1.9</td>
<td>6.7±1.9</td>
<td>7.0±2.1</td>
<td>6.9±2.1</td>
</tr>
<tr>
<td>BioT (ng/dl)</td>
<td>173±50</td>
<td>162±46</td>
<td>158±47</td>
<td>155±48</td>
<td>153±49</td>
</tr>
<tr>
<td>Estradiol (ng/dl)</td>
<td>2.05±0.55</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>2.13±0.58</td>
</tr>
<tr>
<td>Free E₂ (ng/dl)</td>
<td>0.034±0.008</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>0.036±0.009</td>
</tr>
<tr>
<td>BioE₂ (ng/dl)</td>
<td>1.31±0.34</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>1.31±0.36</td>
</tr>
<tr>
<td>LH (IU/l)</td>
<td>6.2 (4.3–8.7)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>8.3 (4.8–9.2)</td>
</tr>
<tr>
<td>FSH (IU/l)</td>
<td>9.0 (6.3–16.2)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>10.3 (6.9–17.8)</td>
</tr>
</tbody>
</table>

Data presented as estimated mean±s.d., unless *non-Gaussian distribution; data presented as median (first to third quartiles). ng/dl may be converted to nmol/l by multiplying by 0.0347 for testosterone and to pmol/l by multiplying by 36.76 for estradiol. NA, not available.

Over the 4-year follow-up period, a modest decrease in BMI was noted (mean Δ = −0.10±1.37 kg/m²). After adjusting for baseline age, subjects presenting with an increase in BMI (>0 kg/m²; n = 70 or 42.2%) presented a greater decline in TT levels compared with those with a decrease in BMI (<0 kg/m²; n = 96 or 57.8%); −58.4 vs −17.9 ng/dl respectively (P = 0.019).

No differences in changes of FT or BioT levels were noted between these groups (P >0.16). This reflected the mean decrease in SHBG levels in subjects presenting with an increase in BMI, compared with increasing SHBG levels in subjects with a decrease in BMI: −1.7 vs +2.8 nmol/l respectively (P = 0.015). Additional adjustment for baseline T or SHBG levels did not alter these findings (data not shown).

Baseline predictors of changes in T and SHBG levels

Lifestyle- and health-related factors, body composition, insulin, and IGF-1 Age at baseline was not associated with the evolution of T or SHBG levels over time (all P >0.50; data not shown), neither were indicators of quality of life, psychological and functional well-being, medical history, or number of medications (either as a continuous variable or as more or less than three prescribed medications), physical activity, or frequency of alcoholic consumptions at baseline (all P >0.20; data not shown). Adjusting for baseline age and BMI, current or former smoking at baseline was associated with a faster yearly decline of FT levels with 0.13 ng/dl (P = 0.037) compared with subjects who never smoked; similar trends for TT (P = 0.12) and BioT (P = 0.09) did not reach statistical significance (Table 3). These observations held true when additionally adjusting for baseline T levels (data not shown). Among smokers, the number of pack year smoked was not associated with changes in testosterone levels (P >0.50).

BMI at baseline was not associated with changes in testosterone or SHBG levels, neither was absolute nor...
Calcium intake was not associated with changes in testosterone or SHBG levels (data not shown). The CYP19 gene was associated with subsequent changes in TT, FT, BioT, or SHBG levels (data not shown). Baseline glucose, insulin, leptin, IGF-1, or IGF-BP3 levels were not associated with changes in testosterone or SHBG levels (all $P > 0.33$; data not shown).

**Sex steroids and gonadotropins** After controlling for age and BMI, baseline $E_2$, LH, and FSH levels were associated with a faster decline in testosterone levels over time. However, for $E_2$ and LH this association was no longer significant after adjusting for the predictive effects of baseline testosterone levels, whereas a significant trend was still observed for higher baseline FSH levels being associated with a faster decline in T levels (of 0.40, 0.007, and 0.15 ng/dl per IU/l increase in FSH levels for TT, FT, and BioT respectively; Table 3). The evolution of BioT levels associated with median, upper, and lower quartiles of baseline FSH levels is illustrated in Fig. 3. No associations with the changes in SHBG levels were found (data not shown).

**Genetic polymorphisms** Neither the CAG repeat length of the AR nor the TTTA repeat length of the CYPI9 gene was associated with subsequent changes in TT, FT, BioT, or SHBG levels (data not shown). The polymorphisms of the ERa were significantly associated with the evolution of testosterone levels over time. Specifically, after controlling for baseline age and BMI, a slower yearly average decline of TT levels was observed with 19.0 ng/dl ($P = 0.009$) for carriers of the (‘TA,TA’) haplogenotype and with 15.0 ng/dl ($P = 0.024$) for carriers of the (‘TA,X’) haplogenotype as compared with those carrying the (‘X,X’) haplogenotype. Similarly, a slower yearly average decline of BioT levels was observed with 7.6 ng/dl ($P = 0.007$) for carriers of the (‘TA,TA’) haplogenotype and 5.2 ng/dl ($P = 0.041$) for

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Table 3 Baseline predictors of subsequent changes in T levels, using linear mixed-effects modeling for longitudinal data analysis.

<table>
<thead>
<tr>
<th>Baseline predictors</th>
<th>TT</th>
<th>FT</th>
<th>BioT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yearly average change</td>
<td>$-9.5$</td>
<td>$-0.16$</td>
<td>$-6.1$</td>
</tr>
<tr>
<td>$E_R$ haplogenotypes $^a$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(‘TA,TA’)</td>
<td>$+19.0$</td>
<td>$+0.26$</td>
<td>$+7.56$</td>
</tr>
<tr>
<td>(‘TA,X’)</td>
<td>$+15.0$</td>
<td>$+0.18$</td>
<td>$+5.22$</td>
</tr>
<tr>
<td>(‘X,X’)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Current and former smokers</td>
<td>$-6.26$</td>
<td>$-0.13$</td>
<td>$-2.57$</td>
</tr>
<tr>
<td>Never smokers</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Calcium intake</td>
<td>$+0.013$</td>
<td>$+0.0003$</td>
<td>$+0.006$</td>
</tr>
<tr>
<td>$E_2$ $^b$</td>
<td>$-7.13$</td>
<td>$-7.23$</td>
<td>$-5.43$</td>
</tr>
<tr>
<td>LH $^a$</td>
<td>$+0.458$</td>
<td>$+0.07$</td>
<td>$+0.67$</td>
</tr>
<tr>
<td>FSH $a$</td>
<td>$-0.076$</td>
<td>$-0.007$</td>
<td>$-0.11$</td>
</tr>
</tbody>
</table>

*$^b$ estimates for the interaction terms (baseline variables $\times$ year), indicating whether the time evolution depends on baseline variables. Values of $\beta^*$ indicate how much the yearly average evolution in testosterone levels changes per unit increase in the baseline predictor value. All models are adjusted for baseline age and BMI.

$^a$Models additionally adjusted for baseline testosterone levels.

$^b$Models with baseline $TE_2$, $FE_2$, and $BioE_2$ for TT, FT, and BioT respectively.

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Figure 3 Mean evolution of BioT levels over time between median, upper, and lower quartiles according to baseline FSH levels.
carriers of the (‘TA,X’) haplogenotype when compared with those carrying the (‘X,X’) haplogenotype. For FT, a significantly slower decline was found for carriers of the (‘TA,TA’) haplogenotype of 0.26 ng/dl per year; P = 0.026) when compared with those carrying the (‘X,X’) haplogenotype (Table 3). This indicates a slower decline in testosterone levels in carriers of a ‘TA’ allele compared with subjects without a ‘TA’ allele. None of the genetic polymorphisms were associated with changes in SHBG levels (data not shown). Adjusting for the predictive effects of baseline testosterone or E2 levels did not alter these observations (data not shown).

Discussion

In this study we present 4-year longitudinal data on testosterone levels in community-dwelling elderly men and discuss predictors of the observed changes. We demonstrated that even in elderly subjects with already decreased testosterone levels, a further decline in testosterone levels can still be observed. Furthermore, in accordance with previous reports (4–7, 18) the decrease is least apparent for TT and most accentuated for calculated BioT levels, due to the relatively stable SHBG levels. Consistent with the results of the Massachusetts Male Aging Study (MMAS), the observed longitudinal changes in testosterone levels were of greater magnitude than the baseline cross-sectional trends (4). In addition, in these elderly men the magnitude of these longitudinal changes was comparable to three previous longitudinal studies covering a broader age range (4, 16, 19), and slightly higher than the results obtained by the Baltimore Longitudinal Study on Aging (BLSA) and by Zmuda et al. (17, 18).

In our cross-sectional analyses, age was negatively associated with FT and BioT and positively with SHBG, LH, and FSH levels. These results are consistent with those reported in other studies (7, 15). However, within the considered older age group, age at baseline was not predictive of subsequent changes in testosterone or SHBG levels, indicating a decrease in testosterone production over time which is independent of age. This is in agreement with the results of the Multiple Risk Factor Intervention Trial (MRFIT study) (17) and with the previously discussed observation of a similar magnitude of decline among different populations and age ranges, but at variance with the results of the BLSA, where age at baseline was negatively associated with TT levels over time (18). Further, health status at baseline was predictive of longitudinal changes in neither testosterone nor SHBG levels. However, the inclusion criteria we applied yielded a population of healthy elderly men, and it cannot therefore be concluded that co-morbidity is not an important determinant of testosterone levels (1).

Consistent with previous findings (3, 7, 12, 15), BMI at baseline was negatively associated with TT, FT, BioT, and SHBG levels. Moreover, those who gained weight over the 4-year period presented a greater decline in TT, explained by a decrease in SHBG levels, compared with those with a decrease in BMI. These results suggest that changes in BMI over time modulate age-related changes in TT and SHBG, but in our study population neither FT nor BioT levels were affected. This is in agreement with the findings of the BLSA and MMAS in elderly men (18, 30) and the results of the CARDIA Male Hormone Study in a younger population (31).

However, in this study no baseline body composition parameters (BMI, fat, or lean mass as assessed by bioelectrical impedance) were predictive of subsequent changes in testosterone or SHBG levels. This is consistent with the results of the MRFIT study (17), but in contrast with the results from the larger BLSA and MMAS cohorts, where obesity at baseline predicted a greater decline over time in TT and SHBG, and even in FT levels in the MMAS, (12, 18). It remains unclear whether body composition influences sex steroid hormone concentrations over time or whether, conversely, sex steroid hormone concentrations alter body composition; most likely this relationship is bidirectional.

Regarding the influence of genetic polymorphisms considered in this study, we observed an association of ERα polymorphisms with changes in testosterone levels, indicating a slower decline in testosterone levels in carriers of a ‘TA’ allele compared with non-carriers. At variance with the longitudinal results from Krithivas et al. (13), no effect of AR CAG repeat length on the evolution in testosterone levels was found. However, it should be noted that their study population was clearly younger with a time interval of 8 years between visits, which could result in a greater absolute decline in testosterone levels.

In this study, baseline E2, LH, and FSH levels were predictive of a subsequent decline in testosterone levels. However, when controlling for baseline testosterone levels, only FSH levels remained significantly associated with a decline in testosterone levels. This observation could be explained by the fact that both in the case of primary hypogonadism and of aging in healthy men, the rise in FSH levels is steeper than that of LH levels (4, 16, 32), and by the greater ultradian variability of LH levels. Moreover, we have already reported on the negative association between testicular volume and FSH levels in this elderly population, with FSH levels being an independent determinant of total testicular volume (33). As a consequence, in elderly patients presenting with relatively high FSH levels despite having low-normal serum testosterone levels, biochemical follow-up in the next years may be considered.

As a whole, these findings point toward a substantial testicular factor in the observed decline in testosterone production, whereas the association with E2 levels and the ERα polymorphisms is suggestive of estrogen-related processes and might be related to changes in the neuroendocrine regulation of testosterone production in elderly men, which are characterized by relative deficiency of LH secretion (1). Indeed, E2 levels did not...
change in our study population, despite the overall decline in testosterone levels, which suggests increased aromatase activity (34), and circulating E2 is known to be a major player in LH negative feedback regulation in men (35). Nevertheless, a comparative study in young and older men of the effects of aromatase inhibition has failed to disclose a greater restraining action of E2 on LH and testosterone levels in the elderly men when compared with young men (36).

Notwithstanding the observation of a clear age-related decline in serum testosterone levels, this does not necessarily mean that the same holds true at a tissue level. Belanger et al. have already suggested that the observed decline in androgen levels could be partially compensated by an increase in steroid-converting enzymes in peripheral tissues, especially the 5α-reductase activity resulting in tissue dihydrotestosterone levels unaffected by aging (14). On the other hand, there is also evidence of decreased androgen tissue concentrations (1) and of reduced androgen sensitivity, with reports of decreased numbers of AR in various tissues in elderly men (37, 38). However, up to now no practical and clinically useful biological marker of androgen activity at tissue level is available. Therefore, one has to further rely on serum testosterone levels as an indirect and less than optimal parameter of androgen activity at tissue level.

This is the first study to report on yearly based, longitudinal changes in testosterone levels in a fairly homogenous population of community-dwelling men over 70 years of age at baseline, with 20% of subjects over 80 years. Notably, this age group is often under-represented in the available literature but is steadily increasing in the general population. The strength of this study is the yearly time interval for longitudinal assessment of serum testosterone levels and it is a population, rather than clinic or complaint-based, study. Moreover, most men were in relatively good state of health, suggesting that the studied longitudinal changes largely reflect effects related to aging per se; adjusting for illnesses and medication produced little, if any, change in the observed overall effects of aging. Mixed-effects modeling analysis for longitudinal data treats every measurement as a separate outcome, but accounts for the interdependence of repeated measurements from the same individual. Finally, all samples from the same subject were analyzed in a single assay run, eliminating the possibility of within-subject variation due to interassay variability.

A limitation of our study is that it is based on single time-point hormonal measurements for each visit. Nevertheless, it has been shown previously that single time-point estimates are a valid approach for population studies (39). Further, bioelectrical impedance analysis has been shown to have a suboptimal accuracy and is limited by various factors for assessing body composition, which could possibly mask associations of baseline body composition with subsequent changes in testosterone levels. Another possible concern is the clearly higher TT and SHBG levels at visit 1 compared with the subsequent visits. This could point toward a selection bias, with dropout between visit 1 and 2 of subjects with higher testosterone levels. However, dropout analysis showed that dropout was associated with lower testosterone levels at the previous visit. While mixed-effects model analyses naturally allow for dropout to be associated with the outcome history (40), and thus with lower testosterone levels at the previous visit, we could not account for the possibility that dropout was associated with higher/lower testosterone levels at the current (or future) visits. Another hypothesis could be a seasonal effect on testosterone levels, since blood sampling at visit 1 was mainly performed from March till June, whereas for the subsequent visits this was done from July till August. However, from the study by Brambilla et al. it appeared that seasonal variation is unlikely to be an important source of variation in serum sex steroid levels in men (41).

Finally, the present study was performed in a well-characterized and specific subset of the population consisting of generally healthy men of high age with a relatively short follow-up period, and the results should therefore not be extrapolated to the general population. In the general population, factors related to co-morbidity are likely to accentuate the observed age-related hormonal changes.

In conclusion, a decline in serum testosterone levels, especially in the non-SHBG-bound and FT fractions, was shown to occur in generally healthy community-dwelling men aged 71–86 years with already decreased testosterone levels compared with healthy young men. Moreover, this decline appeared to be independent of age at baseline, indicating a rather constant decline in testosterone production with aging. Further, subjects who gained weight during follow-up displayed a greater decline in TT and SHBG levels, whereas baseline body composition was not predictive of subsequent changes in testosterone levels. The predictive value of FSH levels appears to confirm the involvement of testicular changes as an important factor for the decline in testosterone production and might warrant future follow-up in case of low-normal testosterone levels. In addition, the predictive effects of E2 and the ERα polymorphisms suggest a contribution of estrogen-related processes in the decline of serum testosterone levels, which might relate to the known involvement of changes in the neuroendocrine regulation of testosterone production in elderly men.

Declaration of interest
The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

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