Is the effect of testosterone on body composition modulated by the androgen receptor gene CAG repeat polymorphism in elderly men?

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Abstract

Objective: The androgen receptor (AR) gene contains a CAG repeat polymorphism coding for a polyglutamine chain, the length of which is inversely correlated with AR transcriptional activity. We explored whether this polymorphism modulates the activities of testosterone (T) related to body composition in elderly men.

Design: We performed cross-sectional analyses using data from a 4-year follow-up study in community-dwelling men aged 75–89 years (n=159).

Methods: Body composition was assessed by dual-energy X-ray absorptiometry and its relation with T and the AR gene CAG repeat length was assessed by multiple linear regression analyses, adjusting for confounding and exploring effect modification.

Results: AR gene CAG repeat length was not directly related to body composition, either with or without adjustment for confounding variables like age, weight, total T or sex hormone binding globulin (SHBG) levels. However, exploration of effect modification showed that CAG repeat length modulated the relation between T and body composition (standardized regression coefficients of interaction term: $\beta = 0.12$, $P < 0.01$ and $\beta = -0.09$, $P < 0.05$ for fat-free mass and fat mass respectively). These results were confirmed using similar models and data of mean T, SHBG and weight of the 2 years preceding body composition assessment instead of data of the same year ($\beta = 0.09$, $P < 0.05$ and $\beta = -0.09$, $P < 0.05$ respectively).

Conclusion: These findings suggest that the AR gene CAG polymorphism contributes, albeit modestly, to the between-subject variation of T action on body composition in community-dwelling elderly men.

Introduction

The androgen receptor (AR) gene contains a polymorphic trinucleotide CAG microsatellite repeat sequence in its first exon. This repeat sequence encodes for a functionally important polyglutamine chain in the NH2-terminal transcriptional activation domain of the AR gene. The normal range of this repeat sequence is between 9 and 37 repeats, with an average length of 22–23 repeats (1–3). Since the AR gene is located on the long arm of the X-chromosome (Xq11-12) (4) only one copy exists in men. Patients with Kennedy’s disease (spinal and bulbar muscular atrophy), with an AR gene CAG repeat length exceeding the normal range, have decreased androgen sensitivity. Besides neurodegenerative features, these subjects display increased prevalence of gynecomastia, reduced virilization, elevated serum luteinizing hormone and estradiol levels and low sperm counts (5–7). Both in vitro experiments (8, 9) as well as epidemiological and clinical studies have provided evidence that variability within the normal range of AR gene CAG repeat length modulates androgen action (10).

It is well established that both endogenous and exogenous testosterone (T) influence body composition. In particular, T has a positive influence on fat free mass (FFM), whereas it is associated with lower fat mass (FM). Replacement doses of T increase FFM, muscle size and strength in hypogonadal men (11). In accordance with these observations, men receiving androgen deprivation therapy for prostate cancer suffer from decreasing muscle mass and increasing amounts of body fat (12).

In men, aging is associated with a progressive decline of T production, which results from both testicular changes and altered neuroendocrine regulation of LH secretion (13). Concomitantly, age-related changes in body composition are observed. Several studies describe an increase in (abdominal) fat mass together with a
decline of muscle and bone mass (14–17). This aging-associated loss of skeletal muscle mass results in functional impairment and physical disability in the elderly (18), whilst abdominal fat accumulation contributes to a higher risk of the metabolic syndrome, type 2 diabetes (19) and coronary heart disease (20–22). Despite an increasing interest in the role of sex steroids on body composition, it remains unclear to what extent the relative androgen deficiency in elderly men intervenes in the age-related changes of their body composition. Moreover, it is likely that genetic factors such as the AR gene CAG repeat polymorphism might modulate the T effects on body composition.

Earlier reports on the role of the AR gene CAG repeat length in determining body composition in men were not univocal. Zitzmann et al. (23) reported a positive, independent correlation between the CAG repeat length and body fat content in healthy 20–50 year old men, while Walsh et al. (24) found a positive association between CAG repeat length and fat free mass in men aged 55–93 years. Another study found a negative association between CAG repeat length (≤ 23) and waist-to-hip ratio (3).

We assessed whether AR gene CAG repeat length plays a substantial role with respect to the relation between serum T and body composition in a population of community-dwelling men over 70 years of age. If this genetic factor intervenes, its modulatory action may have to be taken into account to explain more clearly the role of androgens in the determination of body composition in elderly men.

Subjects and methods

Study subjects

The present cross-sectional study includes part of a study population of ambulatory community-dwelling elderly men, living in the semi-rural community of Merelbeke (Belgium). The latter cross-sectional and follow-up population study was designed to investigate the process of aging, focusing on effects of hormonal changes on bone metabolism and body composition. The study was approved by the ethics review board of the Ghent University Hospital. All participants gave written informed consent for participation in this study. Baseline characteristics, exclusion and inclusion criteria have been described extensively in previous publications (25–27).

In 1996, 352 men enrolled in the study and in 2000, 209 were re-evaluated. Reasons for loss to follow-up were institutionalization, withdrawal of consent, death and occurrence of diseases or treatments considered to affect androgen levels. Among these 209 subjects, 50 were additionally excluded because of disorders or treatments affecting hormonal or body composition status (n = 32) or because of missing data (n = 18). The present analysis is based on data obtained in the year 2000 (body composition) and the years 1998–2000 (hormonal levels and body weight).

Hormonal assays

Each following year, serum was obtained between 0800 and 1000 h after overnight fasting and was stored at −80 °C until batch analysis. To avoid effects of seasonal variation, blood collection was completed over a period of two months during summer. Commercial radioimmunoassays with I125-labeled tracers were used to determine serum levels of total T (Medgenix, Fleurus, Belgium) and sex hormone binding globulin (SHBG; Orion Diagnostica, Espoo, Finland). All samples from the same subject were assayed in a single assay run using duplicate measurements. The intra- and inter-assay coefficients of variation were 4.8 and 5.1% for T, and 3.6 and 6.6% for SHBG respectively. Serum bioavailable (BioT) and free testosterone (FT) were calculated from serum total T, SHBG and albumin concentrations using a previously validated equation derived from the mass action law (28).

Determination of AR gene CAG repeat length

Genomic DNA was extracted from EDTA-treated blood using a commercial kit (Qiagen Midi Kit, Qiagen Inc). We used PCR to amplify exon 1 of the AR gene with primers 5′AGCCTGTGAACCTCTCGACG3′ (sense) and 5′CTGCCCTACAAACTTCCTTGGC3′ (antisense). After ethanol precipitation, the amplified fragment was directly sequenced on a ABI Prism 310 sequencer (ABI Prism, Perkin-Elmer Applied Biosystems, Foster City, CA, USA), using BigDye Terminator Cycle Sequencing Reaction Kit (ABI Prism, Perkin-Elmer Applied Biosystems). Fragment length size was determined running GeneScan-400HD Analysis Software (ABI Prism, Perkin-Elmer Applied Biosystems) (29).

Body composition

Every year, 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 in meters squared (m²). Waist-to-hip ratio (WHR) was calculated as the ratio of waist circumference over hip circumference. The waist circumference was defined as the abdominal circumference located midway between the lower rib margin and the iliac crest. The hip circumference was defined as the widest circumference around the buttocks.

Whole body soft tissue composition assessment using dual-energy X-ray absorptiometry (DEXA) with
a Hologic QDR 4500A device (Hologic Inc., Bedford, MA, USA) was performed in the year 2000. Standardization of the densitometer was performed weekly using a body composition calibration phantom. The coefficient of variation (CV) for phantom measurements was below 1%. The use of DEXA in estimating body composition in elderly men has been validated in several studies (30, 31).

Statistical analysis
All variables were checked for normality of distribution by the Kolmogorov–Smirnov one-sample test for goodness-of-fit. When necessary, analysis was done on logarithmically transformed data. Unless otherwise stated, all variables are expressed as mean ± S.D.

Univariate regression coefficients were used to show associations between hormonal and anthropometrical parameters. To explore the effect of the AR gene CAG repeat length on body composition, multiple linear regression models were constructed with body composition parameters as dependent variables and weight, T and SHBG levels, and CAG repeat length as independent variables. Subsequently, modulation of CAG repeat length on the relationship between T and body composition was explored by implementing interaction terms into the models. An interaction term was constructed by multiplying the two main components T levels and CAG repeat length (using centered data). The association between T and FFM or RSMi was no longer positive in this elderly study population (n = 159). Median age in 2000 was 78 (76–81) years. Mean BMI and WHR values suggested moderate overweight in our study population, although the whole range was represented. Prevalence of sarcopenia among these elderly men was estimated at 25.7% using the relative skeletal muscle index (RSMi) with a cut-off value of 2.2 ng/dl respect-ively). In case of non-Gaussian distribution, data are presented as mean (interquartile range).

Results
Clinical characteristics and hormonal levels
Table 1 summarizes the baseline characteristics of the study population (n = 159). Mean BMI and WHR values suggested moderate overweight in our study population, although the whole range was represented. Prevalence of sarcopenia among these elderly men was estimated at 25.7% using the relative skeletal muscle index (RSMi) with a cut-off value of 7.26, as described by Baumgartner et al. (17). Furthermore, a strong positive correlation was observed between FM and FFM (β = 0.57, P < 0.001, Table 2).

Mean total T level was in the low normal range for young men. Approximately one-third of the subjects had T levels below the cut-off value for the young adult reference range (<320 ng/dl). Mean bioavailable T (BioT) or free T (FT) levels were relatively lower than total T levels (156 ± 49 and 7.0 ± 2.2 ng/dl respectively) and close to the lower limit of the normal range for young men. All hormonal parameters displayed a normal Gaussian distribution, except for SHBG. The mean T level from the 2 years (1998, 1999) preceding DEXA assessment of body composition was 410 ± 140 ng/dl.

Relations between CAG repeat length, hormone concentrations, age and body composition
The range of AR gene CAG repeat length was 15–31 with a median length of 21 repeats and showed a bimodal distribution. Univariate regression analysis revealed no significant associations between AR gene CAG repeat length as a continuous variable and hormonal or body composition parameters (Table 2). A clear negative association was observed between total T levels and indices of adiposity (β = −0.35, P < 0.001 and β = −0.40, P < 0.001 for BMI and FM respectively), either unadjusted or after adjustment for age. Remarkably, the association between total T levels and FFM or RSMi was negative in this elderly study population (β = −0.23, P < 0.01 and β = −0.18, P < 0.05 respectively). This finding, which may seem unexpected, is probably explained by confounding factors since this association changed substantially when SHBG levels or total body weight were considered. In the latter case, the association between T and FFM or RSMi was no longer statistically significant (data not shown), while adjusting for both SHBG levels and total body weight led to a positive association between total T levels and FFM (β = 0.15, P < 0.01) or RSMi (β = 0.21, P < 0.01). As these results indicate a confounding role of SHBG and total body weight, these variables are taken into account in the next section.
account in further analyses explaining the relationship between T, CAG repeat length and body composition parameters.

BioT and FT levels were negatively associated with all indices of adiposity and were not associated with FFM or RSMi. Adjustment for total body weight led to a positive association with FFM or RSMi while the negative associations with indices of adiposity remained significant (data not shown). SHBG levels were negatively associated with all indices of body composition and positively with total T levels. In this narrow age range, T levels were not associated with aging. Both total body weight and FFM were negatively associated with age, while FM or other body composition parameters were not associated with age.

Models exploring the modulation of T activity on body composition by CAG repeat length

To explore modulation of the relationship between T levels and body composition by AR gene CAG repeat length, multiple linear regression models were constructed with FFM and FM as dependent variables, and CAG repeat length, weight, T and SHBG levels as independent variables. For both FFM and FM, cross-sectional data from the year 2000 were used to construct these models. As a validation, these analyses were repeated with FFM and FM from the year 2000 as dependent variables, using mean hormone and weight values from the 2 years preceding body composition assessment (i.e. 1998 and 1999) as independent variables.

Table 3 shows the results of the different models for FFM using data from 2000. Model I, with CAG repeat length, T and SHBG levels as independent variables, showed no significant association between CAG repeat length and FFM. However, this model revealed a confounding effect of SHBG levels, since the negative association between T and FFM (Table 2) was no longer statistically significant. Model II, including an interaction term to investigate possible modulation of T activity by CAG repeat length revealed a significant interaction between these two variables ($\beta=0.21$, $P<0.01$). In model III, adjusting for total body weight showed no significant association between CAG repeat length and FFM, but displayed a positive trend between T levels and FFM ($\beta=0.07$, $P<0.1$). Model IV, including the interaction term while adjusting for total body weight again revealed the modulating effect on the relationship between T and FFM by CAG repeat length ($\beta=0.12$, $P<0.01$). Adjusting for both SHBG levels and total body weight (model V) revealed a positive association between T levels and FFM ($\beta=0.15$, $P<0.01$), while it did not change the observed modulating effect on the relationship between T and FFM by CAG repeat length (model VI; $\beta=0.12$.

<table>
<thead>
<tr>
<th>Age</th>
<th>Weight</th>
<th>BMI</th>
<th>FM</th>
<th>FFM</th>
<th>RSMi</th>
<th>T</th>
<th>SHBG</th>
<th>CAG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>–</td>
<td>–0.16†</td>
<td>–0.07</td>
<td>–0.12</td>
<td>–0.19†</td>
<td>–0.14</td>
<td>0.01</td>
<td>0.11</td>
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<tr>
<td>Weight</td>
<td>–</td>
<td>0.90*</td>
<td>0.88*</td>
<td>0.88*</td>
<td>0.67*</td>
<td>–0.34*</td>
<td>–0.33*</td>
<td>0.09</td>
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<tr>
<td>BMI</td>
<td>–</td>
<td>–</td>
<td>0.70*</td>
<td>0.73*</td>
<td>–0.35*</td>
<td>–0.36*</td>
<td>0.13</td>
<td></td>
</tr>
<tr>
<td>FM</td>
<td>–</td>
<td>0.57*</td>
<td>0.42*</td>
<td>–0.40*</td>
<td>–0.25†</td>
<td>0.10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FFM</td>
<td>–</td>
<td>–</td>
<td>0.79*</td>
<td>–0.23†</td>
<td>–0.35*</td>
<td>0.04</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RSMi</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–0.18†</td>
<td>–0.39*</td>
<td>0.04</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T</td>
<td>–</td>
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<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Entries are standardized regression coefficients. *$P<0.001$; †$P<0.01$; ‡$P<0.05$. ND, not done.

Table 3 Results of multiple linear regression models exploring the effect on fat free mass of AR gene CAG repeat length, serum testosterone levels and considered confounders in community-dwelling elderly men ($n=159$) using cross-sectional data from 2000.

<table>
<thead>
<tr>
<th>$aR^2$</th>
<th>Model I</th>
<th>Model II</th>
<th>Model III</th>
<th>Model IV</th>
<th>Model V</th>
<th>Model VI</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\delta^2$</td>
<td>10.6%</td>
<td>14.6%</td>
<td>77.1%</td>
<td>78.4%</td>
<td>78.2%</td>
<td>79.4%</td>
</tr>
<tr>
<td>CAG</td>
<td>0.03</td>
<td>0.02</td>
<td>–0.04</td>
<td>–0.04</td>
<td>–0.03</td>
<td>–0.03</td>
</tr>
<tr>
<td>Testosterone</td>
<td>–0.04</td>
<td>–0.05</td>
<td>0.07</td>
<td>0.07</td>
<td>0.15†</td>
<td>0.14†</td>
</tr>
<tr>
<td>SHBG</td>
<td>–0.32*</td>
<td>–0.30†</td>
<td>–</td>
<td>–</td>
<td>–0.14†</td>
<td>–0.13†</td>
</tr>
<tr>
<td>Weight</td>
<td>–</td>
<td>–</td>
<td>0.91*</td>
<td>0.89*</td>
<td>0.88*</td>
<td>0.87*</td>
</tr>
<tr>
<td>Interaction (T×CAG)</td>
<td>–</td>
<td>–</td>
<td>0.21†</td>
<td>–</td>
<td>0.12†</td>
<td>–</td>
</tr>
</tbody>
</table>

Entries are standardized regression coefficients. *$P<0.001$; †$P<0.01$; ‡$P<0.05$. $aR^2$ = adjusted $R$ square. Explanatory variables are displayed in the first column. In model I controlling for serum T and SHBG levels. Model II showed that investigating modulation revealed a significant interaction between T and CAG repeat length. Model III adjusting for total body weight. In model IV including the interaction term while adjusting for total body weight again revealed the modulating effect by CAG repeat length. Model V adjusting for both SHBG levels and total body weight. Finally, adjusting for both SHBG and total body weight did not change the observed modulating effect by CAG repeat length on the relationship between T and FFM (model VI).
The total variance explained by this last model was 79.4% and the explained variance of the interaction term ranged between 1.2 and 4.0% throughout the different models. With respect to the direction of this interaction, the obtained regression equation suggests that a decline in T level leads to a greater decrease of FFM in subjects with a longer AR gene CAG repeat length when compared with those with a shorter repeat length. Regarding modulation of T activity on RSMi by CAG repeat length, similar results as for FFM were found (data not shown).

When replacing T and SHBG by BioT or FT levels in these models, we found similar results confirming the modulating effect by AR gene CAG repeat length on T activity regarding FFM ($\beta$ for the interaction term = 0.12 with $P<0.01$ with either BioT or FT, data not shown). We repeated these analyses also for FFM assessed by DEXA in 2000 with the use of mean values from the 2 years preceding body composition assessment as independent variables (mean T, mean SHBG and mean weight from 1998 and 1999; data not shown) to explore possible foregoing influence of T on FFM. This analysis again showed a significant interaction between T and CAG repeat length ($\beta=0.11$, $P<0.01$). The explained variation of the interaction term was 1.1% in the full model.

Regarding FM, similar results were observed as for the models of FFM (data not shown). However, the interaction term between T levels and CAG repeat length became statistically significant only after adjusting for the confounding effect of total body weight ($\beta$ for the interaction term = −0.09, $P<0.05$ and $\beta=−0.09$, $P<0.05$ for data from 2000 and 1998–1999 respectively). The explained variation of the interaction term in the full model was 0.7 and 0.8% respectively. With respect to the direction of the interaction, these results suggest that a decline in T levels leads to a greater rise in FM in subjects with a longer CAG repeat length when compared with those with a shorter length.

Confirmatory analyses of these results using BioT or FT levels in these models yielded similar results and established the observed modulating effect ($\beta$ for the interaction term = −0.09 with $P<0.01$ with either BT or FT, data not shown).

Correcting for age, height, physical activity and/or smoking did not have any influence on these results for either FFM or FM and were therefore omitted from the models (data not shown).

Discussion

Testosterone is known to influence body composition in men with a positive effect on lean body mass while reducing fat mass (32). Although the results of the present study did not reveal a direct contribution of the CAG repeat length of the AR gene on the determination of body composition in community-dwelling elderly men, they do indicate a modulating effect by this polymorphism on the relationship between T and body composition. The unfavorable effects of lower T levels on body composition tend to be more accentuated in elderly men with a longer AR gene CAG repeat sequence when compared with subjects with a short CAG repeat sequence. From a physiological point of view, the latter modulating effect is in line with expectations since AR transcriptional activity and consequently androgen action on target tissues will be influenced concomitantly on the one hand by the AR gene CAG repeat length and on the other hand by circulating and local tissue androgen levels. However, the amplitude of this modulating effect appears limited, which is not surprising since the AR gene CAG repeat polymorphism represents only a small part of the variability of androgen action (33) and determination of body composition is evidently multifactorial, involving besides constitutional factors other hormonal influences such as the somatotropic axis and glucocorticoids, as well as lifestyle-related variables such as diet and exercise.

Our observations are in line with the findings of Zitzmann et al. (23) in younger men, who reported a positive correlation between CAG repeat length and body fat content in 106 healthy men aged 20–50 years. The fact that we only found a significant association between the interaction term and fat mass after controlling for total body weight might be explained by the high prevalence of frailty in our elderly study population. Indeed, 25% of our study subjects were in a state of sarcopenia, as defined by the relative skeletal muscle index described by Baumgartner et al. (17). Another recent report on the relation between body composition and AR gene CAG repeat length described higher fat free mass with greater CAG repeat length in two independent cohorts (24), which seems to be at variance with our results. In contrast with the results reported by Gustafson et al. (3), who described higher WHR in healthy male subjects (age 51–93 years) with both a short CAG repeat length ($\leq23)$ and a rare variant of the GGN polymorphism of the AR, we did not find a relation between CAG repeat length and WHR. However, in the present study the GGN polymorphism of the AR gene was not studied.

The possible underlying mechanisms of the modulated transcriptional activity of the AR being inversely correlated with the number of CAG repeats are yet to be completely unraveled. Possible explanations are not only variations in the basal activity of the AR (9, 34), but also that functional interactions of the polyglutamine stretch with coactivators such as ARA24 and p160, which mediate coactivator-induced transcriptional activity, could be accountable for the decreased transcriptional activity with longer CAG repeat length (34, 35). Furthermore, decreased expression of AR mRNA is described in cells with increasing CAG repeat length (36).
One issue that is rarely commented on is the fact that the influence of CAG repeat length might be different between tissues. Cell specificity of the CAG repeat length effect has already been demonstrated in different cell lines of prostatic and non-prostatic origin and is probably mostly due to the involvement of accessory factors expressed differentially between these cell lines (9). However presently we are not aware of any in vitro data evaluating this issue using muscle or adipose cell lines. Interestingly, in a recent study in mice with replacement of the mouse AR gene by human AR alleles, varying glutamine tract length resulted in subtle phenotypic variation, which included, besides obvious variation in seminal vesicle weight, differences in body fat amount, albeit not unidirectional (33). As the differences between the various strains of CAG repeat length were more pronounced in tissues with higher AR expression and thus higher androgen sensitivity, AR CAG repeat tissue specificity might reflect overall AR tissue expression and differentially expressed accessory factors.

A possible limitation of our study is that it is based on a single time-point hormonal measurement. Nevertheless, it has been shown previously that single time-point estimates are a valid approach for population studies (37). Moreover, analysis of the multiple linear regression models using mean values of T and SHBG from the 2 years preceding the body composition assessment confirms the conclusions. Another limitation is the fact that body composition was assessed by DEXA only at one time point, where longitudinal data might have provided additional insights. Albeit that extreme weight changes could possibly affect sex hormone status, it should be noted that extreme gains or losses of body weight were not exclusion criteria in this study. However, mean weight changes between 1998 and 2000 were only modest within this study population. Only 1.4 and 2.1% of the study subjects had more than 10% weight gain or loss in this 2-year interval respectively. One subject had a weight loss of 20%; analysis of the regression models with and without this individual yielded the same results. Finally, the present study was performed in a well characterized and specific subset of the population consisting of generally healthy men of high age and the results should therefore not be extrapolated to the general population.

In conclusion, our results indicate that the effects of testosterone on both fat free and fat mass are modulated by the length of the CAG repeat polymorphism of the AR gene in a population of healthy elderly, community-dwelling men. Whether this effect of only modest amplitude has practical diagnostic or therapeutic implications in the context of the age-related decline of androgens and late onset male hypogonadism remains to be established.

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