

CLINICAL STUDY

The impact of the CAG repeat polymorphism of the androgen receptor gene on muscle and adipose tissues in 20–29-year-old Danish men: Odense Androgen Study

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Abstract

Background: The number of CAG repeats (CAG_n) within the CAG repeat polymorphism of the androgen receptor gene correlates inversely with the transactivation of the receptor.

Objective: To examine the impact of CAG_n on muscle, fat distribution, and circulating androgen levels. **Design, settings and participants:** Population-based, cross-sectional study of 783 Danish men aged 20–29 years.

Methods: Genotyping was performed in 767 men. Areas of thigh and lower trunk muscle ($muscle_{thigh}$ and $muscle_{lower\ trunk}$), subcutaneous adipose tissues (SAT_{thigh} and $SAT_{lower\ trunk}$), and deep adipose tissues (i.m. and visceral) were measured in 393 men by magnetic resonance imaging (MRI). Lean body mass (LBM) and fat mass (FM) were measured in all men by whole body dual-energy X-ray absorptiometry (DEXA). The absolute areas acquired by MRI were the main outcomes. The absolute DEXA measurements and relative assessments of both modalities were considered as the secondary outcomes.

Results: CAG_n (range: 10–32) correlated inversely with absolute $muscle_{thigh}$ ($r = -0.108$), absolute $muscle_{lower\ trunk}$ ($r = -0.132$), relative $muscle_{thigh}$ ($r = -0.128$), relative $muscle_{lower\ trunk}$ ($r = -0.126$), relative $LBM_{lower\ extremity}$ ($r = -0.108$), and relative LBM_{total} ($r = -0.082$), and positively with relative SAT_{thigh} ($r = 0.137$), relative $SAT_{lower\ trunk}$ ($r = 0.188$), relative $FM_{lower\ extremity}$ ($r = 0.107$), and relative FM_{total} ($r = 0.082$). These relationships remained significant, controlling for physical activity, smoking, chronic disease, and age. CAG_n did not correlate with any circulating androgen.

Conclusions: The CAG repeat polymorphism affects body composition in young men: absolute $muscle_{thigh}$ and absolute $muscle_{lower\ trunk}$ increase as CAG_n decreases. Expressed relatively, muscle areas and LBM increase, while SAT and FM decrease as CAG_n decreases. The polymorphism does not affect deep adipose tissues or circulating androgen levels in young men.

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Introduction

Androgens mediate their effects on target organs via the androgen receptor, which is expressed in many tissues including muscular and adipose tissues (1). The transcriptional activity of the hormone–receptor complex is inversely correlated with the number of CAG repeats (CAG_n) in a sequence of the androgen receptor gene (the CAG repeat polymorphism) (2, 3). Fat mass (FM), insulin, and leptin increase with increasing CAG_n (4). Currently, it is unknown whether the association with FM is ubiquitously present or restricted to subcutaneous adipose tissue (SAT) or visceral adipose tissue (VAT). Moreover, positive relations between CAG_n and fat-free mass were reported in two

cohorts of 294 and 112 males of a broad range of ages (5). Androgen levels are not related to CAG_n in younger men, but decline more rapidly with age in men with lower repeat numbers (5, 6). This has been explained by diminished central androgen receptor activity in older men with longer alleles resulting in a decreased negative feedback on the hypothalamic–pituitary–gonadal (HPG) axis, leading to relatively higher androgen levels (5, 6).

We included a population-based cohort of 783 young men aged 20–29 years to address the primary hypothesis that in young men, CAG_n is inversely correlated with muscle area/mass, and is positively correlated with SAT and VAT. Finally, we examined the circulating levels of androgens in relation to CAG_n .

Methods

Subjects

Odense Androgen Study is a population-based cohort study of 783 Danish men aged 20–29 years. Informed consent was obtained from all subjects. The study population is described in detail elsewhere (7, 8). In brief, 3000 men were randomly drawn from the Danish Central Personal Registry, and they received a questionnaire. Respondents ($n=2042$) were invited and 784 men were included, of which one dropped out. The 783 men matched the county population as regards body mass index, chronic disease, medication, physical activity, tobacco exposure, sociodemography, and socio-economic status (7). Three anabolic steroid users were excluded from the analysis. Genotyping of the CAG repeat polymorphism was performed in 767 men, magnetic resonance imaging (MRI) in 406 men (genotyped: $n=393$), and dual-energy X-ray absorptiometry (DEXA) in all men. The examinations took place from March 2002 to May 2003. The local ethical review board gave approval for the study (#20010198), which was conducted according to the Declaration of Helsinki.

Physical examination and medical history

A physical examination was performed retrieving the following data by questionnaire, by interview, and from electronic hospital records: chronic disease (yes/no); medication (yes/no); smoking (cigarettes/day); anabolic steroid abuse (yes/no); and physical activity in terms of jogging (km/week), cycling (km/week), strength training (yes/no), and other sports (h/week).

Biochemistry

Subjects arrived fasting at 0730–0930 h for venous blood sampling. Total testosterone (TT), androstenedione, dihydrotestosterone (DHT), and estradiol (E_2) were measured by an in-house assay (9, 10) using extraction, celite chromatography, and a final RIA (intra-assay coefficient of variation (CV) values: 8.2, 9.4, 9.1, and 7.4% and inter-assay CV values: 13.8, 11.4, 11.0, and 10.5% respectively). The accuracy of the TT assay was monitored in an external quality assessment program (German Society of Clinical Chemistry): the mean bias in 20 consecutive control samples was +5.4% (95% confidence interval (CI): 1.2–9.7%). Bioavailable testosterone was calculated from a validated formula (11) using TT, SHBG (Immulite 2000, Diagnostic Products Corporation, Los Angeles, CA, USA; intra-assay and inter-assay CV values: 3.0 and 5.0% respectively), and albumin (Roche/Hitachi 917, Roche Diagnostics; intra-assay and inter-assay CV values: 0.7 and 2.0% respectively).

Genotyping

DNA was isolated from whole blood using QIAamp DNA Blood Midi Kits (Qiagen). CAG_n was determined in 767 men. The genotyping was performed with the primers GAGsense: TCCGAATCTGTTCCGAGCGTGC and CAGanti: GCTGTGAAGGTTGCTGTTCCCTCAT. For detection, the sense primer was labeled with 6-FAM. DNA amplification was performed in a total volume of 6 μ l containing 1 \times PCR buffer, 1.5 mM MgCl₂, 200 μ M of each dNTP (Roche), 167 nM of each primer (DNA Technology, Aarhus, Denmark), 0.1 U Taq DNA polymerase (Sigma), and \sim 10 ng template DNA. The annealing temperature of the PCR was 66 °C. PCR products were resolved on the MegaBACE 1000 according to the manufacturer's instructions, and were analyzed using the Fragment Profiler software (Amersham Biosciences). Approximately 10% of the samples were sequenced with the primer CAGseq: GAATCTGTTCCAGAGCGTGCG as a quality control.

Body composition

MRI was performed in the first 406 consecutively included subjects with an open, low field (0.2 Tesla) MR unit (Magnetom Open Viva, Siemens, Erlangen, Germany). Muscle_{thigh}, SAT_{thigh}, and intramuscular adipose tissue (IMAT)_{thigh} were determined in one femoral slice (equidistant from the trochanter major and patella) using a T1-weighted gradient-echo sequence (repetition time: 370 ms, echo time: 15 ms, acquisition matrix: 512 \times 512, field of view: 230 mm). The intraobserver CV values of muscle_{thigh} and SAT_{thigh} were 1.1 and 3.0% respectively. Three slices of the lower trunk (10 mm thick, 20 mm apart, lower slice at the dorsal, intervertebral space of L4/L5) were recorded using an axial, T1-weighted gradient-echo sequence (repetition time: 450 ms, echo time: 15 ms, acquisition matrix: 512 \times 288, field of view: 400 mm). A bias correction algorithm was developed to ensure uniform pixel intensities of adipose tissue throughout all images (12). The area of total lower trunk adipose tissue was assessed from bimodal histograms discriminating between adipose and non-adipose tissues (Adobe Photoshop 7.0; Adobe Systems, Inc.). The intra-abdominal compartment was demarcated, and VAT was quantified. SAT_{lower trunk} was computed by subtracting peri-vertebral and bone marrow adipose tissues and VAT from the area of total abdominal adipose tissue. Muscle_{lower trunk} was computed by subtracting the area of the peritoneal cavity, retro-peritoneal non-muscular tissues, SAT_{lower trunk}, peri-vertebral adipose tissues, and calcified tissue from the total lower trunk area. The reported areas of muscle_{lower trunk}, SAT_{lower trunk}, and VAT represent the mean of the three slices. The intraobserver CV values of muscle_{lower trunk}, SAT_{lower trunk}, and VAT were 4.7, 1.7, and 7.2% respectively. Lean body mass (LBM_{total}),

LBM_{upper extremity}, LBM_{lower extremity}, and LBM_{central} were measured by DEXA using a Hologic 4500A densitometer (Waltham, MA, USA). Body weight (Seca, Roskilde, Denmark) and height measured by a stadiometer were recorded.

Data analysis

The MRI and DEXA measurements were expressed as absolute and relative outcomes. The relative outcomes were generated in order to evaluate the amount of a specific tissue in proportion to the total area (MRI) or in proportion to the total mass (DEXA) of the region of interest. Regarding the expression of the relative outcomes, the method of adjustment using linear regression is more correct than the use of ratios or percentages (13, 14), because the relationships between specific tissue and total area/mass – despite being linear – had non-zero intercepts in all cases; thus, linear regression was used to compute relative muscle_{thigh} as absolute muscle_{thigh} adjusted for the total thigh area. Similarly, relative muscle_{lower trunk} was expressed as absolute muscle_{lower trunk} adjusted for the total lower trunk area. Both SAT_{thigh} and IMAT_{thigh} were adjusted for total thigh area, SAT_{lower trunk} and VAT for total lower trunk area, LBM_{total} and FM_{total} for total body mass, LBM_{lower extremity} and FM_{lower extremity} for total lower extremity mass, LBM_{upper extremity} and FM_{upper extremity} for total upper extremity mass, and both LBM_{central} and FM_{central} were adjusted for total central mass.

To overcome the issue of multiple testing, the six absolute MRI measurements (muscle_{thigh}, muscle_{lower trunk}, SAT_{thigh}, IMAT_{thigh}, SAT_{lower trunk}, and VAT) were considered as the main outcome measures of the study, whereas the eight absolute DEXA measurements (LBM_{total}, LBM_{lower extremity}, LBM_{upper extremity}, LBM_{central}, FM_{total}, FM_{lower extremity}, FM_{upper extremity}, and FM_{central}) as well as the relative outcomes of all 14 MRI and DEXA measurements were given less emphasis as suggested by Altman (15).

The correlation strength between CAG_n and the outcomes were examined using Pearson's correlation analysis. Analyses were performed i) with all men included and ii) excluding men with low TT, defined as TT < 12.5 nmol/l in the present population (7, 8). Any significant effect of genotype on an outcome was further analyzed using linear regression analysis to express the effect (slope) as the change in muscle area or LBM per repeat. Finally, multiple regression analyses were performed to examine the effect after adjustment for physical activity, smoking, chronic disease, and age. TT, DHT, and E₂ were also adjusted for in those analyses that examined muscle area or LBM. All regression analyses were performed using robust s.e.m.'s, and CAG_n was treated as a continuous variable.

CAG_n was nearly normally distributed, but it did not become perfectly Gaussian upon logarithmic, root, power, or reciprocal transformation. Muscle_{thigh}

and LBM_{lower extremity} were normally distributed. Natural logarithm transformations provided Gaussian distributions of the remaining outcomes and all circulating androgens. Means reported are geometric, unless otherwise stated. The level of significance was set at $P < 0.05$. Data were analyzed using Stata Statistical/Data Analysis software version 8.2 (StataCorp, College Station, TX, USA).

Results

CAG_n was distributed around a median of 21 repeats with a minimum and a maximum of 10 and 32 repeats respectively (Fig. 1). The subject characteristics are given in Table 1.

Main outcomes – absolute MRI outcomes

CAG_n was inversely correlated with absolute muscle_{thigh} and muscle_{lower trunk} (Table 1 and Fig. 2A and B). A reduction of ten CAG repeats equaled an increase in muscle_{thigh} and muscle_{lower trunk} of 8.3 and 11.2 cm² respectively. Among the remaining four absolute MRI outcomes, neither (SAT_{thigh} and SAT_{lower trunk}) nor deep adipose tissues (IMAT_{thigh} and VAT) correlated significantly with CAG_n (Table 1).

Secondary outcomes

Regarding the relative outcomes, inverse correlations with CAG_n were found for relative muscle_{thigh}, relative muscle_{lower trunk}, relative LBM_{total}, and relative LBM_{lower extremity} (Fig. 3A–D). A reduction of ten CAG repeats was equivalent with increments in relative muscle_{thigh}, relative muscle_{lower trunk}, relative LBM_{total},

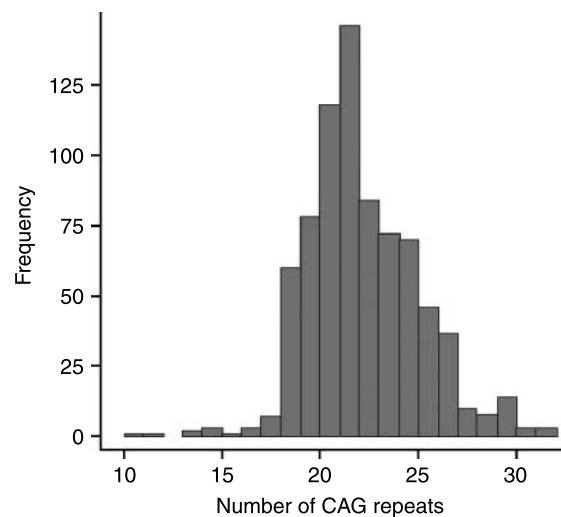


Figure 1 The frequency distribution of the number of CAG repeats in 767 men aged 20–29 years, in whom the CAG repeat polymorphism of the androgen receptor gene was genotyped in the Odense Androgen Study.

Table 1 Subject characteristics of the 767 men aged 20–29 years, in whom the CAG repeat polymorphism of the androgen receptor gene was genotyped.

	<i>n</i>	Geometric mean	2.5 and 97.5 percentiles	Correlation ^a with CAG repeats
Body composition				
Thigh muscle area (cm ²)	393	164	124–207	−0.108*
Lower trunk muscle area (cm ²)	393	189	149–238	−0.132 [†]
Thigh subcutaneous adipose tissue (cm ²)	393	50.6	19.1–118	0.046
Thigh i.m. adipose tissue (cm ²)	393	4.3	1.5–12.8	−0.019
Lower trunk subcutaneous adipose tissue (cm ²)	393	125	37.9–364	0.004
Visceral adipose tissue (cm ²)	393	65.9	30.5–134	−0.057
Total LBM (kg)	767	63.5	50.9–79.4	−0.047
Lower extremity LBM (kg)	767	21.8	16.7–27.2	−0.062
Upper extremity LBM (kg)	767	8.0	6.1–10.6	−0.044
Central LBM (kg)	767	30.3	24.6–37.7	−0.030
Total FM (kg)	767	13.8	6.3–32.1	0.042
Lower extremity FM (kg)	767	5.2	2.3–10.9	0.061
Upper extremity FM (kg)	767	1.59	0.74–3.7	0.032
Central FM (kg)	767	6.0	2.4–16.1	0.036
Body weight (kg)	767	80.9	60.9–110.5	−0.013
Body height (cm)	767	181.6	167.9–194.8	−0.068
Body mass index (kg/m ²)	767	24.6	19.3–33.3	0.021
Sex steroid hormones				
Total testosterone (nmol/l)	767	20.4	11.0–36.2	0.010
Bioavailable testosterone (nmol/l)	767	12.0	7.0–20.1	0.006
Free testosterone (nmol/l)	767	0.46	0.27–0.75	0.016
Dihydrotestosterone (nmol/l)	767	1.58	0.73–3.20	0.014
Androstenedione (nmol/l)	767	3.8	1.8–7.6	0.012
Covariates: lifestyle and disease				
Jogging (km/week)	767	4.3 ^b	0, 0, 10 ^c	0.006 ^d
Cycling (km/week)	767	23.0 ^b	0, 5, 700 ^c	0.006 ^d
Additional activities (h/week)	767	2.8 ^b	0, 0, 30 ^c	−0.074 ^{*,d}
Strength training (yes/no) (%)	767	19.9 ^e	17.2–23.0 ^f	−0.076 ^{*,d}
Physically active (yes/no) (%)	767	75.6 ^e	72.4–78.6 ^f	−0.068 ^d
Chronic disease (yes/no) (%)	767	6.0 ^e	4.4–7.9 ^f	−0.011 ^d

**P*<0.05, [†]*P*<0.01.^aPearson's correlation coefficients.^bArithmetic mean: geometric mean incomputable due to zero-values.^cMinimum, median, and maximum shown due to severely skewed distribution.^dSpearman's rank correlation coefficients.^eProportion of men answering 'yes' (binary variable).^f95% Confidence interval for the proportion of men answering 'yes'.

and relative LBM_{lower extremity} of 6.3, 9.3 cm², 0.96, and 0.49 kg respectively. In addition, significant, positive correlations with CAG_n were found for relative SAT_{thigh}, relative SAT_{lower trunk}, relative FM_{total}, and relative FM_{lower extremity} (Fig. 3E–H). A reduction of ten CAG repeats was equivalent with declines in relative SAT_{thigh}, relative SAT_{lower trunk}, relative FM_{total}, and relative FM_{lower extremity} of 6.8, 19.5 cm², 1.01, and 0.51 kg respectively. Neither the absolute DEXA outcomes (Table 1) nor the relative outcomes of deep adipose tissues (relative IMAT_{thigh} and relative VAT, data not shown) correlated significantly with CAG_n.

Analyses with exclusion of men with low TT

Of the 780 men not taking anabolic steroids, 45 men (5.8%) had TT<12.5 nmol/l. Of these 45 men, 33 were genotyped. All significant correlations reported above remained statistically significant when excluding men with low TT. In these subsets of

analyses, additional, significant correlations were also found between CAG_n and the following relative outcomes: relative LBM_{upper extremity} (*R* = −0.083, *P* = 0.030), relative FM_{upper extremity} (*R*: 0.084, *P* = 0.027), and relative FM_{central} (*R*: 0.085, *P* = 0.039).

Multiple regression analyses

All relative outcomes that correlated significantly with CAG_n in univariate analyses remained significant correlates of CAG_n when controlling for physical activity (jogging, cycling, strength training, and other sports) smoking, chronic disease, medication, and age (Tables 2–6). Consistent with the univariate analyses, a reduction of ten CAG repeats was equivalent with increments in relative muscle_{thigh}, relative muscle_{lower trunk}, relative LBM_{total}, and relative LBM_{lower extremity} of 5.3, 7.0 cm², 0.76, and 0.43 kg respectively (Tables 2 and 3), and was equivalent with declines in relative SAT_{thigh}, relative SAT_{lower trunk},

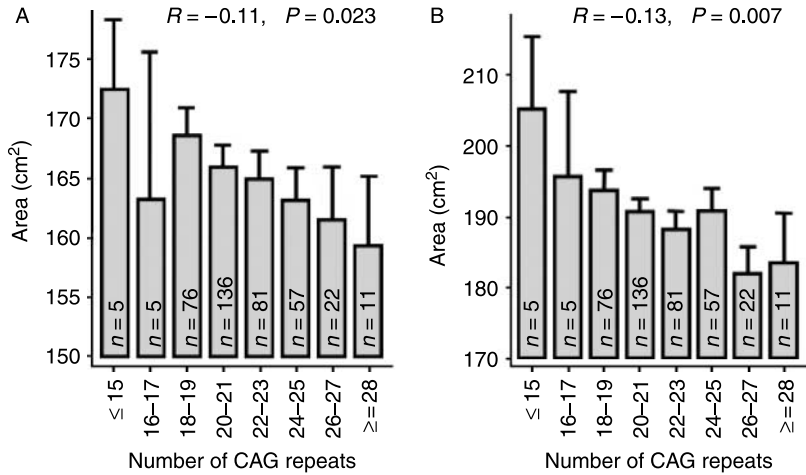


Figure 2 The relationship between the number of CAG repeats and outcomes of body composition in 393 men aged 20–29 years: absolute thigh muscle area (A) and absolute lower trunk muscle area (B).

relative FM_{total}, and relative FM_{lower extremity} of 5.5, 17.0 cm², 0.79, and 0.45 kg respectively (Tables 4 and 5). Again, CAG_n did not correlate with the outcomes of deep adipose tissues, relative IMAT_{thigh} and relative VAT (Table 6).

Covariates

The most consistent positive correlate of all relative outcomes of muscle area and LBM as well as inverse correlate of all relative outcomes of adipose tissue

and FM was ‘other sports’: compared with men not undertaking this activity, 10 h/week of other sports corresponded to enlargements of 11.1, 14.0 cm², 2.0, and 0.83 kg in relative muscle_{thigh}, relative muscle_{lower trunk}, relative LBM_{total}, and relative LBM_{lower extremity} respectively. This was paralleled by reductions in relative SAT_{thigh}, relative SAT_{lower trunk}, relative FM_{total}, and relative FM_{lower extremity} of 10.9, 15.8 cm², 2.2, and 0.92 kg. The only covariate correlating significantly with relative IMAT_{thigh} was jogging, which was also a significant correlate of the

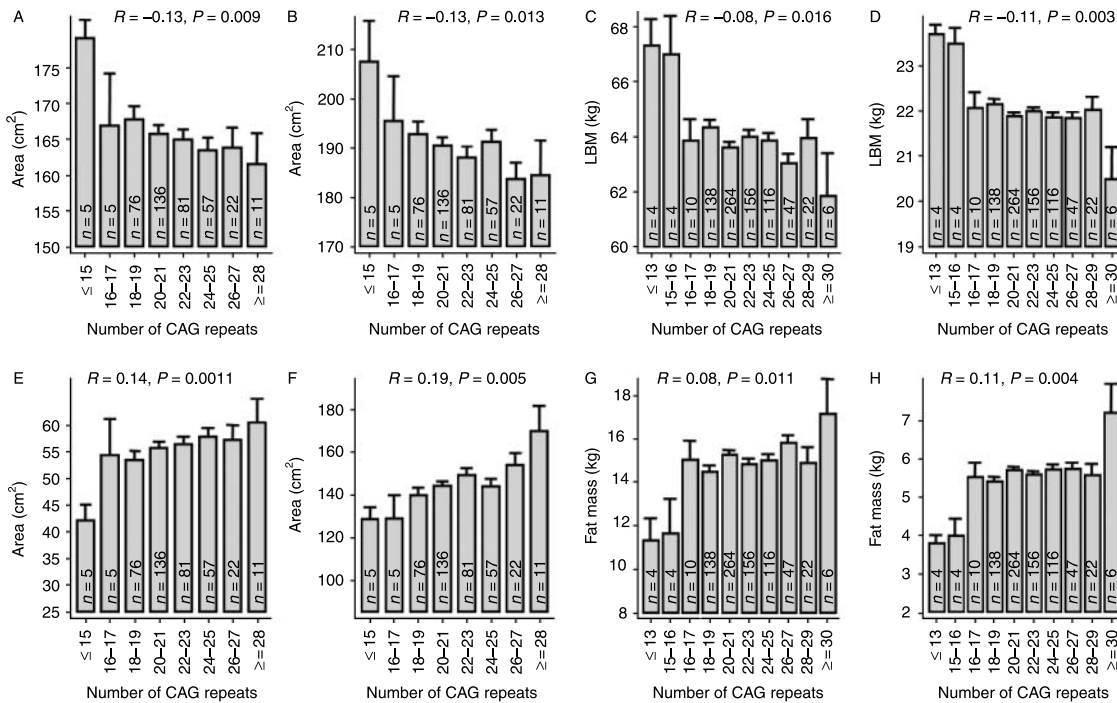


Figure 3 The relationship between the number of CAG repeats and outcomes of body composition in 20–29-year-old men: relative thigh muscle area (A), relative lower trunk muscle area (B), relative total lean body mass (C), relative lower extremity lean body mass (D), relative thigh subcutaneous adipose tissue (E), relative lower trunk subcutaneous adipose tissue (F), relative total fat mass (G), and relative lower extremity fat mass (H). n = 393 in A–B–E–F and n = 767 in C–D–G–H.

Table 2 Relative muscle area assessed by magnetic resonance imaging: contribution of the CAG repeat polymorphism, sex steroid hormones, physical activity, smoking, chronic disease, and age to the variation in relative outcomes of thigh and lower trunk muscle areas in 393 young men in the Odense Androgen Study.

	Relative thigh muscle area		Relative lower trunk muscle area	
	Coef. (cm ²)	β -coefficient	Coef. (cm ²)	β -coefficient
CAG repeats (number)	-0.57	-0.111*	-0.75	-0.102*
Total testosterone (nmol/l)	-0.12	-0.057	0.027	0.009
Total dihydrotestosterone (nmol/l)	4.84	0.219 [§]	4.63	0.147*
Total estradiol (pmol/l)	0.005	0.013	-0.01	-0.024
Jogging (km/week)	0.09	0.073	0.04	0.025
Cycling (km/week)	-0.003	-0.008	-0.06	-0.106*
Fitness center (yes/no)	0.45	0.013	2.55	0.051
Other sports (h/week)	1.11	0.342	1.41	0.306
Smoking (cigarettes/day)	-0.24	-0.131 [†]	-0.12	-0.044
Chronic disease (no medication)	-14.12	-0.100 [‡]	-14.89	-0.074*
Chronic disease (medically treated)	-2.20	-0.028	0.61	0.005
Age (years)	0.40	0.095	-0.23	-0.033
		R^2 : 0.232		R^2 : 0.154

Conventional coefficients and normalized β -coefficients. * $P < 0.05$, [†] $P < 0.01$, [‡] $P < 0.001$, [§] $P < 0.0001$, ^{||} $P < 10^{-6}$ (all apply for both coefficients).

other outcome of deep adipose tissue, VAT. Jogging also correlated positively with relative LBM_{total} and relative LBM_{lower extremity} and inversely with relative FM_{total} and relative FM_{lower extremity}. Chronic disease without concomitant medication had a negative impact on relative muscle_{thigh}, relative muscle_{lower trunk}, and relative LBM_{lower extremity}. Smoking was significantly correlated with decreased relative muscle_{thigh}, and noticeably, relative VAT was 12.0 cm² higher in 29-year-old men than in 20-year-old men. The effect of CAG_n on any outcome did not change significantly when the analyses were performed separately in physically active men and in physically inactive men.

CAG_n and circulating androgens

No significant relationships were found between CAG_n and TT, bioavailable testosterone, DHT, androstenedione,

or sex hormone binding globulin (SHBG; $P = 0.78$, $P = 0.86$, $P = 0.69$, $P = 0.69$, and $P = 0.80$). CAG_n did correlate positively with E₂ ($R = 0.097$, $P = 0.007$). In an additional multivariate model incorporating CAG_n, SHBG, and TT as independent variables and E₂ as the dependent variable, E₂ correlated positively with CAG_n ($R = 0.094$, $P = 0.003$) and TT ($R = 0.518$, $P < 10^{-25}$), but inversely with SHBG ($R = -0.213$, $P < 10^{-5}$).

Discussion

This is the first large population-based study of the effects of the CAG repeat polymorphism of the androgen receptor gene on muscle area, LBM, and adipose tissue in young men. The effect of this common allelic polymorphism on muscle size could be demonstrated in a male population aged 20–29 years, and could not be attributed to any of the covariates examined,

Table 3 Relative lean body mass assessed by dual-energy X-ray absorptiometry: contribution of the CAG repeat polymorphism, sex steroid hormones, physical activity, smoking, chronic disease, and age to the variation in relative outcomes of total and lower extremity lean body mass in 767 young men in the Odense Androgen Study.

	Relative total LBM		Relative lower extremity LBM	
	Coef. (kg)	β -coefficient	Coef. (kg)	β -coefficient
CAG repeats (number)	-0.078	-0.067*	-0.044	-0.098 [†]
Total testosterone (nmol/l)	0.045	0.088*	0.008	0.041
Total dihydrotestosterone (nmol/l)	0.55	0.103 [†]	0.22	0.107 [†]
Total estradiol (pmol/l)	-0.002	-0.015	-0.0001	-0.002
Jogging (km/week)	0.031	0.101 [†]	0.012	0.101 [†]
Cycling (km/week)	0.0001	0.002	0.001	0.029
Fitness center (yes/no)	0.52	0.063	-0.017	-0.005
Other sports (h/week)	0.20	0.258 [‡]	0.081	0.272 [‡]
Smoking (cigarettes/day)	-0.004	-0.011	-0.006	-0.039
Chronic disease (no medication)	-1.24	-0.049	-1.00	-0.100*
Chronic disease (medically treated)	-0.34	-0.021	-0.27	-0.043
Age (years)	0.034	0.029	0.027	0.060
		R^2 : 0.147		R^2 : 0.158

Conventional coefficients and normalized β -coefficients. * $P < 0.05$, [†] $P < 0.01$, [‡] $P < 10^{-12}$ (all apply for both coefficients).

Table 4 Relative subcutaneous adipose tissue assessed by magnetic resonance imaging: contribution of the CAG repeat polymorphism, physical activity, smoking, chronic disease, and age to the variation in relative outcomes of subcutaneous adipose tissues (SAT) in 393 young men in the Odense Androgen Study.

	Relative thigh SAT		Relative lower trunk SAT	
	Coef. (cm ²)	β-coefficient	Coef. (cm ²)	β-coefficient
CAG repeats (number)	0.55	0.111*	1.70	0.164 [†]
Jogging (km/week)	-0.05	-0.041	-0.003	-0.001
Cycling (km/week)	0.01	0.030	0.09	0.104*
Fitness center (yes/no)	-0.77	-0.026	-4.55	-0.065
Other sports (h/week)	-1.09	-0.348 [‡]	-1.58	-0.244 [‡]
Smoking (cigarettes/day)	0.21	0.115*	0.09	0.023
Chronic disease (no medication)	14.73	0.108 [†]	11.34	0.040*
Chronic disease (medically treated)	2.42	0.032	-7.96	-0.050
Age (years)	-0.46	-0.096*	-1.10	-0.110*
		R ² : 0.20		R ² : 0.12

Conventional coefficients and normalized β-coefficients. *P<0.05, [†]P<0.01, [‡]P<0.0001 (all apply for both coefficients).

including sex steroid concentrations. Two main outcomes (thigh muscle area and lower trunk muscle area) correlated significantly and inversely with CAG_n. Additionally, inverse correlations were also found for the relative outcomes of thigh muscle area, lower trunk muscle area, lower extremity LBM, and total LBM. In men with normal TT levels, upper extremity LBM also correlated inversely with CAG_n.

Our study takes advantage of not being susceptible to neuroregulatory defects in the feed-forward and feed-back systems of the HPG axis, which has been reported in healthy aging men (16–18). Moreover, age per se as well as the increased prevalence of chronic diseases, use of medication, and obesity with age will affect circulating androgens negatively (19–23). This may mask an effect of the CAG repeat polymorphism at older ages, because the effects are best observed in eugonadal men (24) and because adjustment for measures such as disease and medication are analytically challenging.

The participants were randomly drawn from the Danish Central Personal Registry (25, 26), and they matched the background population of 20–29-year-old men as regards sociodemography, body mass index, physical activity, smoking, alcohol intake, medication, and prevalence of chronic diseases (7). The subjects were carefully interviewed and examined to adjust for any confounding impact of lifestyle or disease. The narrow age interval limits the potential bias of cohort effects. The young age limits the exposure to potential environmental confounders, and provides the optimal setting for examinations of physiological relationships in a period in life when peak muscle mass is attained (27, 28). Thus, the results indicate that the CAG repeat polymorphism may be related to variations in accretion of muscular tissue, while studies of older men may also reflect variations in the age-related loss of muscle.

Only one previous study (5) examined the relationship between CAG_n and fat-free mass; our results are in disagreement with the results of Walsh *et al.* (5),

Table 5 Relative fat mass assessed by dual-energy X-ray absorptiometry: contribution by the CAG repeat polymorphism, physical activity, smoking, chronic disease, and age to the variation in relative outcomes of total and lower extremity fat mass in 767 young men in the Odense Androgen Study.

	Relative total fat mass		Relative lower extremity fat mass	
	Coef. (kg)	β-coefficient	Coef. (kg)	β-coefficient
CAG repeats (number)	0.079	0.064*	0.045	0.093 [†]
Jogging (km/week)	-0.031	-0.096 [†]	-0.012	-0.097 [†]
Cycling (km/week)	0.000	0.002	-0.001	0.020
Fitness center (yes/no)	-0.51	-0.058	0.031	-0.009
Other sports (h/week)	-0.22	-0.275 [‡]	-0.092	-0.289 [‡]
Smoking (cigarettes/day)	0.002	0.004	0.005	0.031
Chronic disease (no medication)	1.44	0.053	1.09	0.103*
Chronic disease (medically treated)	0.35	0.039	0.35	0.052
Age (years)	-0.029	-0.027	-0.029	-0.061
		R ² : 0.12		R ² : 0.14

Conventional coefficients and normalized β-coefficients. *P<0.05, [†]P<0.01, [‡]P<10⁻¹² (all apply for both coefficients).

Table 6 Relative deep adipose tissue assessed by magnetic resonance imaging: contribution of the CAG repeat polymorphism, physical activity, smoking, chronic disease, and age to the variation in relative outcomes of deep adipose tissues in 393 young men in the Odense Androgen Study.

	Relative thigh i.m. adipose tissue		Relative visceral adipose tissue	
	Coef. (cm ²)	β -coefficient	Coef. (cm ²)	β -coefficient
CAG repeats (number)	0.005	0.006	-0.19	-0.028
Jogging (km/week)	-0.021	-0.108 [†]	-0.17	-0.114*
Cycling (km/week)	-0.005	-0.072	-0.02	-0.042
Fitness center (yes/no)	0.082	0.014	-2.00	-0.044
Other sports (h/week)	-0.049	-0.092	-0.12	-0.030
Smoking (cigarettes/day)	0.005	0.018	-0.13	-0.056
Chronic disease (no medication)	0.94	0.040	11.81	0.065
Chronic disease (medically treated)	0.96	0.073	4.01	0.039
Age (years)	0.069	0.084	1.20	0.186 [‡]
		R^2 : 0.05		R^2 : 0.06

Conventional coefficients and normalized β -coefficients. * $P < 0.05$, [†] $P < 0.01$, [‡] $P < 0.001$ (all apply for both coefficients).

who found that absolute and relative fat-free mass correlated positively with CAG_n in two cohorts of 294 men (55–93 years) and 112 men (19–90 years) respectively. The age difference between our cohort and the cohorts studied by Walsh *et al.* is obvious. Their findings were discussed in context with aging: in our young cohort, we found no relationship between CAG_n and circulating androgens, which were assayed after extraction and chromatography to give high precision and accuracy. Neither did Van Pottelbergh *et al.* (29) in a study of 273 community-dwelling healthy men, aged between 71 and 86 years, find a relationship between CAG_n and circulating androgens. However, in the study by Walsh *et al.*, total and bioavailable testosterone were lower in men with fewer repeats at older ages, suggesting a gene-by-age interaction. Also, Krithivas *et al.* reported that longitudinal, intra-individual declines in total and bioavailable testosterone with age were significantly, inversely correlated with CAG_n in the Massachusetts Male Aging Study (6). Reduced central androgen receptor activity in men with longer alleles was hypothesized to diminish the negative feedback on the HPG axis, leading to higher androgen levels (5, 6). Thus, the direct, positive effects of having few CAG repeats on muscle size in young men (toward which our data point) may be overruled by a concomitant decline in androgen levels in elderly men with few repeats. The positive relationship between CAG_n and E₂ in our cohort is interesting, but it needs to be replicated in other cohorts. In addition, the relationship between CAG_n and E₂-dependent parameters should be examined. Zitzmann *et al.* have proposed that relationships between CAG_n and any outcome are most likely to be identified in men with normal levels of circulating androgens (24). Our additional observation of a negative correlation between CAG_n and the relative outcome of upper extremity LBM in men with normal TT levels supports this view.

Using two modalities, we also investigated eight outcomes of adiposity (SAT_{thigh}, IMAT_{thigh}, SAT_{lower trunk}, and VAT assessed by MRI and FM_{total}, FM_{lower extremity}, FM_{upper extremity}, and FM_{central} assessed by DEXA) in relation to CAG_n; positive correlations were seen between CAG_n and SAT_{thigh}, SAT_{lower trunk}, FM_{total}, and FM_{lower extremity} upon adjustment of these parameters for total thigh area, total lower trunk area, body weight, and total lower extremity mass respectively. The relationships remained statistically significant after correction for covariates. It may be a direct effect of increased AR activity in men with shorter alleles, because the AR has been shown to mediate the inhibitory effects of TT and DHT on mesenchymal stem cell differentiation toward adipocytes (30). Our results are in agreement with Zitzmann *et al.* (2003), who found the same relationship with FM in a cross-sectional study involving 106 healthy 20–50-year-old men. The median age of their cohort (28 years) was very close to that of our cohort (25.7 years).

The differences in size and mobilization processes between subcutaneous and visceral adipocytes have previously been put in context with variations in circulating androgens (31). The significant impact of CAG_n on (SAT_{thigh} and SAT_{lower trunk}) and lacking impact on deeper adipose tissues (IMAT_{thigh} and VAT) are interesting findings: they may indicate that actions of androgens on male adipose tissues are mainly associated with favorable impacts on subcutaneous compartments. This is in accordance with interventional studies of testosterone replacement therapy, in which subcutaneous compartments diminished (32, 33), while only one small, older study reported diminished amounts of VAT (34). The interpretation of cross-sectional data is uncertain, but our study strongly indicates that VAT increases considerably from age 20 to 29 years, and that, on average, VAT is further increased in middle-aged men (21). Therefore, the lack of impact of CAG_n on VAT in our study could also reflect

that these young men have yet to encounter the point in life where they can no longer convert a positive energy balance into deposition of fat in subcutaneous stores. Our data do not rule out the possibility that the CAG repeat polymorphism may also modify VAT, once the deposition into deeper adipose tissues accelerates later in life.

The statistically significant results obtained in this study are – in our opinion – also physiologically relevant with ~4–5% increments in thigh and lower trunk muscle areas and 13–16% reductions in SAT areas per ten CAG repeat decreases. Nevertheless, this apparent genetic effect was not as strong as the effect of a lifestyle with many weekly hours spent in sports. We have investigated 14 absolute and 14 relative outcomes of body composition. All relationships with CAG_n – with respect to the trend – are in congruence; inverse relationships with all outcomes of muscle areas and LBM and – vice versa – positive relationships with all outcomes of SAT areas and FM. Our data indicate that having short CAG repeats may be a genetic advantage in terms of muscle accretion. However, we are not aware of any data demonstrating an effect of this polymorphism on muscle function.

In conclusion, we have demonstrated a significant effect of the CAG repeat polymorphism of the androgen receptor gene on thigh and lower trunk muscle areas in young men. This study is the first to demonstrate an inverse relationship between the number of CAG repeats and muscle area. The results need to be confirmed in other young, male cohorts.

Declaration of interest

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

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