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ARTICLE *in* JOURNAL OF ANDROLOGY · SEPTEMBER 2011

Impact Factor: 1.69 · DOI: 10.2164/jandrol.111.014415 · Source: PubMed

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Journal of Andrology, Vol. 33, No. 4, July/August 2012

Androgen Receptor Gene Polymorphisms and the Fat-Bone Axis in Young Men and Women

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ABSTRACT: Androgen receptor (AR) CAG_n (polyglutamine) and GGN_n (polyglycine) repeat polymorphisms determine part of the androgenic effect and may influence adiposity. The association of fat mass, and its regional distribution, with the AR CAG_n and GGN_n polymorphisms was studied in 319 and 78 physically active nonsmoker men and women (mean ± SD: 28.3 ± 7.6 and 24.8 ± 6.2 years old, respectively). The length of CAG and GGN repeats was determined by polymerase chain reaction and fragment analysis, and confirmed by DNA sequencing of selected samples. Men were grouped as CAG short (CAG_S) if harboring repeat lengths ≤21, the rest as CAG long (CAG_L). The corresponding cutoff CAG number for women was 22. GGN was considered short (GGN_S) if GGN ≤23, the rest as GGN long (GGN_L). No association between AR polymorphisms and adiposity or the hormonal variables was observed in men. Neither was there a difference in the studied variables between men harboring CAG_L + GGN_L, CAG_S + GGN_S, CAG_S + GGN_L, and CAG_L + GGN_S combinations. However, in women, GGN_n was linearly related to the percentage of body fat (r = 0.30, $P < .05$), the percentage of fat in the trunk (r = 0.28, $P < .05$), serum leptin concentration (r = 0.40, $P < .05$), and serum osteocalcin concentration (r = 0.32, $P < .05$). In men, free testosterone was inversely associated with adiposity and serum leptin concentration, and positively with osteocalcin, even after accounting for differences in CAG_n, GGN_n, or both. In summary, this study shows that the AR repeat polymorphism has little influence on absolute and relative fat mass or its regional distribution in physically active men. In young women, GGN length is positively associated with adiposity, leptin, and osteocalcin.

Key words: Obesity, testosterone, leptin, osteocalcin, steroids.
J Androl 2012;33:644–650

Testosterone, osteocalcin, and leptin are 3 of the main hormones regulating fat mass in humans. The association between these hormones and fat mass may be modulated by polymorphic variations in the androgen receptors (AR; Zitzmann et al, 2003; Guadalupe-Grau et al, 2010b, 2011; Nielsen et al, 2010).

The AR gene contains a polyglutamine tract encoded by CAG repeats and a polyglycine tract (GGN) encoded by $(GGT)_3GGG(GGT)_2(GGC)_n$. In vitro experiments have shown that elimination of the CAG tract in both human and rat AR resulted in elevated transcriptional activation activity, whereas an expansion of the CAG repeat in human AR caused a linear decrease of transactivation function (Chamberlain et al, 1994), and reduced transcriptional potential (Tut et al, 1997; Lee and Chang, 2003). The length of the GGN tract (number of glycine residues) is linearly and inversely associated with AR protein content in cell cultures (Ding et al, 2005), and longer GGN tracts result in a linearly reduced AR activity per cell (Ding et al, 2005). Body fat accumulation and its regional distribution are in part determined by sex hormones. In men, fat mass and abdominal obesity are associated with reduced circulating androgens (Khaw and Barrett-Connor, 1992; Phillips et al, 2003), whereas testosterone has an antiobesity effect (Mauras et al, 1998; Rolf et al, 2002; Woodhouse et al, 2004). In women, increased circulating androgens have been associated with both abdominal obesity (Hauner et al, 1994; Garaulet et al, 2000) and reduced visceral fat (Turcato et al, 1997).

Supported by Ministerio de Educación y Ciencia (DEP2006-56076-C06-04/ACTI) and FEDER, Gobierno de Canarias (PI2005/177), FUNCIS (PI/10/07), Consejería de Educación, Cultura y Deportes del Gobierno de Canarias (2006/179 0001 and FEDER), Proyecto Interreg IIIB BIOPOLIS, Fundación del Instituto Canario de Investigación del Cáncer (FICIC), Cabildo de Gran Canaria, Cabildo de Tenerife and La Caja de Canarias, and Proyecto Estructurante "Integración de los grupos de investigación en Ciencias de la Salud," ULPGC, Gobierno de Canarias.

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Received for publication June 8, 2011; accepted for publication September 16, 2011.
DOI: 10.2164/jandrol.111.014415

Previous studies in men from Germany (Zitzmann et al, 2003) and Denmark (Nielsen et al, 2010) reported a positive association between CAG_n and fat mass assessed with bioimpedance (Zitzmann et al, 2003), dual-energy x-ray absorptiometry (DXA; Nielsen et al, 2010), and magnetic resonance imaging (MRI; Nielsen et al, 2010). It has been suggested that these findings need to be confirmed in other cohorts (Nielsen et al, 2010). Moreover, the potential interactions between CAG and GGN repeat polymorphism combinations and fat mass have not been studied.

The osteoblasts' produced hormone osteocalcin is also a negative regulator of fat mass (Ferron et al, 2008). A negative association between osteocalcin with fat mass and serum leptin has been reported in cross-sectional studies (Guadalupe-Grau et al, 2009; Kindblom et al, 2009; Pittas et al, 2009); and osteocalcin blunts the expected increase in leptin concentration with fat mass accumulation in humans (Guadalupe-Grau et al, 2010a). Serum osteocalcin levels are also associated with testosterone levels in women (Guadalupe-Grau et al, 2009; Ahn et al, 2010), and in males osteocalcin stimulates testosterone production by acting on the Leydig cells (Oury et al, 2011).

Therefore, osteocalcin may act as a confounder, masking a potential relationship between AR polymorphism and fat mass phenotype in humans. Likewise, physical activity and fitness (Nielsen et al, 2010; Serrano-Sanchez et al, 2010; Wang et al, 2010) may also influence the relationship between AR polymorphism and the fat mass phenotype.

The aim of this study was to determine if AR polymorphisms are associated with fat mass and its regional distribution in young men and women, assessing how this relationship is influenced by osteocalcin and cardiorespiratory fitness ($\dot{V}o_2max$). We hypothesized that men and women with high CAG and GGN repeat lengths would have increased fat mass after accounting for free testosterone, osteocalcin, and $\dot{V}o_2max$ as confounding variables.

Materials and Methods

Subjects

Three hundred nineteen Caucasian men and 78 women agreed to participate in this study. Men and women were (mean ± SD) 28.3 ± 7.6 and 24.8 ± 6.2 years old, and their respective body weight, height, and percentage of body fat were 77.8 ± 10.2 and 59.8 ± 7.5 kg, 176.8 ± 5.6 and 164.5 ± 5.9 cm, and 18.8% ± 7.4% and 28.0% ± 6.4% in men and women, respectively. They were recruited from physically active university students, sports clubs, and local police officers in Gran Canaria (Spain). Recruitment started in February 2003 and extended to March 2010. All subjects underwent a medical examination prior to inclusion in the study. Inclusion criteria were age ranging from 18 to 60, body mass index <35, and participation in sports or other type of physical activities with a minimum frequency of 1 time per week. Subjects smoking, taking any medications, or having any chronic disease, hypertension, or orthopedic limitations were excluded. Less than 5% of all volunteers had to be excluded, mostly because of smoking or chronic diseases. The study was performed in accordance with the Helsinki Declaration of 1975 as regards the conduct of clinical research, being approved by the Ethical Committee of the University of Las Palmas de Gran Canaria. All volunteers provided their written informed consent before participation in the study.

Tests

On the test day, between 7:30 and 8:30 AM, subjects reported to the laboratory under fasting conditions. After each subject had lain in the supine position for at least 5 minutes, a 20-mL blood sample was obtained from an antecubital vein. Then the subject's body composition was determined. In some subjects, an additional test was carried out on a different day to assess their $\dot{V}o_2max$, as previously reported (Guadalupe-Grau et al, 2011).

Body Composition

Whole-body composition was assessed by DXA (QDR-1500, software version 7.10; Hologic Corp, Waltham, Massachusetts) as previously reported (Perez-Gomez et al, 2008). Upper and lower limb lean mass (kg) was calculated from regional analysis of the whole body scan (Sanchís-Moysi et al, 2009; Serrano-Sanchez et al, 2010), which gives a valid and a reliable estimate of muscle mass in the extremities (Kim et al, 2002).

Serum Free Testosterone, Leptin, and Osteocalcin Determinations

Serum leptin, free testosterone, and osteocalcin were determined by enzyme-linked immunosorbent assay (ELx800 Universal Microplate Reader; Bioteck Instruments Inc, Winooski, Vermont), using reagent kits from Linco Research (No. EZHL-80SK; Linco Research, St Charles, Missouri), IBL (Hamburg, Germany), and Nordic Bioscience Diagnostics (Herlev, Denmark), respectively. Low-end sensitivity was 0.05 ng/mL for total leptin, 0.17 pg/mL for free testosterone, and 0.5 ng/mL for osteocalcin, respectively. Intra-assay and interassay coefficients of variation were 3.8% and 4.4% for leptin, 6.1% and 7.8% for free testosterone, and 6.7% and 6.7% for osteocalcin (Guerra et al, 2008; Guadalupe-Grau et al, 2009).

CAG and GGN Repeat Polymorphisms

DNA was extracted from blood samples (200 μL) using High Pure PCR Template Preparation Kits (Roche Applied Science, Mannheim, Germany). To determine the length of the CAG and GGN repeats the corresponding regions located on exon 1 of the AR gene (Genbank accession No. M27423) were amplified using 2 pairs of primers whose sequences have been

previously reported (Rodriguez et al, 2006). One primer from each pair was marked with fluorescent dye (FAM and VIC; Applied Biosystems, Warrington, United Kingdom). Amplification was performed in a 25-μL reaction volume, containing 50 ng of genomic DNA, 200 μM of each deoxynucleotide triphosphate, 1 × Fast Start Taq DNA polymerase buffer (Roche Applied Science), 1 × GC-rich solution buffer (Roche Applied Science), and 1 U of Fast Start Taq DNA polymerase (Roche Applied Science). The concentration of each pair of primers was 1.2 and 1.5 μM for the amplification of the CAG and GGN repeats, respectively. Polymerase chain reaction (PCR) conditions were 30 cycles of 95°C for 45 seconds, 56°C for 30 seconds, and 72°C for 30 seconds for CAG amplification, and 30 cycles of 95°C for 1 minute, 55°C for 2 minutes, and 72°C for 2 minutes for GGN amplification. Each PCR was initiated with a denaturation step at 95°C for 5 minutes and terminated with an extension step at 72°C for 5 minutes. The PCR product was diluted 1:100 in distilled water and 1 μL of the dilution was mixed with 10 μL of formamide and 0.3 μL of GeneScan 500 LIZ Size Standard (Applied Biosystems), denatured at 98°C for 5 minutes, and cooled on ice. Fragment separation was performed by automated capillary electrophoresis, using an ABI Prism 3100 Genetic Analyzer (Applied Biosystems), and the length was determined with Gene Scan Analysis Software (version 3.7; Applied Biosystems). Internal standards supplied by the manufacturer were used for quality control. We blindly repeated the genotype analysis in 54 of the samples, and the results were totally coincident. The fragment size was confirmed by sequencing 48 DNA samples harboring different size alleles for both repeats by using the Big Dye Terminator Sequencing Kit (Applied Biosystems) at University of Las Palmas Sequencing Facility. Genotyping was performed specifically for research purposes based on the hypothesis that the aforementioned polymorphisms may influence leptin, osteocalcin, testosterone, and fat mass. The researchers performing the genotyping were totally blinded to the subjects' identities.

Statistical Analysis

All variables were checked for normal distribution using the test of Shapiro-Wilk. When necessary, the analysis was done on logarithmically transformed data. Because the AR gene is located in the X chromosome and 1 of the 2 alleles is randomly inactivated in women (Lyon, 1999), we decided to calculate the mean of 2 alleles as representative of each pair in women (Gonzalez et al, 2007; Jaaskelainen et al, 2008). The influence of CAG and GGN repeat lengths on body composition, hormones, and $\dot{V}o_2max$ was determined taking CAG and GGN repeat lengths as either continuous or dichotomous variables with allele cutoff thresholds. The relationship between CAG and GGN as continuous variables with body composition, hormones, and physical fitness variables was examined using linear regression analysis. The median value that resulted in the most balanced grouping was used as a cutoff threshold (Hickey et al, 2002; Rodriguez-Gonzalez et al, 2009). Thus, men were grouped as CAG short (CAG_S) if harboring repeat lengths ≤21 and CAG long (CAG_L) if harboring repeat lengths >21. Women were grouped as CAG_S if harboring repeat lengths ≤22 and CAG_L if harboring repeat lengths >22. Men and women were assigned to the GGN short (GGN_S) group if harboring repeat lengths of ≤23; otherwise they were included in the GGN long (GGN_L) group. These median CAG and GGN values are identical to those reported for the population of Gran Canaria (Rodriguez-Gonzalez et al, 2009) and similar to those observed in other Caucasian cohorts (Ruhayel et al, 2004; Shah et al, 2008; Stanworth et al, 2008). In addition, men were also grouped if having any of the following haplotype combinations: $CAG_S + CGN_L$, $CAG_L + CGN_S$, $CAG_L + CGN_L$, and $CAG_S + CGN_S$.

To determine if the association between CAG and GGN repeat numbers and body composition variables is influenced by free testosterone, partial correlation coefficients were calculated after adjustment for free testosterone. The association between GGN repeat length with adiposity (percentage of body fat) and serum leptin concentration in women was assessed by calculating the partial correlation coefficients adjusted for free testosterone, osteocalcin, and $\dot{V}o_2max$.

The impact of AR was assessed separately in men and women. Comparisons between short and long polymorphic variants were carried out using Student's *t* test. Lean mass was corrected for differences in height by dividing muscle mass by height squared (Lietzke, 1956).

Results

The median CAG repeat length was 21 in men and 22 in women, ranging from 13 to 35, and from 9 to 33 repeats, respectively. Men and women had a median GGN repeat length of 23, ranging from 12 to 28 and from 15 to 26, respectively.

In men and women, CAG_S and CAG_L groups had similar body composition and similar concentrations of leptin, osteocalcin, and testosterone (Table 1). Likewise, in men and women, leptin, osteocalcin, and testosterone concentrations were similar in GGN_S and GGN_L groups (Table 2). However, women with a GAG_L repeat length polymorphism were about 3 cm taller than their GAG_S counterparts.

There was no relationship between CAG and GGN repeat length and either body composition or hormonal variables in men, nor between CAG repeat length and body composition or hormonal variables in women.

In women, the length of GGN repeat AR repeat polymorphism was linearly related to the logarithm of the percentage of body fat ($\log \% \text{ fat} = 1.7 \times \log GGN - 0.87$, $r = 0.30$, $P < .05$), the logarithm of the percentage of fat in the trunk ($\log \% \text{ trunk fat} = 2.5 \times \log GGN - 2.15$, $r = 0.28$, $P < .05$), the logarithm of serum leptin concentration ($\log \text{leptin} = 6.2 \times \log GGN - 7.46$, $r = 0.40$, $P < .05$), and the logarithm of serum osteocalcin concentration ($\log \text{osteocalcin} = 2.3 \times \log GGN - 1.87$, $r = 0.32$, $P < .05$). The association of GGN repeat length with adiposity and serum leptin concentration remained statistically significant after accounting for free testosterone, osteocalcin, and $\dot{V}o_2max$, in women (Table 3).

Table 1. *Body composition, anthropometrics, physical activity, fitness, and hormones in men and women with CAG_s and CAG_L androgen receptor polymorphisms (mean ± SD)*

	Men				Women			
	CAG_S	No.	CAG_L	No.	CAG_S	No.	CAG_L	No.
Age, y	27.5 ± 7.5	175	28.9 ± 7.5	144	24.0 ± 5.3	46	26.3 ± 7.4	31
Height, cm	176.4 ± 5.4	175	177.3 ± 5.8	144	163.3 ± 5.5[a]	47	166.4 ± 6.0	31
Body mass, kg	76.9 ± 9.8	174	78.9 ± 10.7	141	59.1 ± 6.9	47	60.7 ± 8.3	31
Body fat, %	18.6 ± 6.8	174	19.1 ± 8.0	141	27.1 ± 5.6	47	29.3 ± 7.3	31
Lean body mass, kg	59.2 ± 5.8	174	60.2 ± 5.4	141	40.6 ± 3.8	47	40.2 ± 3.1	31
Whole body fat mass, kg	14.7 ± 6.7	174	15.7 ± 8.5	141	16.2 ± 4.9	47	18.3 ± 6.8	31
Trunk fat mass, kg	6.8 ± 4.1	174	7.4 ± 5.2	141	5.9 ± 2.7	48	7.4 ± 4.5	31
Trunk fat, %	17.6 ± 8.8	174	18.4 ± 10.2	141	20.7 ± 6.9	47	24.4 ± 10.0	31
Sports history, y	8.1 ± 6.3	160	8.2 ± 5.3	135	8.0 ± 8.3	40	6.8 ± 7.9	27
$\dot{V}o_2$max, $mL \cdot kg^{-1} \cdot min^{-1}$	47.2 ± 7.9	160	46.5 ± 7.9	129	40.8 ± 8.5	38	38.7 ± 7.2	26
Leptin, $ng \cdot mL^{-1}$	4.7 ± 4.7	155	5.0 ± 4.8	133	13.1 ± 6.9	36	15.1 ± 8.3	30
Osteocalcin, $ng \cdot mL^{-1}$	21.5 ± 7.3	159	21.6 ± 8.8	128	18.4 ± 6.2	37	21.1 ± 7.2	29
Free testosterone, $pg \cdot mL^{-1}$	20.9 ± 11.1	149	21.0 ± 9.6	127	3.9 ± 1.9	34	3.4 ± 1.9	29

Abbreviations: CAG_L, CAG long; CAG_S, CAG short.

[a] $P < .05$ compared with CAG_L (same gender).

In men, the logarithm of free testosterone was inversely associated with adiposity and serum leptin concentration, and positively with osteocalcin (Table 4). These associations remained unchanged after accounting for differences in CAG, GGN, or both CAG and GGN repeat length.

Adiposity and serum concentrations of free testosterone, leptin, and osteocalcin were similar in men harboring the allelic combinations $CAG_L + GGN_L$, $CAG_S + GGN_S$, $CAG_S + GGN_L$, and $CAG_L + GGN_S$.

Discussion

In contrast with our hypothesis, no association was observed in our young men between AR length polymorphic variations and adiposity or serum leptin concentration. Moreover, this lack of association remained after adjusting for osteocalcin, free testosterone, and cardiorespiratory fitness ($\dot{V}o_2$max). In women, however, a consistent association between GGN repeat length with adiposity and serum leptin concentration was observed. This association was even stronger after accounting for osteocalcin, free testosterone, and $\dot{V}o_2$max as confounders. As in men, the CAG repeat length was not associated with adiposity or serum leptin concentration in women.

Thus, our results indicate that, in young men, the AR polymorphism is not associated with whole body fat mass, its relative amount, or its regional distribution. This finding is in contrast with a previous study in 106 men from Germany, who had a similar mean age (28 years) compared with ours, in which the CAG repeat length was associated with the percentage of body fat

Table 2. *Body composition, anthropometrics, physical activity, and fitness in men and women with GGN_s and GGN_L androgen receptor polymorphisms (mean ± SD)*

	Men				Women			
	GGN_S	No.	GGN_L	No.	GGN_S	No.	GGN_L	No.
Age, y	27.9 ± 7.0	197	28.5 ± 8.3	122	25.8 ± 7.4	34	24.3 ± 5.2	43
Height, cm	176.7 ± 5.6	197	177.1 ± 5.6	122	165.1 ± 6.4	35	164.0 ± 5.4	43
Body mass, kg	77.2 ± 9.7	194	78.8 ± 11.0	121	60.0 ± 8.8	35	59.6 ± 6.2	43
Body fat, %	18.4 ± 7.1	194	19.5 ± 7.8	121	27.7 ± 8.0	35	28.3 ± 4.7	43
Lean body mass, kg	59.5 ± 5.8	194	59.8 ± 5.4	121	40.6 ± 3.8	35	40.3 ± 3.4	43
Whole body fat mass, kg	14.6 ± 6.8	194	16.0 ± 8.5	121	17.1 ± 7.4	35	17.0 ± 4.2	43
Trunk fat mass, kg	6.8 ± 4.3	194	7.5 ± 5.1	121	6.8 ± 4.7	36	6.3 ± 2.3	43
Trunk fat, %	17.5 ± 9.3	194	18.6 ± 9.7	121	22.2 ± 10.7	35	22.1 ± 6.0	43
Sports history, y	7.9 ± 6.0	177	8.5 ± 5.8	118	7.0 ± 8.5	31	8.0 ± 7.8	36
$\dot{V}o_2$max, $mL \cdot kg^{-1} \cdot min^{-1}$	47.3 ± 7.8	171	46.3 ± 8.0	118	40.4 ± 7.3	33	39.5 ± 8.8	31
Leptin, $ng \cdot mL^{-1}$	4.8 ± 4.6	177	5.0 ± 5.0	111	13.7 ± 8.3	30	14.3 ± 7.1	36
Osteocalcin, $ng \cdot mL^{-1}$	21.4 ± 8.2	175	21.7 ± 7.8	112	18.3 ± 5.7	30	20.7 ± 7.4	36
Free testosterone, $pg \cdot mL^{-1}$	21.7 ± 11.0	173	19.6 ± 9.3	103	3.8 ± 2.1	30	3.5 ± 1.7	33

Abbreviations: GGN_L, GGN long; GGN_S, GGN short.

Table 3. *Relationship of the logarithm of GGN repeat length with adiposity and serum leptin concentration in women after accounting for free testosterone, osteocalcin, and $\dot{V}_{O_2}$max in women (n = 45)*

	Fat Mass[a]	% Body Fat[a]	Trunk Fat[a]	% Trunk Fat[a]	Leptin (ng·mL^{-1})[a]
Logarithm of GGN	−0.34[b]	−0.41[b]	−0.33[b]	−0.39[b]	−0.31[b]

[a] Logarithmically transformed.
[b] $P < .05$.

(assessed with bioimpedance: $r = 0.27$). Likewise, in a study of young Danish men (20–29 years old), a weak ($r = 0.06$) but statistically significant association between the CAG repeat number and the percentage of body fat measured by DXA was reported (Nielsen et al, 2010). In the latter, no association between CAG repeat number and visceral fat measured using MRI in 393 subjects was observed (Nielsen et al, 2010). Stanworth et al (2008) reported, in 232 men with type 2 diabetes, a positive association of CAG repeat number with leptin, waist circumference, and body mass index.

In agreement with our results, no association between the CAG repeat number and the percentage of body fat (assessed with bioimpedance) was reported by Goutou et al (2009) in 170 men (mean age, 42) from Greece. Thus, in young men, the CAG repeat number has little or no influence on adiposity, with small differences between countries, which could be due to genetic and environmental differences. As a novelty, we have shown for the first time that there is no association in young men between the GGN repeat number and adiposity.

The possibility for an interaction between the CAG and GGN repeat polymorphism and fat mass has not been previously studied in young men. The CAG_S + GGN_S microsatellite combination has been associated with stronger androgen signaling (Navarro et al, 2002; Gonzalez Hernandez et al, 2008). However, our results indicate lack of interaction between CAG and GGN repeat numbers and adiposity in men, because men harboring the allelic combinations CAG_L + GGN_L, CAG_S + GGN_S, CAG_S + GGN_L, and CAG_L + GGN_S have similar adiposity phenotypes.

In agreement with previous studies (Gonzalez Hernandez et al, 2008), no relationship was observed in the present investigation between CAG repeat number and serum leptin concentration in either sex. However, in women a strong association between the GGN repeat number and serum leptin concentration was observed, which could not be accounted for by differences in free testosterone. This association may be indirect, that is, due to lower antiobesity effects of testosterone in women with higher GGN_n, resulting in increased fat mass. However, it may be also mediated by a direct inhibitory effect of testosterone-derived androgens in the adipocytes (Pineiro et al, 1999). In fact, testosterone administration in female subjects decreased leptin levels beyond the expected level from the reduction of fat mass (Elbers et al, 1997). The effect of leptin on target cells depends on its circulating free fraction, which is determined by soluble leptin receptor (sOB-R), the main leptin-binding protein in plasma (Chan et al, 2002; Lou et al, 2010). Free plasma testosterone has been reported to be inversely related to sOB-R (Chan et al, 2002; Ara et al, 2006), although the potential role played by androgens and AR in regulation of sOB-R remains unknown.

In agreement with previous studies in middle-aged (Krithivas et al, 1999; Stiger et al, 2008) and aged men (Van Pottelbergh et al, 2001), no association between CAG or GGN length and free testosterone levels were observed in the present investigation.

The regulation of osteocalcin production in man remains unknown (Foresta et al, 2011). AR knockout male mice have disrupted adipogenesis and osteogenesis leading to osteoporosis and late-onset obesity (Kawano et al, 2003; Tsai et al, 2011). Transgenic models indicate that, in males, osteocalcin stimulates testosterone production by acting on the Leydig cells (Oury et al, 2011). In turn, dihydrotestosterone stimulates the expression and release of osteocalcin from human male adipose tissue (Foresta et al, 2011). In agreement, a positive association between osteocalcin and free testosterone was observed in men, which was not significantly influenced by the AR genotypes. In contrast, no association was observed between free testosterone and

Table 4. *Relationship between free testosterone, adiposity, leptin, and osteocalcin in men (n = 259)*

	Fat Mass	% Body Fat	Trunk Fat	% Trunk Fat	Leptin (ng·mL^{-1})	Osteocalcin (ng·mL^{-1})
Free testosterone (pg·mL^{-1})	−0.26[a]	−0.28[a]	−0.28[a]	−0.29[a]	−0.25[a]	0.22[a]
Adjusted for logCAG	−0.27[a]	−0.29	−0.30[a]	−0.30[a]	−0.25[a]	0.22
Adjusted for logGGN	−0.27[a]	−0.29[a]	−0.30[a]	−0.30[a]	−0.25[a]	0.22[a]
Adjusted for logCAG and logGGN	−0.27[a]	−0.29[a]	−0.30[a]	−0.30[a]	−0.25[a]	0.22[a]

[a] $P < .05$.

osteocalcin in women. However, in a recent 9-week strength training study we observed a significant association between changes in free testosterone and changes in serum osteocalcin in women ($r = 57$, n = 23; Guadalupe-Grau et al, 2009). In agreement with a role for androgens in the regulation of serum osteocalcin in women, we found a positive association between the length of the GGN tract and serum osteocalcin in the present study. It remains to be determined whether estradiol interacts with testosterone to regulate osteocalcin levels in women.

In summary, this study shows that the AR repeat polymorphism has little, if any, influence on the absolute and relative fat mass or its regional distribution in physically active nonsmoker men, whereas, in young women, the GGN repeat number is positively associated with adiposity. These findings could indicate that under conditions of reduced androgen levels, as observed in females, an increased number of ARs and, hence, androgen-mediated transcriptional potential due to lower GGN repeat number may favor an androgenic-mediated reduction in adipose mass and serum leptin concentration.

Acknowledgment

The authors thank José Navarro de Tuero for his excellent technical assistance.

References

Ahn KH, Lee SH, Park HT, Kim T, Hur JY, Kim YT, Kim SH. Effect of adiponectin and sex steroid hormones on bone mineral density and bone formation markers in postmenopausal women with subclinical hyperthyroidism. *J Obstet Gynaecol Res*. 2010; 36:370–376.

Ara I, Perez-Gomez J, Vicente-Rodriguez G, Chavarren J, Dorado C, Calbet JA. Serum free testosterone, leptin and soluble leptin receptor changes in a 6-week strength-training programme. *Br J Nutr*. 2006;96:1053–1059.

Chamberlain NL, Driver ED, Miesfeld RL. The length and location of CAG trinucleotide repeats in the androgen receptor N-terminal domain affect transactivation function. *Nucleic Acids Res*. 1994;22:3181–3186.

Chan JL, Bluher S, Yiannakouris N, Suchard MA, Kratzsch J, Mantzoros CS. Regulation of circulating soluble leptin receptor levels by gender, adiposity, sex steroids, and leptin: observational and interventional studies in humans. *Diabetes*. 2002;51:2105–2112.

Ding D, Xu L, Menon M, Reddy GP, Barrack ER. Effect of GGC (glycine) repeat length polymorphism in the human androgen receptor on androgen action. *Prostate*. 2005;62:133–139.

Elbers JM, Asscheman H, Seidell JC, Frolich M, Meinders AE, Gooren LJ. Reversal of the sex difference in serum leptin levels upon cross-sex hormone administration in transsexuals. *J Clin Endocrinol Metab*. 1997;82:3267–3270.

Ferron M, Hinoi E, Karsenty G, Ducy P. Osteocalcin differentially regulates beta cell and adipocyte gene expression and affects the development of metabolic diseases in wild-type mice. *Proc Natl Acad Sci U S A*. 2008;105:5266–5270.

Foresta C, Strapazzon G, De Toni L, Gianesello L, Bruttocao A, Scarda A, Plebani M, Garolla A. Androgens modulate osteocalcin release by human visceral adipose tissue. *Clin Endocrinol*. 2011; doi: 10.1111/j.1365-2265.2011.03997.x [published online ahead of print].

Garaulet M, Perex-Llamas F, Fuente T, Zamora S, Tebar FJ. Anthropometric, computed tomography and fat cell data in an obese population: relationship with insulin, leptin, tumor necrosis factor-alpha, sex hormone-binding globulin and sex hormones. *Eur J Endocrinol*. 2000;143:657–666.

Gonzalez A, Javier Dorta F, Rodriguez G, Brito B, Rodriguez MA, Cabrera A, Diaz-Chico JC, Reyes R, Aguirre-Jaime A, Nicolas Diaz-Chico B. Increased risk of breast cancer in women bearing a combination of large CAG and GGN repeats in the exon 1 of the androgen receptor gene. *Eur J Cancer*. 2007;43:2373–2380.

Gonzalez Hernandez A, Cabrera de Leon A, Dominguez Coello S, Almeida Gonzalez D, Rodriguez Perez MC, Brito Diaz B, Aguirre-Jaime A, Diaz-Chico BN. Serum resistin and polymorphisms of androgen receptor GAGn and GGNn and aromatase TTTAn. *Obesity (Silver Spring)*. 2008;16:2107–2112.

Goutou M, Sakka C, Stakias N, Stefanidis I, Koukoulis GN. AR CAG repeat length is not associated with serum gonadal steroids and lipid levels in healthy men. *Int J Androl*. 2009;32:616–622.

Guadalupe-Grau A, Ara I, Dorado C, Vicente-Rodriguez G, Perez-Gomez J, Cabrero JC, Serrano-Sanchez JA, Santana A, Calbet JA. Osteocalcin as a negative regulator of serum leptin concentration in humans: insight from triathlon competitions. *Eur J Appl Physiol*. 2010a;110:635–643.

Guadalupe-Grau A, Perez-Gomez J, Olmedillas H, Chavarren J, Dorado C, Santana A, Serrano-Sanchez JA, Calbet JA. Strength training combined with plyometric jumps in adults: sex differences in fat-bone axis adaptations. *J Appl Physiol*. 2009;106:1100–1111.

Guadalupe-Grau A, Rodriguez-Gonzalez FG, Dorado C, Olmedillas H, Fuentes T, Perez-Gomez J, Delgado-Guerra S, Vicente-Rodriguez G, Ara I, Guerra B, Arteaga-Ortiz R, Calbet JA, Diaz-Chico BN. Androgen receptor gene polymorphisms lean mass and performance in young men. *Br J Sports Med*. 2011;45:95–100.

Guadalupe-Grau A, Rodriguez-Gonzalez FG, Ponce-Gonzalez JG, Dorado C, Olmedillas H, Fuentes T, Perez-Gomez J, Sanchis-Moysi J, Diaz-Chico BN, Calbet JA. Bone mass and the CAG and GGN androgen receptor polymorphisms in young men. *PLoS ONE*. 2010b;5:e11529.

Guerra B, Fuentes T, Delgado-Guerra S, Guadalupe-Grau A, Olmedillas H, Santana A, Ponce-Gonzalez JG, Dorado C, Calbet JA. Gender dimorphism in skeletal muscle leptin receptors, serum leptin and insulin sensitivity. *PLoS ONE*. 2008;3:e3466.

Hauner H, Bognar E, Blum A. Body fat distribution and its association with metabolic and hormonal risk factors in women with angiographically assessed coronary artery disease. Evidence for the presence of a metabolic syndrome. *Atherosclerosis*. 1994;105:209–216.

Hickey T, Chandy A, Norman RJ. The androgen receptor CAG repeat polymorphism and X-chromosome inactivation in Australian Caucasian women with infertility related to polycystic ovary syndrome. *J Clin Endocrinol Metab*. 2002;87:161–165.

Jaaskelainen J, Korhonen S, Kuulasmaa T, Kurl S, Niskanen L, Vanhala M, Hippelainen M, Voutilainen R, Heinonen S. Androgen receptor gene CAG repeat length in women with metabolic syndrome. *Gynecol Endocrinol*. 2008;24:411–416.

Kawano H, Sato T, Yamada T, Matsumoto T, Sekine K, Watanabe T, Nakamura T, Fukuda T, Yoshimura K, Yoshizawa T, Aihara K, Yamamoto Y, Nakamichi Y, Metzger D, Chambon P, Nakamura K, Kawaguchi H, Kato S. Suppressive function of androgen

receptor in bone resorption. *Proc Natl Acad Sci U S A*. 2003; 100:9416–9421.

Khaw KT, Barrett-Connor E. Lower endogenous androgens predict central adiposity in men. *Ann Epidemiol*. 1992;2:675–682.

Kim J, Wang Z, Heymsfield SB, Baumgartner RN, Gallagher D. Total-body skeletal muscle mass: estimation by a new dual-energy X-ray absorptiometry method. *Am J Clin Nutr*. 2002;76:378–383.

Kindblom JM, Ohlsson C, Ljunggren O, Karlsson MK, Tivesten A, Smith U, Mellstrom D. Plasma osteocalcin is inversely related to fat mass and plasma glucose in elderly Swedish men. *J Bone Miner Res*. 2009;24:785–791.

Krithivas K, Yurgalevitch SM, Mohr BA, Wilcox CJ, Batter SJ, Brown M, Longcope C, McKinlay JB, Kantoff PW. Evidence that the CAG repeat in the androgen receptor gene is associated with the age-related decline in serum androgen levels in men. *J Endocrinol*. 1999;162:137–142.

Lee DK, Chang C. Endocrine mechanisms of disease: expression and degradation of androgen receptor: mechanism and clinical implication. *J Clin Endocrinol Metab*. 2003;88:4043–4054.

Lietzke MH. Relation between weightlifting totals and body weight. *Science*. 1956;124:486–487.

Lou PH, Yang G, Huang L, Cui Y, Pourbahrami T, Radda GK, Li C, Han W. Reduced body weight and increased energy expenditure in transgenic mice over-expressing soluble leptin receptor. *PLoS ONE*. 2010;5:e11669.

Lyon MF. X-chromosome inactivation. *Curr Biol*. 1999;9:R235–R237.

Mauras N, Hayes V, Welch S, Rini A, Helgeson K, Dokler M, Veldhuis JD, Urban RJ. Testosterone deficiency in young men: marked alterations in whole body protein kinetics, strength, and adiposity. *J Clin Endocrinol Metab*. 1998;83:1886–1892.

Navarro D, Luzardo OP, Fernandez L, Chesa N, Diaz-Chico BN. Transition to androgen-independence in prostate cancer. *J Steroid Biochem Mol Biol*. 2002;81:191–201.

Nielsen TL, Hagen C, Wraae K, Bathum L, Larsen R, Brixen K, Andersen M. 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. *Eur J Endocrinol*. 2010;162:795–804.

Oury F, Sumara G, Sumara O, Ferron M, Chang H, Smith CE, Hermo L, Suarez S, Roth BL, Ducy P, Karsenty G. Endocrine regulation of male fertility by the skeleton. *Cell*. 2011;144:796–809.

Perez-Gomez J, Rodriguez GV, Ara I, Olmedillas H, Chavarren J, Gonzalez-Henriquez JJ, Dorado C, Calbet JA. Role of muscle mass on sprint performance: gender differences? *Eur J Appl Physiol*. 2008;102:685–694.

Phillips GB, Jing T, Heymsfield SB. Relationships in men of sex hormones, insulin, adiposity, and risk factors for myocardial infarction. *Metabolism*. 2003;52:784–790.

Pineiro V, Casabiell X, Peino R, Lage M, Camina JP, Menendez C, Baltar J, Dieguez C, Casanueva F. Dihydrotestosterone, stanozolol, androstenedione and dehydroepiandrosterone sulphate inhibit leptin secretion in female but not in male samples of omental adipose tissue in vitro: lack of effect of testosterone. *J Endocrinol*. 1999;160:425–432.

Pittas AG, Harris SS, Eliades M, Stark P, Dawson-Hughes B. Association between serum osteocalcin and markers of metabolic phenotype. *J Clin Endocrinol Metab*. 2009;94:827–832.

Rodriguez-Gonzalez G, Ramirez-Moreno R, Perez P, Bilbao C, Lopez-Rios L, Diaz-Chico JC, Lara PC, Serra-Majem L, Chirino R, Diaz-Chico BN. The GGN and CAG repeat polymorphisms in the exon-1 of the androgen receptor gene are, respectively, associated with insulin resistance in men and with dyslipidemia in women. *J Steroid Biochem Mol Biol*. 2009;113:202–208.

Rodriguez G, Bilbao C, Ramirez R, Falcon O, Leon L, Chirino R, Falcon O Jr, Diaz BP, Rivero JF, Perucho M, Diaz-Chico BN, Diaz-Chico JC. Alleles with short CAG and GGN repeats in the androgen receptor gene are associated with benign endometrial cancer. *Int J Cancer*. 2006;118:1420–1425.

Rolf C, von Eckardstein S, Koken U, Nieschlag E. Testosterone substitution of hypogonadal men prevents the age-dependent increases in body mass index, body fat and leptin seen in healthy ageing men: results of a cross-sectional study. *Eur J Endocrinol*. 2002;146:505–511.

Ruhayel Y, Lundin K, Giwercman Y, Hallden C, Willen M, Giwercman A. Androgen receptor gene GGN and CAG polymorphisms among severely oligozoospermic and azoospermic Swedish men. *Hum Reprod*. 2004;19:2076–2083.

Sanchís-Moysi J, Idoate F, Olmedillas H, Guadalupe-Grau A, Alayón S, Carreras A, Dorado C, Calbet JAL. The upper extremity of the professional tennis player: muscle volumes, inter-arm asymmetry and muscle fiber type distribution. *Scand J Med Sci Sports*. 2009;20:524–534.

Serrano-Sanchez JA, Delgado-Guerra S, Olmedillas H, Guadalupe-Grau A, Arteaga-Ortiz R, Sanchis-Moysi J, Dorado C, Calbet JA. Adiposity and age explain most of the association between physical activity and fitness in physically active men. *PLoS ONE*. 2010;5:e13435.

Shah NA, Antoine HJ, Pall M, Taylor KD, Azziz R, Goodarzi MO. Association of androgen receptor CAG repeat polymorphism and polycystic ovary syndrome. *J Clin Endocrinol Metab*. 2008;93: 1939–1945.

Stanworth RD, Kapoor D, Channer KS, Jones TH. Androgen receptor CAG repeat polymorphism is associated with serum testosterone levels, obesity and serum leptin in men with type 2 diabetes. *Eur J Endocrinol*. 2008;159:739–746.

Stiger F, Brandstrom H, Gillberg P, Melhus H, Wolk A, Michaelsson K, Kindmark A. Association between repeat length of exon 1 CAG microsatellite in the androgen receptor and bone density in men is modulated by sex hormone levels. *Calcif Tissue Int*. 2008;82: 427–435.

Tsai MY, Shyr CR, Kang HY, Chang YC, Weng PL, Wang SY, Huang KE, Chang C. The reduced trabecular bone mass of adult ARKO male mice results from the decreased osteogenic differentiation of bone marrow stroma cells. *Biochem Biophys Res Commun*. 2011;411:477–482.

Turcato E, Zamboni M, De Pergola G, Armellini F, Zivelonghi A, Bergamo-Andreis IA, Giorgino R, Bosello O. Interrelationships between weight loss, body fat distribution and sex hormones in pre- and postmenopausal obese women. *J Intern Med*. 1997; 241:363–372.

Tut TG, Ghadessy FJ, Trifiro MA, Pinsky L, Yong EL. Long polyglutamine tracts in the androgen receptor are associated with reduced trans-activation, impaired sperm production, and male infertility. *J Clin Endocrinol Metab*. 1997;82:3777–3782.

Van Pottelbergh I, Lumbroso S, Goemaere S, Sultan C, Kaufman JM. Lack of influence of the androgen receptor gene CAG-repeat polymorphism on sex steroid status and bone metabolism in elderly men. *Clin Endocrinol*. 2001;55:659–666.

Wang HY, Hu Y, Wang SH, Shan QL, Li YC, Nie J, Yi LY, Bao DP, Xu CY, He ZH. Association of androgen receptor CAG repeat polymorphism with Vo_2max response to hypoxic training in North China Han men. *Int J Androl*. 2010;33:794–799.

Woodhouse LJ, Gupta N, Bhasin M, Singh AB, Ross R, Phillips J, Bhasin S. Dose-dependent effects of testosterone on regional adipose tissue distribution in healthy young men. *J Clin Endocrinol Metab*. 2004;89:718–726.

Zitzmann M, Gromoll J, von Eckardstein A, Nieschlag E. The CAG repeat polymorphism in the androgen receptor gene modulates body fat mass and serum concentrations of leptin and insulin in men. *Diabetologia*. 2003;46:31–39.