Journal of Steroid Biochemistry & Molecular Biology 113 (2009) 202–208

Contents lists available at ScienceDirect

Journal of Steroid Biochemistry and Molecular Biology

journal homepage: www.elsevier.com/locate/jsbmb

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

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ARTICLE INFO

Article history:
Received 27 July 2008
Received in revised form
18 November 2008
Accepted 22 December 2008

Keywords:
Low density lipoprotein-cholesterol
Androgen receptor
CAG repeat polymorphism
GGN repeat polymorphism
Insulin
Insulin resistance

ABSTRACT

The human androgen receptor (AR) gene possesses two trinucleotide repeats of CAG and GGN in exon-1. The GGN repeat affects the amount of AR protein translated, while the CAG repeat affects the efficiency of AR transcriptionaly. In this study, we have genotyped these polymorphic tracts in a representative sample of 557 Caucasian adult individuals (314 women and 243 men) from the Canary Islands, Spain (the ENCA Study), and investigated their association with fasting serum levels of lipids, glucose and insulin. The number of CAG repeats in women (expressed as the average length of the two alleles) was inversely correlated with serum levels of LDL-cholesterol (Spearman rho = −0.179; $P < 0.01$). Women with an average number of CAG repeats in the upper tertile showed significantly lower levels of LDL-cholesterol than those grouped in the lower and middle tertile, after adjusting for age, body mass index, waist-to-hip ratio, smoking and alcohol drinking. The number of GGN repeats in men was correlated with fasting insulin levels (Spearman rho = −0.206; $P < 0.01$), the homeostasis model assessment of insulin resistance (HOMA-IR; Spearman rho = −0.230; $P < 0.01$) and the McAuley index of insulin sensitivity (Spearman rho = 0.194; $P < 0.01$). Men with a number of GGN repeats in the upper tertile showed lower levels of insulin and HOMA and a higher level of the McAuley index than those grouped in the lower and middle tertile, after adjusting for the variables listed above. These results support the hypothesis that the longer alleles of the CAG and GGN polymorphisms in the exon-1 of the AR gene, indicative of lower androgenic signaling, respectively protect women from developing dyslipemia and men from developing insulin resistance.

1. Introduction

The genomic effects of androgens are exerted via the androgen receptor (AR). The AR gene is located on the X-chromosome (q11.2-q12), and contains eight exons [1]. The exon-1 contains a CAG repeat tract (encoding for polyglutamine) and a GGN repeat tract (encoding for polyglycine) of polymorphic length, close to the region encoding the transactivation-1 domain of the AR protein [2]. Short CAG repeat tracts enhance the AR transcriptional activity [3–9], while short GGN tracts increase the amount of AR protein available inside the cell [10].

In healthy males, the efficiency of some reproductive functions seems to be inversely related to the length of the CAG tract [8,11–14]. In addition, the length of the CAG tract has been found to be inversely associated with the incidence of prostate cancer [15,16]. In women with breast cancer, short CAG tracts in the AR gene have been associated with a higher frequency of more aggressive tumors and positive lymph node involvement [17,18].

The possible role of the CAG repeat length polymorphism on metabolic parameters related to cardiovascular diseases has also been investigated. Shorter CAG repeats of the AR gene, indicative of stronger androgenic activity, have been associated with lower serum levels of high density lipoprotein cholesterol (HDL-c) [19,20] and a higher risk of heart disease in men [21]. There is also increasing evidence linking the excess of androgen and the development of insulin resistance. In men, free testosterone has been found to be correlated with insulin resistance, as measured by the homeostatic model of assessment of insulin resistance (HOMA-IR), an effect that seems to be modulated by the AR CAG length [22]. There

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0960-0760/$ – see front matter © 2009 Published by Elsevier Ltd.
doi:10.1016/j.jsbmb.2008.12.009

are no reports on the possible effects of the CAG repeat in metabolic parameters in women. A clear association of AR polymorphisms with the risk of developing cardiovascular diseases or IR in the general population is still controversial.

The polyglycine tract in the AR protein is encoded by an invariant six-glycine tract (GGT/GGG) followed by a polymorphic GGC repeat (CGT)3 GGG(GGT)2(GGC)*n*). Most authors refer to the polyglycine tract as being encoded by a GGN repeat, which is equivalent to the GGC repeat plus six triplets [18]. To our knowledge, there are no reports on the possible metabolic role of the GGN repeat either in men or women.

Specific, AR-mediated effects of androgen in women are sometimes difficult to assess because of the fact that they are capable of aromatizing into estrogens and also due to further genomic action mediated by the estrogen receptor (ER). It is generally accepted that people carrying short CAG and/or GGN tracts are subjected to stronger androgenic signaling [18]. Thus, studies on CAG and GGN polymorphisms in the general population can help to better define subgroups that are at an increased risk of developing syndromes attributable to androgenic stimulus. In addition, these types of studies can also help to better define the true physiological and pathological effects of androgen in women.

In an attempt to gain an insight into the influence of these polymorphisms on the androgen relationship to metabolic parameters in the general population, we have studied their distribution in a relatively wide sample of adult Caucasian individuals, representative of the population of the Canary Islands, Spain.

2. Materials and methods

2.1. Experimental subjects

The Canary Islands are situated in the Atlantic Ocean, near the North-Western Sahara coast. They have belonged to Spain since the fifteenth century and their population, at present, is over 96% Caucasian. Between 1997 and 1998 a nutritional survey (Encuesta Nutricional de Canarias, ENCA) was performed on a representative sample of 1747 individuals aged 5–76 years, randomly selected from the census lists of 32 municipalities within the islands. The study was approved by the ethical committee of the Canarian Public Health Service. After giving informed consent, participants filled out a survey questionnaire with information regarding their dietetic habits, socio-demographic information as well as their personal and familial history of diabetes, hypertension, cancer and cardiovascular, hepatic and kidney disease. Current use of drugs, smoking and alcohol consumption was also recorded. Subsequently, all participants underwent a physical examination where height, weight, waist, hip perimeters and blood pressure were all measured. The survey was conducted by trained professionals (dieticians, nurses and physicians). Out of the 1747 individuals enrolled in the study, 782 underwent blood extractions after an overnight fast for laboratory analyses. Of these, we first excluded those individuals who could not provide any blood samples for DNA isolation procedures ($n = 58$); then, we excluded anyone younger than 18 years of age ($n = 167$). Thus, our study population was comprised of 557 individuals (243 men and 314 women) aged 18–76 years. Diabetes was defined as fasting plasma glucose $\geq$7.0 mmol/l and/or current use of insulin or oral hypoglucemic agents [23]

2.2. Biochemical analyses

All biochemical analyses were performed in the laboratory of biochemistry of the Hospital Universitario Insular de Gran Canaria, using proper quality controls to asses a variation coefficient inter and intra assays (data not shown).

Blood samples were drawn from participants after an overnight fast. Levels of serum glucose, total cholesterol, triglycerides and HDL-c levels were determined by spectrophotometric methods, using a Dimension RxL autoanalyzer (Dade-Behring, Liederbach, Germany). Serum levels with low density lipoprotein-cholesterol (LDL-c) were calculated using the Friedewald formula [24]. Serum insulin levels were determined by radioimmunoanalysis (Diagnostic Products Corporation, Los Angeles CA). Insulin sensitivity was estimated according to either the homeostasis model assessment of insulin resistance (fasting insulin (μU/ml) $\times$ fasting plasma glucose (mmol/l)/22.5)) or the formula proposed by McAuley ($\exp(2.63 - 0.28) \times \ln(\text{fasting insulin}) - 0.31 \times \ln(\text{fasting triglyceride (mmol/l)})$) [25].

Serum concentrations of IGF-I and IGFBP-3 were determined in duplicate by the use of enzyme immunoassays (Diagnostic Systems Laboratories, Webster, TX). The molar weight of IGFBP-3 is 42 kDa and that of IGF-I is 7.7 kDa. Thus, to get the molar ratio we calculated the molar concentrations of IGFBP-3 (μg/l/42 = nmol/l) and the molar concentrations of IGF-I (μg/l/7.7 = nmol/l).

2.3. Determination of the number of CAG and GGN repeats in the AR gene

DNA was isolated from EDTA blood samples using a DNA Isolation Kit (Puregene, Gentra Systems, Minneapolis, USA). To determine the length of the CAG and GGN repeats we amplified the corresponding regions located on the exon-1 of the AR gene (GenBank accession no. M27423) using two pairs of primers whose sequence has been previously reported [26,27]. One primer from each pair was marked with fluorescent dye (FAM or VIC). Amplification was performed in a 25 μl reaction volume, containing 50 ng of genomic DNA, 200 μM of each deoxynucleotide triphosphate, 1 $\times$ Fast Start Taq DNA polymerase Buffer (Roche Applied Science, Mannheim, Germany), 1 $\times$ 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. PCR conditions were: 30 cycles of 95 °C for 45 s, 56 °C for 30 s and 72 °C for 30 s for CAG amplification; 30 cycles of 95 °C for 1 min, 55 °C for 2 min and 72 °C for 2 min for GGN amplification. Each PCR was initiated with a denaturation step at 95 °C for 5 min and terminated with an extension step at 72 °C for 5 min. 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, Warrington, UK), denatured at 98 °C for 5 min 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). We blindly repeated the genotype analysis in 54 of the samples and the results were completely coincident. The size of the fragments was confirmed by sequencing 48 DNA samples harbouring different size alleles for both repeats using the Big Dye Terminator Sequencing Kit (Applied Biosystem). After analyzing the sequencing results we observed that the capillary electrophoresis-based ABI PRISM 3100 Genetic Analyzer gave anomalous results when internal standards supplied by the manufacturer were used. Thus, the actual length of the CAG and GGN repeats was underestimated by 4 and 2 repeat units, respectively. Therefore, and according to other authors [28,29], the allele size estimation in the capillary electrophoresis system was corrected by using a ladder which was created with the actual size obtained after sequencing.

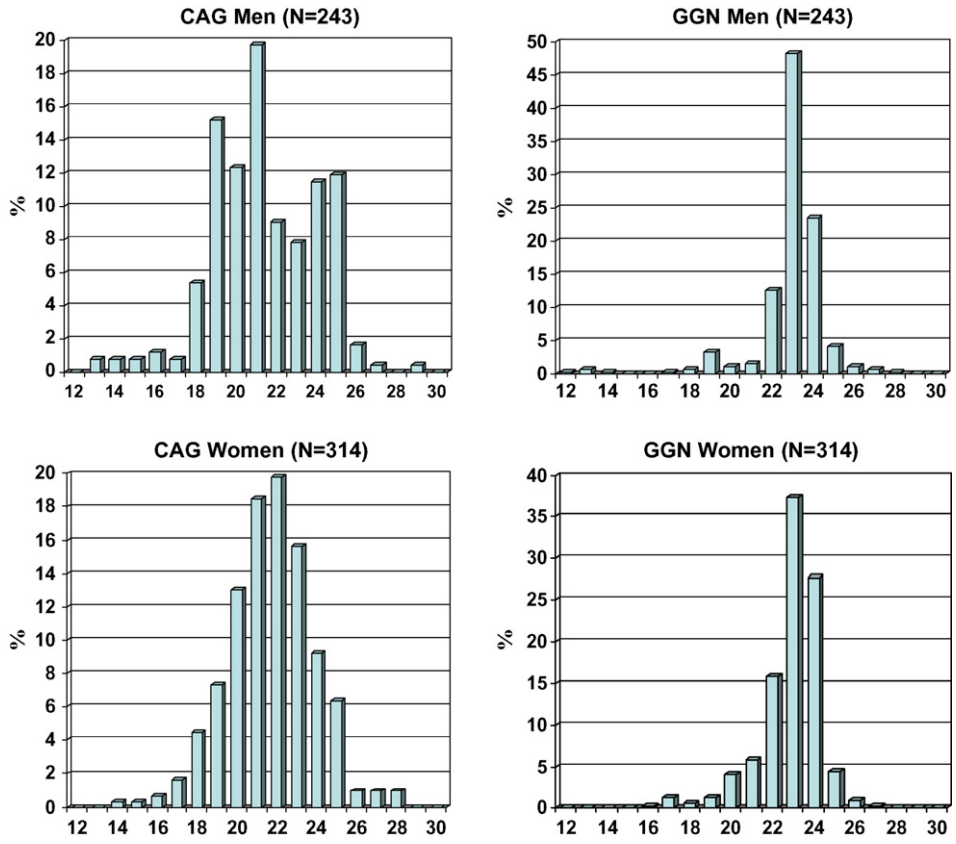

Fig. 1. Frequency distribution of the CAG and GGN repeats in the whole series. The number of CAG and GGN repeats in men and women were not normally distributed. The median number of CAG repeats was 21 in men and 21.5 women. The GGN repeats showed a median value of 23 in both sexes.

2.4. Statistics

Statistical analyses were performed with SPSS for WINDOWS, version 13 (SPSS Inc., Chicago). Before further analyses, the normality of distribution of variables was checked with the Kolmogorov–Smirnov test. A logarithmic transformation was performed for variables that were not normally distributed. Since both CAG and GGN repeats were not normally distributed, the Spearman correlation coefficient (rho) was used to test the strength of the association between the number of these repeats and diverse continuous variables. Differences between data subsets were analyzed using either analysis of variance or analysis of covariance, both with the Bonferroni post hoc test. All tests were considered significant at the 0.05 level. Levels of statistical significance are shown in the figures as asterisks (*: $P < 0.05$; **: $P < 0.01$).

3. Results

Since the AR gene is located on the X-chromosome, men display a unique allele of the gene, whereas women carry two alleles, one of them being randomly silenced by methylation in each individual cell. Thus, the number of CAG or GGN repeats in women is expressed as the average number of repeats found in the two alleles.

Considered individually or as their average, the number of CAG and GGN repeats in men and women was not normally distributed (Kolmogorov–Smirnov test). The number of CAG and GGN repeats in men and women (Fig. 1). The median number of CAG repeats was 21 (range: 13–29) in men and 21.5 (range: 14–28) in women. The GGN repeats showed a median value of 23 in both sexes (range: 12–28 in men and 16–27 in women).

Data from anthropometric, demographic, and biochemical variables in the whole population and within each sex group are given in Table 1. Men had significantly higher plasma levels of glucose and triglycerides than women, and greater waist-to-hip and IGF-I-to-IGFBP-3 ratios. In contrast, men exhibited significantly lower levels of HDL-c, IGFBP-3, and the McAuley index than women.

Spearman correlation coefficients (rho) between the number of CAG or GGN repeats and the rest of the variables analyzed are given in Table 2. In the whole series, the number of CAG repeats did not significantly correlate with the rest of the variables analyzed. However, the average number of CAG repeats in women was significantly and negatively correlated with plasma levels of LDL-c (rho = −0.179;

Table 1
Descriptors of the study population.

Variable	Men (243)	Women (314)	*P*
Age (years)	46.9 ± 15.3	44.8 ± 15.0	0.114
BMI (kg/m^2)	26.6 ± 4.6	26.6 ± 5.7	0.982
Waist/hip ratio	0.95 ± 0.10	0.82 ± 0.09	<0.001
[a]LDL-c (mmol/l)	3.61 ± 1.22	3.51 ± 1.15	0.319
[a]HDL-c (mmol/l)	1.25 ± 0.40	1.46 ± 0.47	<0.001
[a]Triglycerides (mmol/l)	1.42 ± 0.76	1.28 ± 0.68	0.012
[b]Glucose (mmol/l)	4.71 ± 0.77	4.56 ± 0.76	0.033
[b]Insulin (mU/l)	11.9 ± 6.5	11.8 ± 6.8	0.457
[b]HOMA	2.55 ± 1.63	2.45 ± 1.65	0.220
[b]McAuley index	6.80 ± 1.56	7.11 ± 1.66	0.031
IGF-I (μg/l)	174.7 ± 69.9	168.2 ± 62.5	0.412
IGFBP-3 (μg/l)	3929 ± 972	4184 ± 882	0.001
IGF/BP-3	25.0 ± 10.0	22.3 ± 7.9	0.003

The *P* values indicate the differences observed between men and women, which were computed using either the Student's *t*-test for continuous variables or the Chi-square test for categorical variables.

[a] Excluding 35 participants who were receiving hypolipidemic medication (14 men and 21 women).

[b] Excluding 45 participants considered diabetic (25 men and 20 women).

Table 2
Spearman correlation coefficients between the number of CAG and GGN repeats and diverse anthropometric and biochemical variables.

Variable	CAG repeats		GGN repeats	
	Men	Women	Men	Women
Age (years)	−0.062	0.077	−0.055	−0.002
BMI (kg/m^2)	0.027	0.029	−0.008	−0.039
Waist/hip ratio	0.004	0.026	0.033	−0.059
[a]LDL-c (mmol/l)	0.004	−0.179**	−0.030	0.084
[a]HDL-c (mmol/l)	−0.083	0.007	0.124	0.089
[a]Triglycerides (mmol/l)	−0.020	−0.058	−0.075	0.067
[b]Glucose (mmol/l)	−0.072	−0.056	−0.083	−0.004
[b]Insulin (mU/l)	0.110	0.044	−0.206**	−0.043
[b]HOMA	0.076	0.009	−0.230**	−0.024
[b]McAuley index	−0.078	−0.005	0.194**	−0.032
IGF-I (μg/l)	0.103	−0.171**	0.036	−0.048
IGFBP-3 (μg/l)	0.005	−0.051	0.070	−0.095
IGF/BP-3	0.055	−0.178**	0.002	0.007

** $P < 0.01$.
[a] Excluding 35 participants who were receiving hypolipidemic medication (14 men and 21 women).
[b] Excluding 45 participants considered diabetic (25 men and 20 women).

$P = 0.002$), IGF-I (rho = −0.171; $P = 0.003$), and the IGF-I/BP3 molar ratio (rho = −0.178; $P = 0.002$).

Table 3 summarizes the effect of the average number of CAG repeats in women (grouped in tertiles) on mean serum levels of LDL-c, IGF-I and on the IGF/BP-3 molar ratio, the three variables that were significantly correlated with the polymorphism, expressed either as unadjusted values, or after their adjustment for several confounders, such as age, BMI, waist-to-hip ratio, smoking and alcohol drinking. This tendency was observed both in premenopausal and postmenopausal women. Thus, women grouped in the upper tertile showed significantly lower levels of both LDL-c, IGF-I and IGF/BP-3 molar ratio when the values were analyzed without adjustments. However, after adjusting for the variables listed above, only the difference in the levels of LDL-c between groups remained significant.

The number of GGN repeats in the whole series (Table 2) was only weakly correlated with insulin (rho = −0.104; $P = 0.020$) and HOMA (rho = −0.098; $P = 0.029$). However, these correlations, and also the GGN correlation with the McAuley index substantially improved when only men were included in the analysis (insulin: rho = −0.206, $P = 0.02$; HOMA: rho = −0.230, $P = 0.001$; McAuley index: rho = 0.194, $P = 0.004$).

Table 4 summarizes the effect of the average number of GGN repeats in men (excluding those considered diabetic), grouped in tertiles, (GGN < 23; GGN = 23, or GGN > 23) on mean levels of insulin, HOMA and the index proposed by McAuley, the three variables that were significantly correlated with the polymorphism, expressed either as unadjusted values, or after adjustment for the same confounders stated above. In both cases, men grouped in the upper tertile showed significantly lower values of insulin and HOMA and higher values of the McAuley index than those grouped in the other tertiles.

4. Discussion

This study was carried out in 557 individuals recruited for the ENCA study, a sample considered to be representative of the general population of the Canary Islands, Spain [30–33]. The frequency distribution of the CAG alleles found in this study is similar to other Caucasian populations [18]. The GGN frequencies are also similar to those found in other Caucasian populations which have been studied. The slight differences may be attributable to variations in sample size.

An original result derived from this study has been the clear influence of the CAG repeat polymorphism of the AR gene on LDL-c level in women. Plasma levels of LDL-c are regulated in part by the clearance of LDL by the hepatic LDL receptor (LDLR), a cell surface receptor that binds and internalizes LDL, thus reducing plasma LDL-c [34].

Table 3
The association between the average number of CAG repeats grouped in tertiles, serum levels of LDL-c, IGF-I and the IGF-I/BP-3 ratio in women.

Variable	Average number of CAG repeats	Number of participants	Unadjusted values mean (95% CI)	*P*	Adjusted values mean (95% CI)	*P*
[a]LDL-c (mmol/l)	≤20	81	3.64 (3.45–3.89)		3.70 (3.35–4.04)	
	20.5–22	113	3.72 (3.43–3.86)		3.99 (3.73–4.25)	
	>22	97	3.22 (2.98–3.46)	0.013	2.96 (2.59–3.33)	<0.001
IGF-I (μg/l)	≤20	87	172.8 (163.3–190.3)		172.2 (155.1–189.3)	
	20.5–22	120	173.7 (162.2–185.2)		173.2 (159.7–186.7)	
	>22	107	155.2 (142.7–167.7)	0.033	162.8 (144.1–181.5)	0.654
IGF/BP-3	≤20	87	23.65 (21.72–25.59)		23.17 (20.84–25.51)	
	20.5–22	120	23.17 (21.64–24.70)		22.63 (20.80–24.47)	
	>22	107	20.52 (19.09–21.96)	0.015	20.64 (18.10–23.18)	0.309

[a] Excluding women receiving hypolipidemic medication ($n = 21$). Adjustments were made for age, BMI, waist-to-hip ratio, menopausal status, smoking and alcohol drinking.

Table 4
The association between the number of GGN repeats, serum levels of insulin, HOMA and the McAuley indexes in men considered non diabetic ($n = 218$).

Variable	GGN repeats	*N*	Unadjusted values		Adjusted values	
			Mean (95% CI)	*P*	Mean (95% CI)	*P*
Insulin (mU/l)	<23	47	11.50 (10.13–13.04)		11.72 (10.39–13.21)	
	=23	106	10.88 (9.95–11.89)		10.74 (9.92–11.63)	
	>23	65	9.22 (8.34–10.20)	0.016	9.37 (8.41–10.43)	0.021
HOMA	<23	47	2.37 (2.03–2.76)		2.40 (2.09–2.77)	
	=23	106	2.32 (2.10–2.56)		2.28 (2.08–2.50)	
	>23	65	1.81 (1.61–2.03)	0.007	1.87 (1.64–2.12)	0.014
McAuley index	<23	47	6.55 (6.06–7.03)		6.43 (6.02–6.84)	
	=23	106	6.64 (6.35–6.93)		6.68 (6.41–6.96)	
	>23	65	7.35 (6.95–7.75)	0.004	7.34 (6.97–7.71)	0.003

Adjustments were made for age, BMI, waist-to-hip ratio, smoking and alcohol drinking.

The LDLR is induced by estrogens, whereas androgens counteract that effect of estrogens [34,35]. Given that shorter CAG tracts lead to an increase of transcriptional AR activity [1], it is conceivable that they also increase the suppressor effect of androgens on the estrogen-induced LDLR. This might explain the association of shorter AR CAG tracts with the higher level of LDL-c found in women here in this study. Neither our study nor that of Zitzmann et al. [19] found any relationship between the AR CAG tract length and LDL-c in men. This suggests that the estrogenic effect on LDLR in men might not be strong enough to be significantly modulated by the CAG polymorphism of the AR gene.

The shorter CAG repeat of the AR gene has been found to be associated with more severe cardiovascular disease and reduced endothelial response to ischemia in men [21]. The results here reported suggest that shorter CAG tracts might also be harmful for arteriosclerosis in women, since they increase the LDL-c.

Another original result derived from this study has been the strong relationship between the GGN polymorphism of the AR gene and insulin resistance in men. Type 2 diabetes is more prevalent in men than in women [36], which suggests that the differences could reside in endogenous sex hormones. However, the causal relationship of androgen to insulin resistance is still controversial [37].

Castrated male rats exposed to supraphysiological doses of testosterone have increased insulin resistance, whereas physiological testosterone replacement improves insulin [38]. In female rats, moderate increases of testosterone concentration are followed by a marked decrease in insulin sensitivity [39–41].

Two large studies in healthy men have shown an inverse correlation between testosterone and insulin levels [41–43]. Clinical studies have reported an association of insulin resistance with low serum testosterone concentrations in men, suggesting that this could be a predictor of type 2 diabetes [44–47]. Conversely, normal puberty is associated with an increase in insulin resistance in men [48] and testosterone replacement therapies seem to reduce insulin resistance and visceral adiposity in type 2 diabetic men [36,49,50]. Therefore, there are some data linking androgen levels and insulinemia in men, but many aspects are yet to be elucidated.

The negative association described here between the GGN polymorphism of the AR gene and insulin resistance indicates that the higher androgenic signaling due to shorter GGN repeats is associated with markers of insulin resistance (i.e., higher insulin concentration, higher HOMA and lower McAuley indexes). Unfortunately, it was not possible to obtain data regarding serum testosterone levels from the ENCA study. However, our results suggest that GGN polymorphisms can be considered as modulators of testosterone capability to carry out actions affecting the complex mechanism leading to insulin resistance.

Large number of observations shows, a mutual relationship between androgens, body fat distribution, and insulin sensitivity and also involved in the regulation of HDL-c and triglyceride metabolism [51,52].

Morbidly obese and insulin resistant men frequently have low serum levels of testosterone that increase upon weight loss [53]. Estradiol levels show the opposite changes to testosterone with obesity and weight loss. It has been suggest that obesity cause hypostestosteronemia by increased aromatization of testosterone to estradiol in the adipose tissue. On the other hand, hypogonadal men are frequently obese with increased levels of leptin and insulin [54]. Substitution of testosterone in hypogonodal men decreased HDL-c levels.

Treatment with supraphysiological doses of testosterone in normal men decreased HDL-c by about 20%. Conversely, biochemical suppression of endogenous testosterone increase HDL-c [55,56]. Exogenous testosterone administration only produced a fall in HDL-c in the presence of aromatase inhibitors. These observations and the finding of low HDL-c in men with aromatase deficiency or strogen resistancy suggest that physiological tissue level of estradiol play a role in maintaining the physiological levels of HDL-c in men.

In women, mutual interrelationships have also been observed between testosterone, adipose tissue, and insulin sensitivity. On one hand, insulin sensitivity contributes to the pathogenesis of hyperandrogenemia in polycystic ovary syndrome (PCOS). Insulin stimulates androgen ovary synthesis via its cognate receptor [57]. Since the ovaries remain sensitive to insulin, while other tissues as fat and muscle are resistant [58]. In support of this, treatment of insulin resistance in woman with PCOS, significantly decreased serum insulin levels as well as testosterone [59]. Concomitantly, plasma levels of HDL-c increased. These data imply that hyperinsulinemia contributes to the ovarian hyperandrogenism in PCOS. Vice versa, lowering androgen levels using GnRH agonist and AR blockade in hyperandrogenic women were also found to improve insulin sensitivity and lipid profile [60–62].

As we explained above, large CAG repeat tracts decrease the AR transcriptional activity while large GGN tracts decrease the amount of AR protein available inside the cell, both contributes to fall the androgen gene expression trough AR pathway.

Taken into account the physiological testosterone effects in the regulation of cholesterol, and insulin resistance, either in men or women, we can conclude that: longer CAG alleles in the exon-1 of the AR gene lead to a lower LDL-c level in women, whereas longer GGN alleles lead to lower insulin resistance in men. These results support the hypothesis that AR gene polymorphisms that produce lower androgenic signaling protect women from developing dyslipemia, and men from developing insulin resistance.

Acknowledgements

This work was supported by grants from the Carlos III Health Institute (Instituto de Salud Carlos III) (RTICCC C03/10), Fundación Canaria de Investigación y Salud (FUNCIS), Cabildo Insular de Tenerife and the Instituto Canario de Investigación del Cáncer (ICIC). Authors gratefully acknowledge the editing work of Ms Emer M. Pigott.

Fellowships: Germán Rodríguez-González is a recipient of a fellowship from the University of Las Palmas de Gran Canaria; Raquel Ramírez-Moreno is a recipient of a fellowship from the Canary Island Government and Cristina Bilbao is a recipient of fellowships from the Canarian Cancer Research Institute (Instituto Canario de Investigación del Cancer); Laura López-Ríos is a recipient of a fellowship from the Interisland Council of Gran Canaria (Cabildo Insular de Gran Canaria). We also gratefully acknowledge the support of the island councils (Cabildo de Tenerife and Cabildo de Gran Canaria).

References

[1] G. Rodriguez, C. Bilbao, R. Ramirez, O. Falcon, L. Leon, R. Chirino, O. Falcon Jr., B.P. Diaz, J.F. Rivero, M. Perucho, B.N. Diaz-Chico, J.C. Diaz-Chico, Alleles with short CAG and GGN repeats in the androgen receptor gene are associated with benign endometrial cancer, Int. J. Cancer 118 (6) (2006) 1420–1425.

[2] G. Jenster, H.A. van der Korput, J. Trapman, A.O. Brinkmann, Identification of two transcription activation units in the N-terminal domain of the human androgen receptor, J. Biol. Chem. 270 (13) (1995) 7341–7346.

[3] D. Ding, L. Xu, M. Menon, G.P. Reddy, E.R. Barrack, Effect of a short CAG (glutamine) repeat on human androgen receptor function, Prostate 58 (1) (2004) 23–32.

[4] Q. Wang, T.S. Udayakumar, T.S. Vasaitis, A.M. Brodie, J.D. Fondell, Mechanistic relationship between androgen receptor polyglutamine tract truncation and androgen-dependent transcriptional hyperactivity in prostate cancer cells, J. Biol. Chem. 279 (17) (2004) 17319–17328.

[5] S.M. Edwards, M.D. Badzioch, R. Minter, R. Hamoudi, N. Collins, A. Ardern-Jones, A. Dowe, S. Osborne, J. Kelly, R. Shearer, D.F. Easton, G.F. Saunders, D.P. Dearnaley, R.A. Eeles, Androgen receptor polymorphisms: association with prostate cancer risk, relapse and overall survival, Int. J. Cancer 84 (5) (1999) 458–465.

[6] N.L. Chamberlain, E.D. Driver, R.L. Miesfeld, The length and location of CAG trinucleotide repeats in the androgen receptor N-terminal domain affect transactivation function, Nucl. Acids Res. 22 (15) (1994) 3181–3186.

[7] M.P. Schoenberg, J.M. Hakimi, S. Wang, G.S. Bova, J.I. Epstein, K.H. Fischbeck, W.B. Isaacs, P.C. Walsh, E.R. Barrack, Microsatellite mutation (CAG24 → 18) in the androgen receptor gene in human prostate cancer, Biochem. Biophys. Res. Commun. 198 (1) (1994) 74–80.
[8] T.G. Tut, F.J. Ghadessy, M.A. Trifiro, L. Pinsky, E.L. Yong, Long polyglutamine tracts in the androgen receptor are associated with reduced trans-activation, impaired sperm production, and male infertility, J. Clin. Endocrinol. Metab. 82 (11) (1997) 3777–3782.
[9] J. Beilin, E.M. Ball, J.M. Favaloro, J.D. Zajac, Effect of the androgen receptor CAG repeat polymorphism on transcriptional activity: specificity in prostate and non-prostate cell lines, J. Mol. Endocrinol. 25 (1) (2000) 85–96.
[10] D. Ding, L. Xu, M. Menon, G.P. Reddy, E.R. Barrack, Effect of GGC (glycine) repeat length polymorphism in the human androgen receptor on androgen action, Prostate 62 (2) (2005) 133–139.
[11] A. von Eckardstein, G. Castro, I. Wybranska, N. Theret, P. Duchateau, N. Duverger, J.C. Fruchart, G. Ailhaud, G. Assmann, Interaction of reconstituted high density lipoprotein discs containing human apolipoprotein A-I (ApoA-I) variants with murine adipocytes and macrophages. Evidence for reduced cholesterol efflux promotion by apoA-I (Pro165 → Arg), J. Biol. Chem. 268 (4) (1993) 2616–2622.
[12] A.T. Dowsing, E.L. Yong, M. Clark, R.I. McLachlan, D.M. de Kretser, A.O. Trounson, Linkage between male infertility and trinucleotide repeat expansion in the androgen-receptor gene, Lancet 354 (9179) (1999) 640–643.
[13] A. Mifsud, C.K. Sim, H. Boettger-Tong, S. Moreira, D.J. Lamb, L.I. Lipshultz, E.L. Yong, Trinucleotide (CAG) repeat polymorphisms in the androgen receptor gene: molecular markers of risk for male infertility, Fertil. Steril. 75 (2) (2001) 275–281.
[14] A. Kukuvitis, I. Georgiou, I. Bouba, A. Tsirka, C.H. Giannouli, C. Yapijakis, B. Tarlatzis, J. Bontis, D. Lolis, N. Papadimas, J. Sofikitis, Association of oestrogen receptor alpha polymorphisms and androgen receptor CAG trinucleotide repeats with male infertility: a study in 109 Greek infertile men, Int. J. Androl. 25 (3) (2002) 149–152.
[15] J.M. Hakimi, M.P. Schoenberg, R.H. Rondinelli, S. Piantadosi, E.R. Barrack, Androgen receptor variants with short glutamine or glycine repeats may identify unique subpopulations of men with prostate cancer, Clin. Cancer Res. 3 (9) (1997) 1599–1608.
[16] D. Navarro, O.P. Luzardo, L. Fernandez, N. Chesa, B.N. Diaz-Chico, Transition to androgen-independence in prostate cancer, J. Steroid Biochem. Mol. Biol. 81 (3) (2002) 191–201.
[17] H. Yu, B. Bharaj, E.J. Vassilikos, M. Giai, E.P. Diamandis, Shorter CAG repeat length in the androgen receptor gene is associated with more aggressive forms of breast cancer, Breast Cancer Res. Treat. 59 (2) (2000) 153–161.
[18] B. Nicolas Diaz-Chico, F. German Rodriguez, A. Gonzalez, R. Ramirez, C. Bilbao, A. Cabrera de Leon, A. Aguirre Jaime, R. Chirino, D. Navarro, J.C. Diaz-Chico, Androgens and androgen receptors in breast cancer, J. Steroid Biochem. Mol. Biol. (2007).
[19] M. Zitzmann, M. Brune, B. Kornmann, J. Gromoll, S. von Eckardstein, A. von Eckardstein, E. Nieschlag, The CAG repeat polymorphism in the AR gene affects high density lipoprotein cholesterol and arterial vasoreactivity, J. Clin. Endocrinol. Metab. 86 (10) (2001) 4867–4873.
[20] M. Zitzmann, E. Nieschlag, Androgens and vascular function, J. Endocrinol. Invest. 26 (8) (2003) 767–769.
[21] M. Alevizaki, A.T. Cimponeriu, M. Garofallaki, H.L. Sarika, C.C. Alevizaki, C. Papamichael, G. Philippou, E.A. Anastasiou, J.P. Lekakis, M. Mavrikakis, The androgen receptor gene CAG polymorphism is associated with the severity of coronary artery disease in men, Clin. Endocrinol. (Oxf.) 59 (6) (2003) 749–755.
[22] M. Mohlig, A. Jurgens, J. Spranger, K. Hoffmann, M.O. Weickert, H.W. Schlosser, T. Schill, G. Brabant, A. Schuring, A.F. Pfeiffer, J. Gromoll, C. Schofl, The androgen receptor CAG repeat modifies the impact of testosterone on insulin resistance in women with polycystic ovary syndrome, Eur. J. Endocrinol. 155 (1) (2006) 127–130.
[23] American Diabetes Association: clinical practice recommendations. Diabetes Care, 23 (Suppl. 1) (2000) S1–116.
[24] W.T. Friedewald, R.I. Levy, D.S. Fredrickson, Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge, Clin. Chem. 18 (6) (1972) 499–502.
[25] K.A. McAuley, S.M. Williams, J.I. Mann, R.J. Walker, N.J. Lewis-Barned, L.A. Temple, A.W. Duncan, Diagnosing insulin resistance in the general population, Diab. Care 24 (3) (2001) 460–464.
[26] H.F. Sleddens, B.A. Oostra, A.O. Brinkmann, J. Trapman, Trinucleotide (GGN) repeat polymorphism in the human androgen receptor (AR) gene, Hum. Mol. Genet. 2 (4) (1993) 493.
[27] B.S. Bharaj, E.J. Vassilikos, E.P. Diamandis, Rapid and accurate determination of (CAG)*n* repeats in the androgen receptor gene using polymerase chain reaction and automated fragment analysis, Clin. Biochem. 32 (5) (1999) 327–332.
[28] D.W. Boorman, Y. Guo, K. Visvanathan, K. Helzlsouer, T.G. O'Brien, Automated fragment analysis method for determining androgen receptor CAG repeat length, Biotechniques 33 (1) (2002) 140–143.
[29] F. Delmotte, N. Simon, J.C. Leterme, Microsatellite allele sizing: difference between automated capillary electrophoresis and manual technique, Biotechniques 31 (4) (2001) 810, 814–816, 818.
[30] P. Henriquez Sanchez, C. Diaz Romero, E. Rodriguez Rodriguez, F. Lopez Blanco, E. Alvarez Leon, J. Diaz Cremades, M.C. Pastor Ferrer, L. Serra Majem, Biochemical assessment of nutritional status in the Canary Islands population (1998), Arch. Latinoam Nutr. 50 (1 Suppl. 1) (2000) 43–54.
[31] L. Serra Majem, A. Armas Navarro, L. Ribas Barba, Food consumption and food sources of energy and nutrients in Canary Islands (1997–98), Arch. Latinoam Nutr. 50 (1 Suppl. 1) (2000) 23–33.
[32] L. Serra Majem, A. Cabrera Leon, A. Sierra Lopez, Conclusions of the Canary Islands Nutrition Survey (1997–98). Foundations for a nutrition policy in Canary Islands, Arch. Latinoam Nutr. 50 (1 Suppl. 1) (2000) 62–70.
[33] M.C. Navarro Rodriguez, P. Lainez Sevillano, L. Ribas Barba, L. Serra Majem, Anthropometric values and cardiovascular risk factors in Canary Islands (1997–98), Arch. Latinoam Nutr. 50 (1 Suppl. 1) (2000) 34–42.
[34] M.S. Brown, J.L. Goldstein, A receptor-mediated pathway for cholesterol homeostasis, Science 232 (4746) (1986) 34–47.
[35] G.E. Croston, L.B. Milan, K.B. Marschke, M. Reichman, M.R. Briggs, Androgen receptor-mediated antagonism of estrogen-dependent low density lipoprotein receptor transcription in cultured hepatocytes, Endocrinology 138 (9) (1997) 3779–3786.
[36] D. Kapoor, S. Clarke, R. Stanworth, K.S. Channer, T.H. Jones, The effect of testosterone replacement therapy on adipocytokines and C-reactive protein in hypogonadal men with type 2 diabetes, Eur. J. Endocrinol. 156 (5) (2007) 595–602.
[37] D. Kapoor, C.J. Malkin, K.S. Channer, T.H. Jones, Androgens, insulin resistance and vascular disease in men, Clin. Endocrinol. (Oxf.) 63 (3) (2005) 239–250.
[38] A. Holmang, P. Bjorntorp, The effects of testosterone on insulin sensitivity in male rats, Acta Physiol. Scand. 146 (4) (1992) 505–510.
[39] C. Livingstone, M. Collison, Sex steroids and insulin resistance, Clin. Sci. (Lond.) 102 (2) (2002) 151–166.
[40] A. Shepherd, M.P. Cleary, Metabolic alterations after dehydroepiandrosterone treatment in Zucker rats, Am. J. Physiol. 246 (2 Pt 1) (1984) E123–128.
[41] D. Simon, P. Preziosi, E. Barrett-Connor, M. Roger, M. Saint-Paul, K. Nahoul, L. Papoz, Interrelation between plasma testosterone and plasma insulin in healthy adult men: the Telecom Study, Diabetologia 35 (2) (1992) 173–177.
[42] E. Barrett-Connor, K.T. Khaw, Endogenous sex hormones and cardiovascular disease in men. A prospective population-based study, Circulation 78 (3) (1988) 539–545.
[43] S.H. Golden, A.S. Dobs, D. Vaidya, M. Szklo, S. Gapstur, P. Kopp, K. Liu, P. Ouyang, Endogenous sex hormones and glucose tolerance status in postmenopausal women, J. Clin. Endocrinol. Metab. 92 (4) (2007) 1289–1295.
[44] E.C. Tsai, A.M. Matsumoto, W.Y. Fujimoto, E.J. Boyko, Association of bioavailable, free, and total testosterone with insulin resistance: influence of sex hormone-binding globulin and body fat, Diab. Care 27 (4) (2004) 861–868.
[45] J.Y. Oh, E. Barrett-Connor, N.M. Wedick, D.L. Wingard, Endogenous sex hormones and the development of type 2 diabetes in older men and women: the Rancho Bernardo study, Diab. Care 25 (1) (2002) 55–60.
[46] R.K. Stellato, H.A. Feldman, O. Hamdy, E.S. Horton, J.B. McKinlay, Testosterone, sex hormone-binding globulin, and the development of type 2 diabetes in middle-aged men: prospective results from the Massachusetts male aging study, Diab. Care 23 (4) (2000) 490–494.
[47] T. Endre, I. Mattiasson, G. Berglund, U.L. Hulthen, Low testosterone and insulin resistance in hypertension-prone men, J. Hum. Hypertens. 10 (11) (1996) 755–761.
[48] S.A. Amiel, R.S. Sherwin, D.C. Simonson, A.A. Lauritano, W.V. Tamborlane, Impaired insulin action in puberty. A contributing factor to poor glycemic control in adolescents with diabetes, N. Engl. J. Med. 315 (4) (1986) 215–219.
[49] P. Marin, S. Holmang, L. Jonsson, L. Sjostrom, H. Kvist, G. Holm, G. Lindstedt, P. Bjorntorp, The effects of testosterone treatment on body composition and metabolism in middle-aged obese men, Int. J. Obes. Relat. Metab. Disord. 16 (12) (1992) 991–997.
[50] C.J. Malkin, P.J. Pugh, T.H. Jones, K.S. Channer, Testosterone for secondary prevention in men with ischaemic heart disease? QJM 96 (7) (2003) 521–529.
[51] P. Bjorntorp, The regulation of adipose tissue distribution in humans, Int. J. Obes. Relat. Metab. Disord. 20 (4) (1996) 291–302.
[52] G. De Pergola, The adipose tissue metabolism: role of testosterone and dehydroepiandrosterone., Int. J. Obes. Relat. Metab. Disord. 24 (Suppl. 2) (2000) S59–63.
[53] R. Leenen, K. van der Kooy, J.C. Seidell, P. Deurenberg, H.P. Koppeschaar, Visceral fat accumulation in relation to sex hormones in obese men and women undergoing weight loss therapy, J. Clin. Endocrinol. Metab. 78 (6) (1994) 1515–1520.
[54] C. Couillard, J. Gagnon, J. Bergeron, A.S. Leon, D.C. Rao, J.S. Skinner, J.H. Wilmore, J.P. Despres, C. Bouchard, Contribution of body fatness and adipose tissue distribution to the age variation in plasma steroid hormone concentrations in men: the HERITAGE Family Study, J. Clin. Endocrinol. Metab. 85 (3) (2000) 1026–1031.
[55] A. von Eckardstein, G. Assmann, High density lipoproteins and reverse cholesterol transport: lessons from mutations., Atherosclerosis 137 (Suppl.) (1998) S7–11.
[56] A. von Eckardstein, G. Assmann, Prevention of coronary heart disease by raising high-density lipoprotein cholesterol? Curr. Opin. Lipidol. 11 (6) (2000) 627–637.
[57] J.E. Nestler, D.J. Jakubowicz, A.F. de Vargas, C. Brik, N. Quintero, F. Medina, Insulin stimulates testosterone biosynthesis by human thecal cells from women with polycystic ovary syndrome by activating its own receptor and using inositolglycan mediators as the signal transduction system, J. Clin. Endocrinol. Metab. 83 (6) (1998) 2001–2005.
[58] A. Dunaif, A. Thomas, Current concepts in the polycystic ovary syndrome, Annu. Rev. Med. 52 (2001) 401–419.
[59] R. Pasquali, M. Filicori, Insulin sensitizing agents and polycystic ovary syndrome, Eur. J. Endocrinol. 138 (3) (1998) 253–254.

[60] E. Dahlgren, K. Landin, M. Krotkiewski, G. Holm, P.O. Janson, Effects of two antiandrogen treatments on hirsutism and insulin sensitivity in women with polycystic ovary syndrome, Hum. Reprod. 13 (10) (1998) 2706–2711.
[61] E. Diamanti-Kandarakis, A. Mitrakou, S. Raptis, G. Tolis, A.J. Duleba, The effect of a pure antiandrogen receptor blocker, flutamide, on the lipid profile in the polycystic ovary syndrome, J. Clin. Endocrinol. Metab. 83 (8) (1998) 2699–2705.
[62] P. Moghetti, F. Tosi, R. Castello, C.M. Magnani, C. Negri, E. Brun, L. Furlani, M. Caputo, M. Muggeo, The insulin resistance in women with hyperandrogenism is partially reversed by antiandrogen treatment: evidence that androgens impair insulin action in women, J. Clin. Endocrinol. Metab. 81 (3) (1996) 952–960.