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RESEARCH ARTICLE

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Androgen receptor polyglutamine repeat length (AR-CAGn) modulates the effect of testosterone on androgen-associated somatic traits in Filipino young adult men

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Abstract

Objectives: The androgen receptor (AR) mediates expression of androgen-associated somatic traits such as muscle mass and strength. Within the human AR is a highly variable glutamine short-tandem repeat (AR-CAGn), and CAG repeat number has been inversely correlated to AR transcriptional activity *in vitro*. However, evidence for an attenuating effect of long AR-CAGn on androgen-associated somatic traits has been inconsistent in human populations. One possible explanation for this lack of consistency is that the effect of AR-CAGn on AR bioactivity in target tissues likely varies in relation to circulating androgen levels.

Materials and Methods: We tested whether relationships between AR-CAGn and several androgen-associated somatic traits (waist circumference, lean mass, arm muscle area, and grip strength) were modified by salivary (waking and pre-bed) and circulating (total) testosterone (T) levels in young adult males living in metropolitan Cebu, Philippines ($n = 675$).

Results: When men's waking T was low, they had a reduction in three out of four androgen-associated somatic traits with lengthening AR-CAGn ($p < .1$), consistent with *in vitro* research. However, when waking T was high, we observed the opposite effect—lengthening AR-CAGn was associated with an increase in these same somatic traits.

Discussion: Our finding that longer AR-CAGn predicts greater androgen-associated trait expression among high-T men runs counter to *in vitro* work, but is generally consistent with the few prior studies to evaluate similar interactions in human populations. Collectively, these results raise questions about the applicability of findings derived from *in vitro* AR-CAGn studies to the receptor's role in maintaining androgen-associated somatic traits in human populations.

KEYWORDS

androgens, body composition, sexual dimorphism, short-tandem repeats

1 | INTRODUCTION

Humans exhibit sexual dimorphism in numerous phenotypic traits whose development and maintenance are regulated by testosterone (T) and other androgens (Bribiescas, 2001; Griggs et al., 1989; Kim et al., 2004; Shen et al., 2004). Through their anabolic effects on skeletal muscle, androgens increase lean muscle mass and strength, and decrease adiposity by inhibiting adipogenesis and stimulating lipolysis (Norman & Litwack, 1997; Vingren et al., 2012). The effect of androgens in target tissues is mediated through the androgen receptor (AR), a nuclear transcription factor derived from the X-chromosome and which contains two short-tandem repeat (STR) motifs that are highly polymorphic in humans (Chang et al., 1995). One of these STRs, a polyglutamine repeat tract (AR-CAGn) is located in exon 1 of the expressed protein, and varies between 8 and 38 in healthy humans. The number of repeats in the AR-CAGn has been inversely associated with AR transcriptional activity *in vitro* (Albertelli, Scheller, Brogley, & Robins, 2006; Callewaert et al., 2003; Chamberlain, Driver, & Miesfeld, 1994; Choong, Kemppainen, Zhou, & Wilson, 1996; Kazemi-Esfarjani, Trifiro, & Pinsky, 1995; Knoke, Allera, & Wieacker, 1999; Tut, Ghadessy, Trifiro, Pinsky, & Yong, 1997).

The central role of the AR in androgen signal transduction, as well as *in vitro* studies connecting AR-CAGn with AR transcriptional activity, suggest that AR-CAGn genotype should affect androgen-associated traits in men. For a given physiological concentration of T, individuals with longer AR-CAGn are predicted to exhibit less pronounced expression of androgen-associated traits. This expectation has been supported for several reproductive traits, and disorders that are tied to androgen levels, including spermatogenesis (Davis-Dao, Tuazon, Sokol, & Cortessis, 2007) and prostate cancer risk (Gu, Dong, Zhang, & Niu, 2012). In contrast, evidence for a contribution of AR-CAGn to androgen-associated somatic traits, such as lean mass, strength, and adiposity, has been less consistent (Table 1). In human population studies, relationships between androgen-associated somatic traits and AR-CAGn have alternatively been positive, negative, or absent. These patterns suggest that there may be limitations when extrapolating *in vitro* findings of receptor function to studies of human populations.

One factor integral to the relationship between AR-CAGn and androgen-associated somatic traits, and that could help explain inconsistency in the literature, are circulating levels of T. Although the effect of AR-CAGn has been shown to vary with androgen levels *in vitro* (Choong et al., 1996; Kazemi-Esfarjani et al., 1995; Tut et al., 1997), surprisingly few *population level studies* have examined both AR-CAGn and T and the interaction between them (Table 1). We are aware of only three studies that have tested for an interaction between AR-CAGn and T as predictors of somatic traits (Table 1). One reported that AR-CAGn had a more suppressive effect on androgen-associated somatic traits when T levels were high (Campbell, Gray, Eisenberg, Ellison, & Sorenson, 2007), while the other two found that the attenuating effect of AR-CAGn on androgen-associated traits decreased with increasing T (Lapauw, Goemaere, Crabbe, Kaufman, & Ruige, 2007; Stiger et al., 2008). All three studies point to phenotypically-relevant effects of the AR-CAGn × T interaction, but also hint at population level differences that may run counter to the expected role of the polymorphism as a mediator of androgen-associated trait expression.

Working with a large sample of young adult men (age 20-22 y) living in metropolitan Cebu, Philippines, we previously reported that waking salivary testosterone (AM-T) predicted fat free mass, arm muscle area and grip strength among men who participate in sports or physically demanding activities (Gettler, Agustin, & Kuzawa, 2010). More recently, we reported that variation in AR-CAGn among these men was only modestly related to circulating levels of T or gonadotropins (Ryan et al., 2017). Building on these findings, here we test for an interaction between AR-CAGn and T on waist circumference, lean mass, arm muscle area, and grip strength ($n = 675$). We examine relationships between each trait and total plasma T, morning (AM-T), and evening (PM-T) bioavailable (salivary) T. We chose these three measures of T for comparability with other studies, and because time of day and physiological fraction of T appear to have important functional consequences for androgen-associated traits (Kuzawa, Georgiev, McDade, Bechayda, & Gettler, 2016; Ryan et al., 2017). To assess the potential aromatization effects of body fat, we also followed up our analyses by checking for an effect of body fat on our measures of T. If AR-CAGn attenuates the androgenic effects of T uniformly throughout testosterone's physiological range, we expect that androgen-associated somatic traits will be consistently lower at a given concentration of T for men with longer AR-CAGn. If the phenotypic effects of longer AR-CAGn are contingent upon circulating T levels in a nonadditive fashion, as some *in vitro* and some population level studies suggest, we should see a significant interaction between these AR-CAGn and circulating T.

2 | METHODS

Data come from participants in the Cebu Longitudinal Health and Nutritional Survey (CLHNS), who live in Metropolitan Cebu, the Philippines (Adair et al., 2011). The present analyses focus on data collected in 2005, when the participants in the study were 20–22 years of age. A total of 675 men had all necessary questionnaire, anthropometric, hormone, and AR-CAGn polymorphism data available and met selection criteria described below. All anthropometric measures were derived using previously described methods (Gettler et al., 2010). This research was conducted under conditions of written informed consent with human subject clearance from the Institutional Review Boards of Northwestern University and the University of North Carolina at Chapel Hill.

2.1 | Plasma total testosterone

Participants were asked to fast overnight for 12 h, and blood samples were collected in community-based clinics the following morning using EDTA-coated tubes. Mean time of blood draw was 07:07 (range 05:40–09:30). After separation, samples were frozen and shipped on dry ice to Northwestern University for analysis. Plasma total testosterone was analyzed with a commercially available enzyme immunoassay (Diagnostic Systems Laboratories #DSL-10-4000, Webster, TX). All

TABLE 1 List of studies investigating AR-CAGn with respect to androgen-associated somatic traits, as well as other reported analyses, main findings, and sample size

Study	Somatic traits predicted	AR-CAGn	Testosterone	AR-CAGn × Testosterone	Population	Sample size
Campbell et al. (2007)	Fat free mass, %Body fat, Waist circum., Suprailliac skinfold	$p < .05$ ⇑	$p < .01$ ⇑	$p < .001$ ⇓	Kenya (Ariaal)	156
	BMI	NS	$p < .001$ ⇑	$p < .01$ ⇓		
	Arm circum.	NS	NS	NS		
La Pauw et al. (2007)[a]	Weight, BMI	NS	$p < .05$ ⇓	NS	Belgium	159
	Fat free mass, Fat mass	NS	$p < .05$ ⇑	$p < .05$ ⇑		
Stiger et al. (2008)	Bone mineral density (BMD; lumbar spine, femoral neck, total body)	$p < .05$⇑[b]	NS	$p < .05$⇑[c]	Sweden	229
De Naeyer et al. (2014)	Muscle mass	NS	$p < .05$ ⇑	–	Belgium	677
	Force	NS	NS	–		
Goutou, Sakka, Stakias, Stefanidis, and Koukoulis (2009)	BMI, waist-to-hip ratio	NS	NS	–	Greece	170
Stanworth, Kapoor, Channer, and Jones (2008)[d]	BMI	$p < .05$ ⇑	$p < .05$ ⇓	–	United Kingdom	233
	Waist circum.	NS	$p < .05$ ⇓	–		
Folland, Mc Cauley, Phypers, Hanson, and Mastana (2012)	Fat free mass, %Body fat	NS	NS	–	United Kingdom	183
	Strength	NS	$p < .05$ ⇑	–		
Ponce-Gonzalez (2012)	Fat mass, %Body fat, Trunk Fat, % trunk fat	NS	$p < .05$ ⇓	-	Spain	319
Nielsen et al. (2010)	Relative thigh and lower trunk muscle area	$p < .05$ ⇓	$p < .05$ ⇓	–	Denmark	393
	Relative subcutaneous fat	$p < .05$ ⇑	–	–		
	Relative lean body mass	$p < .05$ ⇓	$p < .05$ ⇑	–		767
	Relative fat mass	$p < .05$ ⇑	–	–		
Pausova et al. (2010)	Sub-cutaneous fat, weight, waist circum.	NS	–	–	Canada	233
	Visceral adiposity	$p < .05$ ⇓	–	–		
Zitzmann, Gromoll, von Eckardstein, and Nieschlag (2003)	Fat mass	$p < .01$ ⇑	–	–	Germany	106
Ponce-Gonzalez et al. (2016)[e]	BMI, %Body fat, fat mass, lean mass	$p < .05$ ⇓	–	–	Spain	45
	Weight	NS	–	–		
Walsh et al. (2005)	Weight, BMI, appendicular muscle mass	NS	–	–	United States	117
	Fat free mass, relative total fat free mass	$p < .05$ ⇑	–	–		

(*continues*)

TABLE 1 (continued)

Study	Somatic traits predicted	AR-CAGn	Testosterone	AR-CAGn × Testosterone	Population	Sample size
Gonzalez-Hernandez et al. (2008)	BMI, waist-to-hip ratio, waist circum.	NS	–	–	Spain	653
Huhtaniemi et al. (2009)	Height, Weight, Waist, WHR, %BF	NS	–	–	Italy, Belgium, Poland, Sweden, UK, Spain, Hungary, Estonia	2878
Haring et al. (2012)	Waist circum.	NS	–	–	Germany	1859
Skjaerpe, Giwercman, Giwercman, and Svartberg (2008)[f]	BMI, waist circum.	NS	–	–	Norway	172
Guadalupe-Grau et al. (2009)	Lean mass, physical fitness	NS	–	–	Spain	282
Gustafson, Wen, and Koppanati (2003)	Weight, BMI, waist circum., hip circum., waist-to-hip ratio	NS	–	–	United States	99
Hersberger et al. (2005)	BMI	NS	–	–	Switzerland, Germany	544
Mouritsen et al. (2013)[g]	BMI, sum of skinfold thickness	$p < .05$ ⇓	–	–	Denmark	78
Voorhoeve et al. (2011)	Weight, BMI, fat mass, lean mass	NS	–	–	Netherlands	226

[a]Study conducted among elderly (75–89 years).
[b]No significant effect of AR-CAGn on BMD was shown for the subset of obese subjects.
[c]AR-CAGn and T grouped into tertiles for interaction analysis.
[d]Study conducted among men with Type II diabetes.
[e]Changes in BF are for the same men roughly 13 years old to 27 years old.
[f]Study conducted among elderly men (60–80 years).
[g]Comparing short or long AR-CAGn vs. intermediate repeat lengths; study conducted in boys aged 10–12 years old.

samples were assayed in duplicate, and control samples were included with each assay to monitor interassay variation. The laboratory coefficients of variation for low and high controls were, respectively, 13.3 and 5.8%.

2.2 | Salivary testosterone measurement

Saliva and plasma samples were obtained during the same 24-h period. Each participant was provided with instructions and two tubes for saliva collection. The first sample was collected immediately prior to bed (PM-T). After collection, tubes were sealed and kept at room temperature. Participants were instructed to place the second tube next to their bed and to collect the second sample immediately upon waking the following morning (AM-T). At each collection time, the participant was asked to record the time of collection, with average PM-T and AM-T collection times being 22:23 and 06:34, respectively. Tubes were collected later that day, and immediately placed on ice packs in a cooler by an interviewer. Tubes were then transported to a freezer where they were stored at −35 °C until shipment on dry ice to the Laboratory for Human Biology Research at Northwestern University, where they were stored at −80 °C. Samples were thawed, centrifuged, supernatant separated, and aliquoted into smaller tubes for subsequent analysis of individual analytes. Salivary T concentrations were determined in duplicate using an enzyme immunoassay protocol developed and validated for use with saliva samples (Salimetrics #1-2402, State College, PA). Inter-assay coefficients of variation for the manufacturer were 5.6 and 6.7% for high and low controls, respectively, while in our lab the same coefficients of variation were 11.5 and 13.7%.

2.3 | AR-CAGn measurement and quality control

Briefly, the AR-CAGn was amplified using a previously validated protocol (Ackerman et al., 2012) with a fluorescently labeled primer (AR CAGn forward, 5′-NED-GTGCGCGAAGTGATCCAGAA-3′; and reverse, 5′-TAGCCTGTGGGGCCTCTACG-3′). For each PCR, 20 ng of genomic DNA was amplified in a total volume of 9.2 μl in the presence of 200 μm deoxynucleotide triphosphate, 1.5 mm $MgCl_2$, 0.7 U Ampli-*Taq* Gold polymerase, and 0.75 μm of forward primer and 0.75 μm of reverse primer. PCR products were electrophoresed in the presence of an internal size standard (GeneScan 500 ROX) at room temperature on the Applied Biosystems 3130XL Capillary DNA sequence analysis system, and genotypes were assigned using the GENEMAPPER software version 4.0 (Applied Biosystems). Quality control was conducted by screening ambiguous repeat calls, removing males with a second allele

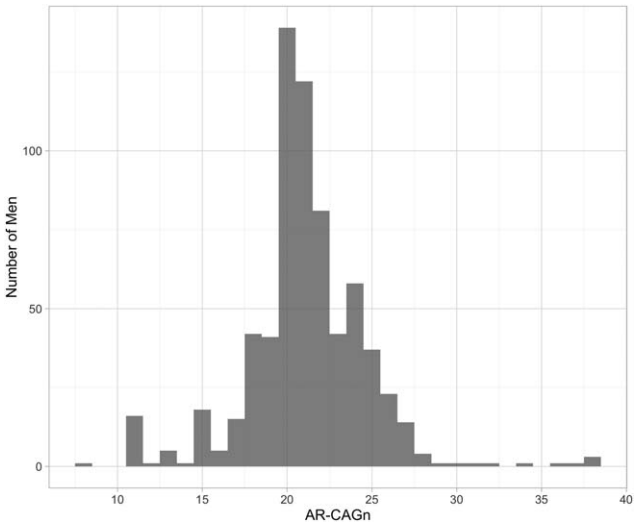

FIGURE 1 Distribution of androgen-receptor polyglutamine repeat length genotype (AR-CAGn) among 675 Filipino young adult men

(AR-CAGn is on the X chromosome), and corroborating male offspring genotype against maternal genotype (Ryan et al., 2017). There was no evidence of population substructure based on associations between AR-CAGn and the first 10 principal components (PCs) of genetic variation in this population (Wu et al., 2012), and so PCs were not included in subsequent analyses. A more detailed description of the AR-CAGn measurement and quality control can be found in Ryan et al. (2017). In this study AR-CAGn varied between 8 and 38 repeats (median = 21). The distribution of repeats is shown in Figure 1.

2.4 | Statistical analyses

Prior to analyses, we examined data for missing values, outliers and extreme observations (Zuur, Ieno, & Elphick, 2010). After removing extreme observations [>5 standard deviations from the mean; BMI (1), total plasma T (5), salivary AM-T (1), and salivary PM-T (1)], we ran analyses on the remaining 675 individual males with complete data for all variables. A more detailed study of the relationships between these measures of testosterone and other hormones in the hypothalamic-pituitary gonadal (HPG) axis can be found in Ryan et al. (2017). For comparability with previous studies, males were first grouped into 'short' (AR-CAGn <21) or 'long' (>21) based on the median split. These groups were used for summary statistics and bivariate comparisons using Chi-square and two-sample *t* tests (Table 2). For all remaining analyses between measures of testosterone and somatic traits we used ordinary least squares (OLS) regression, with both dependent and independent variables modeled as continuous values. Testosterone and AR-CAGn were centered to derive meaningful estimates of the main effects of each (Jaccard & Turrisi, 2003). Because the time of day affects testosterone levels, we used the residuals corrected for time of sample collection for all analyses. Variance inflation factors for all predictor variables were close to 1.0, indicating that collinearity was not a problem in our analyses. Post-test residual inspection and formalized Breusch–Pagan tests (Hothorn et al., 2002) revealed violations of normality and homoscedasticity in residuals for several of the models, assumptions required for OLS. Since transformation did not improve this problem, we refit these models using the heteroscedasticity-corrected covariance matrix from the 'car' package in R

TABLE 2 Characteristics of the full sample and stratified on a median split of AR-CAG length[a]

		Median of AR-CAGn		
	Total sample (n = 675)	Short (CAGn ≤ 21) (n = 406)	Long (CAGn > 21) (n = 269)	p value[b]
Age (years)	21.5 ± 0.3	21.5	21.5	0.291
Employed (%)	58.4	61.3	53.9	0.066
Physical work (%)	33.3	36.0	29.4	0.090
Basketball (%)	30.5	30.0	31.2	0.810
Weightlifting (%)	4.1	3.9	4.5	0.890
Body mass index	20.9 ± 2.9	21.0	20.8	0.242
Waist circumference (cm)	71.9 ± 7.2	72.1	71.5	0.313
Lean mass (kg)	46.6 ± 5.7	46.9	46.2	0.120
Arm muscle area (cm2)	34.8 ± 7.4	35.2	34.2	0.089
Grip strength[c] (kg)	72.7 ± 22.5	72.8	72.7	0.962
AM-T (pg/ml)	193.6 ± 76.7	192.8	194.8	0.743
PM-T (pg/ml)	116.3 ± 52.7	115.7	117.3	0.692
Total T (ng/ml)	7.9 ± 2.8	7.9	7.9	0.752

[a]Mean ± SD.
[b]From two-sided *t* tests or Chi-square.
[c]Compared for the dominant hand only.

TABLE 3 Regression coefficients for AR-CAGn × T interaction from regression models examining androgen-associated somatic traits ($n = 675$)[a]; all models included height, age, and participation in exercise as covariates

	Waist circ.		Lean mass		Arm muscle area		Grip strength	
	β (SE)	*p*	β (SE)	*p*	β (SE)	*p*	β (SE)	*p*
AM-T * AR-CAGn	**0.078 (0.073)**	**0.035**	0.055 (0.059)	0.068	**0.089 (0.074)**	**0.017**	0.041 (0.075)	0.282
PM-T * AR-CAGn	−0.014 (0.073)	0.711	−0.028 (0.059)	0.351	0.014 (0.074)	0.712	0.008 (0.075)	0.833
Total T * AR-CAGn	−0.019 (0.075)	0.616[b]	−0.023 (0.055)	0.409[b]	−0.035 (0.074)	0.354	−0.070 (0.076)	0.069

[a]Standardized regression coefficients reported, significant interactions are bolded.
[b]Coefficients and *p* values derived from heteroscedasticity-corrected covariance matrix.

(Fox & Weisberg, 2011; R Core Development Team, 2011). Unless otherwise indicated, we report results for OLS. Beta coefficients in standard deviations for linear models are reported in Table 3. Because waist circumference, lean mass, arm muscle area, and grip strength all scale with body size, we controlled for height in all models. Following prior work in this population (Gettler et al., 2010) we adjusted for involvement in sports (basketball and weightlifting), as well as having a physically demanding job. All models included age. Although marital status and fatherhood have previously been shown to affect circulating T in this sample (Gettler, McDade, Feranil, & Kuzawa, 2011; Kuzawa et al., 2009), they showed no significant relationships with the somatic traits investigated, nor did employment status, and so all three were excluded to avoid overparameterization. Follow-up models that examined the potential estrogenic effect of body fat (estimated from skinfold thickness; Durnin & Womersley, 1974) on T (dependent variable) included age, activity levels, physically-demanding work, and AR-CAGn.

3 | RESULTS

Men with short (≤21) versus long (>21) AR-CAGn did not differ in age, participation in basketball, weightlifting, or in having a physically demanding job, testosterone levels, or any of the somatic traits investigated (Table 2). There was a significant or borderline significant positive interaction between AR-CAGn and AM-T for three of four traits investigated (Table 3). The relationship between AR-CAGn and waist circumference (Table 3; Figure 2a), lean mass (Table 3; Figure 2b), and arm muscle area (Table 3; Figure 2c) depended on circulating levels of AM-T, although the effect for lean mass was only borderline significant. For all three, the slope of the interaction was positive, indicating that an increase in either T or AR-CAGn was associated with a more positive effect of the other.

The main effects of AM-T and AR-CAGn were not significant for waist circumference ($p = .785$ and $p = .975$, respectively), lean mass ($p = .918$ and $p = .565$, respectively), or arm muscle area ($p = .615$ and $p = .837$, respectively). For grip strength, the main effects for AM-T and AR-CAGn were not significant ($p = .167$ and $p = .539$, respectively), although there was a borderline significant interaction between them (Table 3). AR-CAGn had no effect on the relationship between PM-T or total T and any of the androgen-associated somatic traits (Table 3). After we had established relationships between AR-CAGn, T, and the androgen-associated traits described above, we wished to confirm that body fat itself was not lowering T through the aromatization of T to estrogen (E2). We found no evidence for an effect of percent body fat on AM-T ($p = .209$), PM-T ($p = .758$), but marginally significant evidence for a negative relationship between percent body fat and total plasma T ($p = .066$). Full model outputs are provided in the Supporting Information (Supporting Information Tables S1–S4).

Because receptor–ligand binding kinetics can be nonlinear (Norman & Litwack, 1997), we tested whether AM-T or AR-CAGn exhibited a nonlinear relationship with our androgen-associated somatic outcomes. To do this, we partitioned either AM-T or AR-CAGn into quartiles and ran the same regression models including second order orthogonal polynomials for the other variable (AR-CAGn or AM-T, respectively). In no case did the higher order polynomial regression outperform our standard linear regressions ($p > .05$ for all second order variables), suggesting that a nonlinear relationship was not necessary to model the effects of AM-T and AR-CAGn on androgen-associated somatic traits.

4 | DISCUSSION

Using phenotypic and genetic data from a large sample of young adult Filipino men, we found evidence that the number of glutamine repeats in exon 1 of the androgen receptor (AR-CAGn) affects the expression of several androgen-associated somatic traits. Although the nature of this relationship depended on levels of T, our findings were only partly consistent with expectations. Among men with relatively low T, androgen-associated trait expression was reduced as AR-CAG increased in length, as expected. However, among men with relatively high T levels, longer AR-CAG length predicted increased androgen-associated trait expression. These findings add to a small but growing list of human population studies exploring the interaction between AR-CAG and T, which reveal phenotypic effects that run counter to expectations based upon *in vitro* findings.

In vitro work has demonstrated the central role of the AR-CAGn in modulating the phenotypic effects of androgens. For example, when examining AR transactivational capacity in monkey kidney cells transfected with human AR with varying number of glutamine repeats, Kazemi-Esfarjani et al. (1995) found that the difference between short and long AR-CAGn was most pronounced when levels of an androgen agonist were highest. Choong et al. (1996) report complementary findings: AR transcriptional activity did not vary with AR-CAGn at low

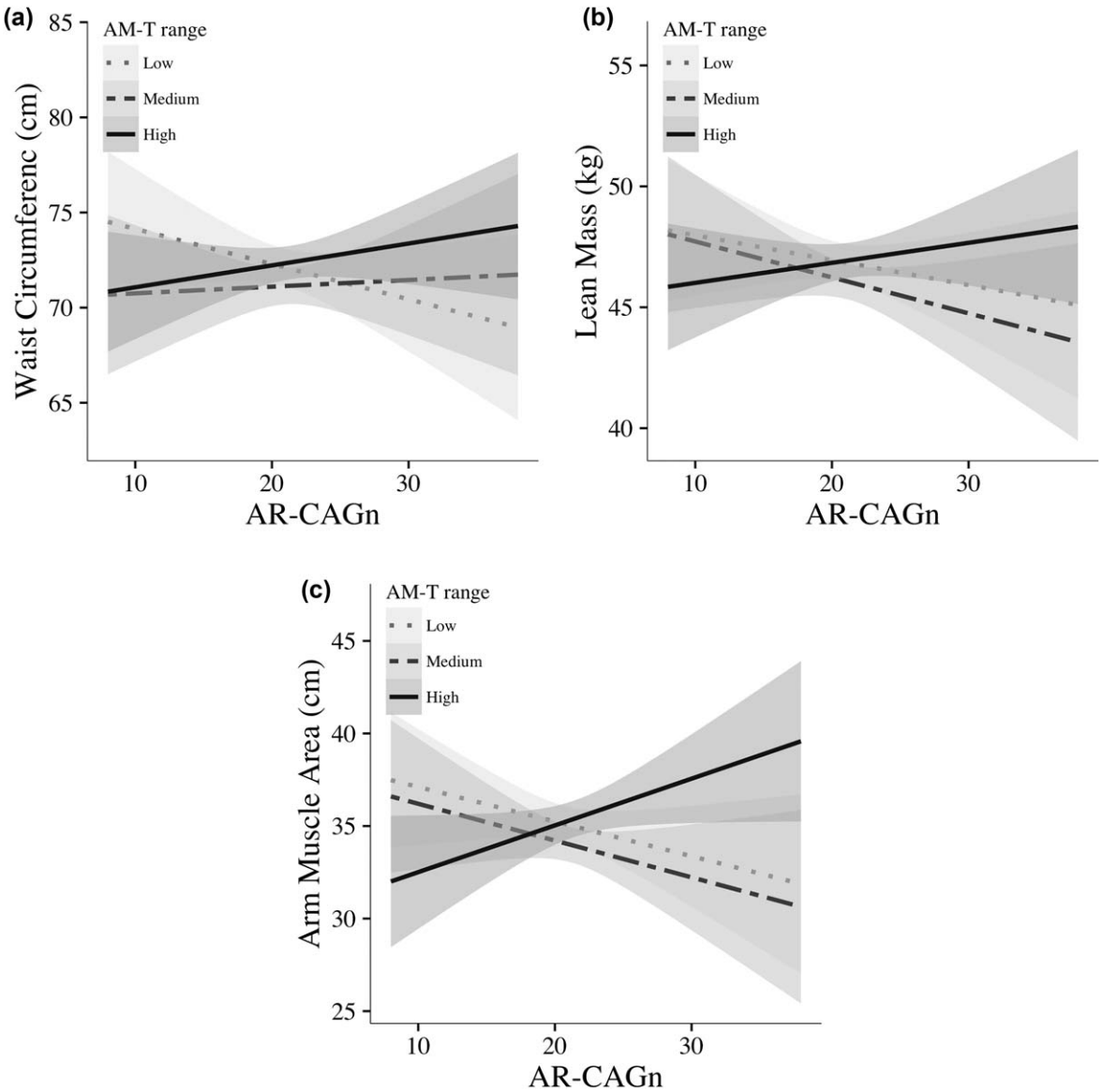

FIGURE 2 Slopes and 95% confidence intervals for the relationship between androgen receptor polyglutamine repeat length (AR-CAGn) and (**a**) waist circumference, (**b**) lean mass, and (**c**) arm muscle area by low (26.1–155 pg/mL), medium (155–214 pg/mL), and high (214–557 pg/mL) waking testosterone (AM-T) levels. Full models examined AM-T as a continuous variable, and included age, height, physically demanding work, and participation in basketball and/or weightlifting as covariates

concentrations of dihydrotestosterone (DHT), but did when DHT levels were high. Tut et al. (1997) also found evidence for an inverse relationship between AR-CAGn and transcriptional activity at high, but not low, doses of DHT *in vitro*. Our finding of an interaction between AR-CAGn and T when describing individual phenotypic traits is therefore not entirely unexpected.

In all of the above examples, the attenuating effect of AR-CAGn on AR transcriptional activity was small or absent when androgen levels were low, and greatest when androgens were high. Although they often examine AR-CAGn ranges longer or shorter than those normally observed in humans, these *in vitro* findings would lead us to predict a negative interaction, whereby longer AR-CAGn has the greatest attenuating effect on androgen-associated traits when T is at its highest. The results of one population level study, conducted with 156 semi-nomadic Ariaal men (20 to >60 years old) in Northern Kenya, match these predictions (Campbell et al., 2007). Campbell and colleagues found a negative interaction between AR-CAGn and T, such that longer AR-CAGn had the greatest negative effect on waist circumference, lean mass, and percent body fat when T levels were highest (Campbell et al., 2007).

In contrast, our results point to a positive interaction between AR-CAGn and AM-T across several androgen-associated somatic traits. Notably, men with longer AR-CAGn exhibited reductions in waist circumference, lean mass, and arm muscle area when AM-T levels were low, but increases in these traits when T were high. While these results are somewhat puzzling, two of the three prior studies that have explored AR-CAG-T interactions as predictors of somatic traits in humans have reported findings similar to ours (Campbell et al., 2007;

Lapauw et al., 2007; Stiger et al., 2008). Similar to our findings in Cebu, men with longer AR-CAGn and high T had higher fat free mass among 159 elderly Danish men (Lapauw et al., 2007), and higher bone mineral density among 229 older Swedish men (Stiger et al., 2008). Although differing from those of Campbell et al. (2007), the findings of these two studies, as well as our own, hint at a biological effect of CAGn that is distinct from that documented in *in vitro* studies.

Why relationships between AR-CAGn and T and androgen-associated traits would vary so markedly between the Ariaal and the other three populations is not clear, but we speculate that differences in nutritional ecology may be important. In theory, the direction of causation linking T and energy stores, as reflected in traits like body fat, may flow in either direction depending on prevailing energetic conditions. In populations with chronically marginal nutrition, such as the Ariaal (Campbell, O'rourke, & Lipson, 2003; Campbell et al., 2007), T levels tend to be lower than in places like the US, which has been interpreted as evidence that low energy intake constrains T production (Bribiescas, 2001; Ellison et al., 2002). In contrast, in populations with abundant nutrition and excess body fat stores, energy is less limiting on the production of T (and its somatic outcomes), levels of which reduce fat stores through the hormone's lipolytic effects. We speculate that this potential for distinct causal relationships between T and energy stores, depending on a population's chronic energy sufficiency, may help explain some of the inconsistencies in the relationship between AR-CAG, T and somatic traits documented previously in human populations. Using estimates of body fat and BMI, the young Filipino men in our study are leaner than men in most Western populations, but very few (<13%) were as lean or leaner than the average of the men studied by Campbell et al. (2007), which could explain why our results are more comparable to the findings of Lapauw et al. (2007) and Stiger et al. (2008).

We found significant or borderline significant interactions between AR-CAGn and bioavailable AM-T, but not with PM-T or total T, suggesting that the interplay between T and AR-CAGn may rely upon both the biological fraction of T and the time of day when it is measured. Our finding that AM-T but not PM-T related to androgen-associated traits adds support to the hypothesis that peak T levels attained during sleep may be particularly relevant to the hormone's anabolic and somatic effects (Kuzawa et al., 2016). Although our measure of total T was also obtained in the morning, we recently showed that salivary T drops rapidly in the first 30 minutes after waking. It is presently not known whether total T follows a similar precipitous post-waking decline, which might be expected to obscure any relationships with somatic traits in this sample.

Past human studies, including in this sample (Gettler et al., 2011; Kuzawa et al., 2009), have shown that T declines markedly as men enter stable pair-bonds or become fathers. This pattern is rare or absent in the males of other mammalian species and has been interpreted as helping facilitate the unusual contribution that human fathers make to offspring care (Gettler, 2014; Gettler et al., 2011; Gray & Anderson, 2010). Alvarado et al. (2015) recently proposed that in humans muscle anabolism has been decoupled from normal physiological variation in T levels, which would allow these T-driven behavioral shifts without negatively impacting a man's ability to engage in physically demanding activities like hunting and farming. Our findings suggest that T levels do affect muscle and related traits, but underscore the complexity of these relationships. In our sample, among men with high T, androgen-associated somatic traits tend to increase as AR-CAGn lengthens, while these same traits decrease with longer AR-CAGn among men with lower T levels. Although speculative, if similar findings are replicated across other human populations, this could indicate that the effects of T on muscle and other traits vary in relation to underlying genetic variation in AR-CAG repeat length.

From an evolutionary perspective, the modulating effect of AR-CAGn on androgen-associated somatic traits is particularly relevant in light of arguments for selection operating at both short and long extremes of AR-CAGn from both disease risk (Ryan & Crespi, 2013) and mating behavior (Butovskaya et al., 2015; Gettler et al., 2017). Recent work by our group has shown that both relationship stability and paternal investment in childcare are lowest among men with either high T and short AR-CAGn, or low T and long AR-CAGn (Gettler et al., 2017). Here, the same extremes of T and AR-CAGn relate to the lowest levels of androgen-associated somatic traits. Because the combination and high T and short AR-CAGn and low T and long AR-CAGn are also associated with risk for prostate cancer and infertility, respectively, selection arising from other traits, diseases, or molecular processes could act to counter-balance these extremes to maintain the high variability in AR-CAGn among humans (Ryan & Crespi, 2013; Shimada et al., 2016).

Our study is not without limitations. First, our comparison is limited to a single assessment of each of the T measurements, while averaging across several days would yield more reliable estimates (Dabbs, 1990). The lower measurement reliability resulting from single measures will limit statistical power, but does not appear to be a factor in our models given that we identified significant relationships for several phenotypes. We also focus on T and the modulatory effect of AR-CAGn on androgen-associated somatic traits, but other proteins and hormones such as sex-hormone binding globulin and estrogen (E2) play important role in these traits (Ponce-González et al., 2012). Indeed, aromatization of T to E2 in adipose tissue may explain inconsistencies in the relationship between AR-CAGn and T on androgen-associated traits across studies (De Naeyer et al., 2014; Eendebak et al., 2016). However, elevated E2 levels arising from aromatization of T in adipose tissue should be relatively low among the men in our study, who are quite lean as compared to men in most Western populations. This conclusion was confirmed by follow-up tests, where there was no relationship between estimates of percent body fat and all measures of T—in particular AM-T, which showed the only consistent interaction with AR-CAGn when predicting our androgen-associated traits. Although not a limitation *per se*, our observed effects sizes, although significant, are relatively small (Sawilowsky, 2009). This greatly lowers the probability of replicating our findings in smaller studies—of the 23 studies listed in Table 1, only 5 had a sample size large enough to detect the mean effect size described here 50% of the time ($n = 568$). Finally, our

findings were strongest at the extremes of AR-CAGn and T, where the fewest individuals contributed to the dataset. This can lead to a disproportionate contribution of certain individuals to the significance of the findings. However our post-test diagnostics revealed no evidence for high leverage data points or influential observations, making such biases in our findings unlikely.

In sum, we find evidence that the effect of AR-CAGn on androgen-associated somatic traits is modified by waking T. This interaction was positive, meaning that when T was low, AR-CAGn had a flat or negative slope with androgen-associated traits. In contrast, when T was high, AR-CAGn was positively associated with these same traits. Although these findings are only partly consistent with expectations based upon *in vitro* findings, they generally agree with the few other studies that have explored T x AR-CAG interactions as predictors of androgen-associated traits in human populations. Our findings thus add to growing evidence for the importance of androgen receptor genetic variation when interpreting the effects of T on somatic traits, while pointing to unexplained complexity in these relationships.

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