The Relationship Between Anogenital Distance and Reproductive Hormone Levels in Adult Men

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Abbreviations and Acronyms

AGD = anogenital distance

FSH = follicle-stimulating hormone

LH = luteinizing hormone

PL = penile length

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Purpose: Anogenital distance is a marker for endocrine disruption in animal studies in which decreased distance has been associated with testicular dysfunction. In this study we investigated whether anogenital distance was associated with reproductive hormone levels in adult men.

Materials and Methods: A total of 116 men (mean age 36.1 ± 8.0 years) were evaluated at an andrology clinic in Houston. Anogenital distance (the distance from the posterior aspect of the scrotum to the anal verge) and penile length were measured using digital calipers. Testis size was estimated by physical examination. Linear regression was used to determine correlations between genital measurements and hormone levels.

Results: Anogenital distance ($r = 0.20$, $p = 0.03$) and penile length ($r = 0.20$, $p = 0.03$) were significantly associated with serum testosterone levels while total testis size was not ($r = 0.17$, $p = 0.07$). No relationship between genital length and luteinizing hormone, follicle-stimulating hormone or estradiol was identified. After adjusting for age the serum testosterone increased by 20.1 ng/dl (95% CI 1.8, 38.4; $p = 0.03$) for each 1 cm increase in anogenital distance. On multivariable models no statistically significant relationship existed between penile length and testosterone levels. Moreover men with hypogonadal testosterone levels (less than 300 ng/dl) had a significantly shorter anogenital distance compared to men with higher testosterone levels (31.6 vs 37.3 mm, $p = 0.02$).

Conclusions: Anogenital distance may provide a novel metric to assess testicular function in men. Assuming that anogenital distance at birth predicts adult anogenital distance, our findings suggest a fetal origin for adult testicular function.

Key Words: testosterone, hormones, genitalia, perineum

In the last half century there has been a reported decline in semen quality and serum testosterone levels with an increased rate in male genital abnormalities and testis cancers.[1–4] While the phenomenon and etiology are uncertain, several investigative groups postulate an environmental factor which disrupts normal endocrine signaling leading to abnormal androgen action and altered genital development.[1] During sexual development the immature genital precursors migrate ventrally via an androgen mediated pathway.[5] A marker for genital development, the AGD, has been examined in animals and humans.[6–9]

A sexually dimorphic measure, AGD was initially used to sex animals.[6,10,11] More recently human studies have

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also shown that boys have a greater perineal length than girls.[9,12–14] Investigators have also used AGD to show that agents which disrupt androgen signaling in animal models can lead to abnormal genital length and even altered testicular function as measured by testosterone and sperm production.[15–18]

In humans 2 recent studies have correlated AGD in men to sperm production. A study of healthy male volunteers demonstrated a positive relationship between anogenital distance and semen concentration, motility and morphology.[19] Another study showed that fertile men had greater anogenital length compared to infertile men.[20] In addition, a similar positive association between anogenital length and sperm count was identified. Assuming that AGD is determined in utero, such studies suggest in utero influences may impact genital development and adult testicular function. To date, to our knowledge no correlation of genital measures to hormone production exists. As testicular and penile development and function are related, we determined if human androgen production is related to anogenital length.

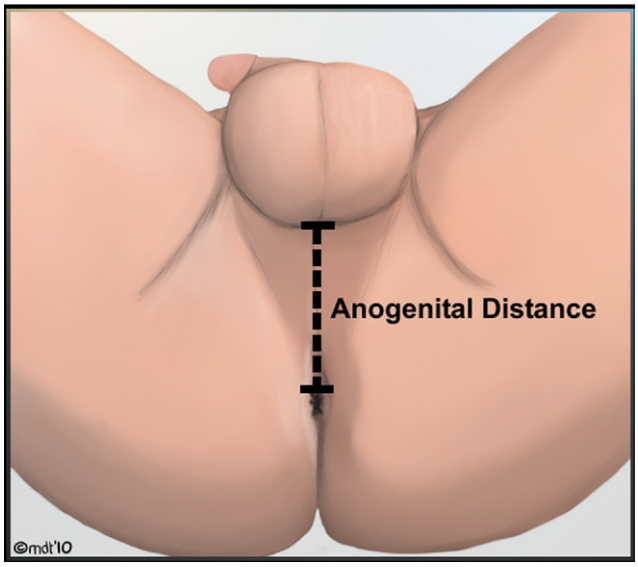

Figure 1. Anogenital distance as measured with men in supine, frog-leg position.

METHODS

Study Population

The methods of cohort assembly have been previously reported.[20] After obtaining institutional review board approval from Baylor College of Medicine, eligible patients were recruited from a urology clinic specializing in reproductive medicine from August 2010 through November 2010. Men with a history of orchiectomy, testicular torsion or prior malignancy were excluded from study. A total of 116 men had serum hormone and genital measurements available for analysis, including 89 evaluated for primary infertility, 16 for secondary infertility, 8 for sexual dysfunction/hypogonadism and 3 for vasectomy. Mean age ± SD was 36.1 ± 8.0 years. Of the cohort 58.6% was white, 13.8% Hispanic and 13.8% black. All men provided written consent for participation.

Genital Measurements

The methods of genital measurement have been described previously.[20] In the supine, frog-leg position with the legs abducted, allowing the soles of the feet to meet, the distance from the posterior aspect of the scrotum to the anal verge was measured using a digital caliper (Neiko USA, Model No. 01407A) (fig. 1). The stretched penile length was measured from the base of the dorsal surface of the penis to the tip of the glans. When comparing measurements among investigators the within subject standard deviation was 4.1 mm for anogenital distance and 5.4 mm for stretched PL. The correlation coefficient was 0.91 for AGD and PL measurements. It is important to note that other investigators have defined anogenital distance from the anus to the anterior base of the penis and the distance from the posterior scrotum to the anus (as was measured in this study) as the anoscrotal distance.[6,11,14] Given the age of the patients measured, the posterior scrotum was measured as the anterior border as it was considered a more comfortable, reliable and reproducible measure. Testicular volume was estimated manually during the physical examination by 1 investigator (LIL) at approximately 25 to 27C.

Hormone Analysis

All hormone assays were processed by a single, experienced laboratory (Laboratory for Male Reproductive Research and Testing, Baylor College of Medicine, Houston, Texas). Testosterone (normal range 200 to 1,000 ng/dl), LH (normal range 6 to 19 mIU/ml), FSH (normal range 4 to 10 mIU/ml) and estradiol (0.5 to 5 ng/dl) values were assessed using an automated, 1-step competitive binding assay with the Beckman Coulter Access® II Immunoassay system. The assays were recalibrated daily with controls that spanned the normal range for all hormones.

Statistical Analysis

ANOVA was used to compare means between groups. In addition, the Wilcoxon rank sum test was also used given the nonparametric distribution of the data with no difference in the overall interpretation or conclusions. Linear regression models and correlation coefficients were used to determine the relationship between genital measures and hormone values. Given the nonparametric distribution of the genital measures (ie AGD and PL), linear regression models were also run with $\log_{10}$ transformed variables with no differences in the overall conclusions. Linear regression coefficients between genital measures, hormone values and anthropomorphic variables were determined, and relationships with $p < 0.2$ were included in the multivariable models. All p values were 2-sided and analyses were performed using Stata® 10.

RESULTS

Anthropomorphic, hormonal and genital measurements are listed in table 1. When stratifying by race

Table 1. *Demographic, anthropomorphic, hormonal and genital characteristics of the cohort*

Mean ht inches (SD)	70.4	(3.2)
Mean wt lb (SD)	210.3	(54.7)
Mean kg/m^2 body mass index (SD)	29.8	(7.5)
No. race (%):		
White	91	(78.5)
Black	16	(13.8)
Asian	9	(7.8)
Mean hormone levels (SD):		
Testosterone (ng/dl)	316.5	(131.6)
FSH (mIU/ml)	8.5	(8.4)
LH (mIU/ml)	4.7	(3.0)
Estradiol (ng/dl)	2.8	(2.9)
Testosterone/LH	88.1	(56.5)
Mean genital measurements (SD):		
AGD (mm)	34.3	(13.3)
PL (mm)	108.5	(22.8)
Total testis vol (ml)	34.1	(8.2)

no significant differences were seen in testosterone, estradiol, LH or FSH levels. AGD ($r = 0.20$, $p = 0.03$) and PL ($r = 0.20$, $p = 0.03$) were significantly associated with serum testosterone levels (fig. 2). Removal of the largest AGD measures (3 or more SD above mean) did not significantly change the conclusions. In contrast, total testis size did not show a statistically significant association with testosterone levels ($r = 0.17$, $p = 0.07$). While AGD and PL showed no significant association with LH, FSH or estradiol, total testis volume was significantly associated with FSH ($r = 0.50$, $p < 0.01$) and LH ($p = 0.43$, $p < 0.01$). All genital measurements seemed to be correlated with each other, as AGD with penile length ($r = 0.20$, $p < 0.01$), AGD with total testicular volume ($r = 0.31$, $p < 0.01$) and penile length with total testicular volume ($r = 0.24$, $p < 0.01$).

In the unadjusted and adjusted models AGD significantly correlated with serum testosterone levels. In fact, for each 1 cm increase in AGD the serum testosterone increased by 20.1 ng/dl (95% CI 1.8, 38.4; $p = 0.03$). In contrast, no statistically significant correlation was seen with penile length, although a trend was seen (table 2). A positive relationship was also identified between the testosterone-to-LH ratio and AGD as well as testis size but not penile length. Moreover when stratifying men by several hypogonadal testosterone levels between 200 and 300 ng/dl, men with lower testosterone levels had significantly shorter anogenital distances than those with higher levels (table 3).

Neither AGD nor penile length correlated with LH, FSH or estradiol. On age adjusted models total testis size was significantly associated with FSH and LH ($p < 0.01$). When stratifying by race, the direction and magnitude of the measures of associations between genital measurements and hormone values remained similar. It should be noted that smaller sample sizes limited the statistical significance for white ($p = 0.1$) but not for black individuals ($p = 0.04$).

DISCUSSION

The current study demonstrated an association between anogenital distance and serum testosterone levels in a cohort of United States adult men evaluated in an andrology practice. In addition, there was a trend toward a similar association between testosterone levels and stretched penile length. We previously confirmed a relationship in this cohort between AGD and semen quality, but to our knowledge the current study represents the first assessment of the relationship between anogenital distance and serum testosterone levels.[19]

In rodents in utero exposure to agents known to disrupt androgen mediated pathways corrupts normal male genital development with a decrease in genital lengths (ie phallus length, AGD) and impaired testosterone and sperm production.[18,21] In humans Swan et al demonstrated that mothers exposed to higher levels of endocrine disruptors gave

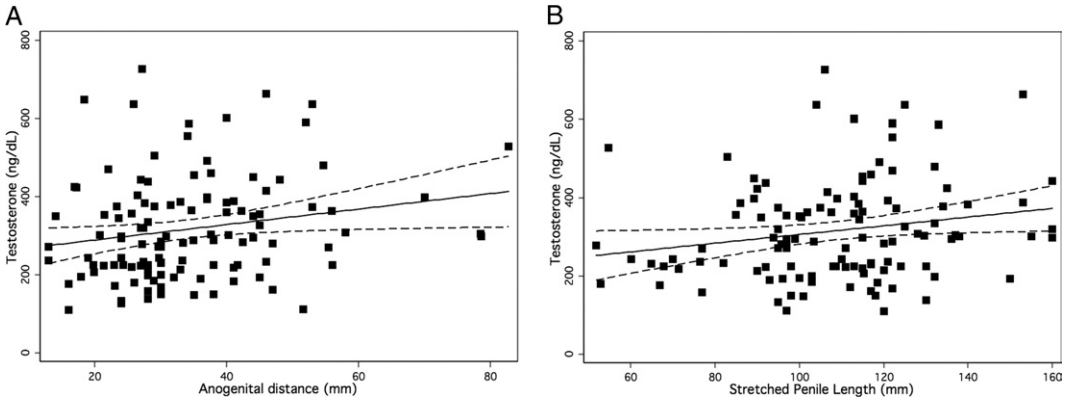

Figure 2. Scatterplot displaying relationship between (*A*) anogenital distance and serum testosterone levels ($r = 0.20$, $p = 0.03$), and (*B*) stretched penile length and serum testosterone levels ($r = 0.20$, $p = 0.03$).

Table 2. *Multivariable linear regression model of the relationship between genital measurements and hormone parameters*

	β (95% CI) Unadjusted	p Value	β (95% CI) Adjusted*	p Value
Testosterone:				
AGD	1.98 (0.18, 3.78)	0.03	2.01 (0.18, 3.84)	0.03
Penile length	1.11 (0.08, 2.14)	0.03	0.95 (−0.13, 2.02)†	0.08
Testicular vol	2.70 (−0.26, 5.65)	0.07	2.87 (−0.22, 5.95)	0.07
FSH:				
AGD	−0.07 (−0.20, 0.06)	0.30	−0.06 (−0.19, 0.07)	0.34
Penile length	0.00 (−0.07, 0.07)	0.98	0.00 (−0.07, 0.07)	0.97
Testicular vol	−0.55 (−0.73, −0.37)	<0.01	−0.55 (−0.73, −0.36)	<0.01
LH:				
AGD	−0.03 (−0.07, 0.02)	0.21	−0.03 (−0.07, 0.02)	0.26
Penile length	0.01 (−0.02, 0.03)	0.66	0.00 (−0.02, 0.03)	0.69
Testicular vol	−0.16 (−0.23, −0.10)	<0.01	−0.15 (−0.22, −0.09)	<0.01
Estradiol:				
AGD	−0.02 (−0.07, 0.03)	0.49	−0.03 (−0.08, 0.02)	0.28
Penile length	−0.01 (−0.03, 0.02)	0.71	0.00 (−0.03, 0.02)	0.80
Testicular vol	−0.02 (−0.09, 0.05)	0.62	−0.03 (−0.10, 0.04)	0.36
Testosterone/LH:				
AGD	0.98 (0.12, 1.83)	0.03	0.98 (0.12, 1.85)	0.03
Penile length	0.22 (−0.25, 0.68)	0.35	0.23 (−0.27, 0.74)†	0.36
Testicular vol	2.47 (1.18, 3.76)	<0.01	2.59 (1.27, 3.92)	<0.01

* Adjusted for age unless otherwise noted.
† Adjusted for age and body mass index.

birth to sons with shorter perineal length, linking environmental exposure and human genital development.[8,9] Hsieh et al demonstrated shorter anogenital distances in boys with genital anomalies (ie hypospadias and cryptorchidism), further establishing a link between normal genital development and perineal length in humans.[6]

A relationship between genital measures and testicular function was recently shown by 2 groups who related sperm production to anogenital distance in adult men. Mendiola et al examined healthy volunteers, and found a positive relationship between semen parameters and anogenital distance.[19] Our group simultaneously found differences in perineal lengths between infertile and fertile men.[20]

Several rodent studies have established a critical gestational masculinization programming window in which endocrine disruptors can permanently alter genital development, growth and function.[21,22] The current report establishes a similar relationship between genital development and hormone levels in humans. Indeed, Andersson et al demonstrated that infertile men have lower serum testosterone levels than fertile men, establishing that infertile men seem to have global testicular impairment.[23]

Welsh et al showed that if the genital lengths of a male rat were altered with flutamide in utero, they could not be rescued by postnatal testosterone treatment, suggesting that adult genital length may be firmly established in utero.[22] As the testicular dysgenesis syndrome hypothesizes an environmental cause for declining testicular function, increasing testis cancer and increasing genital anomalies, the current study links subtle defects in genital development to impaired adult function, suggesting that gestational exposure may have a critical role in testicular function, supporting a fetal origin of the testicular dysgenesis syndrome.

It is important to note that of the 4 hormones assessed in the current report (ie testosterone, estrogen, LH and FSH) only testosterone is synthesized in the testis. Thus, it is perhaps not surprising that a relationship with genital distance was only found for testosterone. While our laboratory does not routinely test for other testis derived hormones, it would be interesting to determine if antimüllerian hormone and inhibin levels were also correlated to genital lengths.

Certain limitations warrant mention. In a referral center for male infertility it was not always possible to blind observers to the men's diagnoses or fatherhood status which theoretically can lead to observer bias. However, hormone levels were not known at the time of genital measurement. Thus, investigators were blind to hormone measurements.

Table 3. *Comparison of AGD in men stratified by hypogonadal testosterone using the Wilcoxon rank sum test*

Testosterone cutoff (ng/dl)	Less than Cutoff		Cutoff or Greater		p Value
	No.	Mean AGD (SD)	No.	Mean AGD (SD)	
200	21	30.7 (2.1)	95	35.0 (1.4)	0.18
220	27	30.4 (1.8)	89	35.4 (1.5)	0.09
240	42	30.5 (1.5)	74	36.4 (1.7)	0.02
260	45	30.1 (1.4)	71	36.9 (1.7)	0.01
280	50	30.2 (1.4)	66	37.3 (1.8)	<0.01
300	62	31.6 (1.5)	54	37.3 (2.0)	0.02

In addition, although there is a known diurnal variation in hormone levels in men, phlebotomy occurred throughout the day and time of blood draw was not captured in data acquisition. As there would likely be a random distribution across genital lengths, such measurement variations would likely lead to a regression to the null. Nevertheless, an association was found. In addition, only men referred to and evaluated in our clinic were eligible for enrollment. Therefore, it is possible that our patient population does not represent all men.

Nevertheless, our study represents the first analysis to show an association between perineal length and androgen levels in men. Coupled with recent data examining adult AGD and sperm production, AGD may predict normal genital development in men and, therefore, could provide a novel metric to assess testicular function. If AGD at birth predicts adult AGD, our findings suggest that serum testosterone levels in adulthood depend on factors operating in the fetal period.

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