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

Michael L. Eisenberg,* Tina K. Jensen, R. Chanc Walters, Niels E. Skakkebaek and Larry I. Lipshultz†

From the Department of Urology, Stanford University School of Medicine, Palo Alto, California (MLE), Department of Environmental Medicine, Institute of Public Health, University of Southern Denmark, Winsloewsparken, Odense (TKJ), and University Department of Growth and Reproduction, Rigshospitalet, Copenhagen (NES), Denmark, and Division of Male Reproductive Medicine and Surgery, Scott Department of Urology, Baylor College of Medicine, Houston, Texas (RCW, LIL)

Abbreviations and Acronyms

AGD = anogenital distance

FSH = follicle-stimulating hormone

LH = luteinizing hormone

PL = penile length

Submitted for publication June 3, 2011.

Study received institutional review board approval.

* Correspondence: Department of Urology, Stanford University School of Medicine, 300 Pasteur Drive, Palo Alto, California 94305 (telephone: 650-723-4537; FAX: 650-498-5346; e-mail: eisenberg@stanford.edu).

† Financial interest and/or other relationship with Allergan Pharmaceuticals, Auxilium Pharmaceuticals, AMS, Pfizer and Repros Therapeutics.

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

0022-5347/12/1872-0594/0
THE JOURNAL OF UROLOGY®

Vol. 187, 594-598, February 2012
Printed in U.S.A.
DOI:10.1016/j.juro.2011.10.041

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.

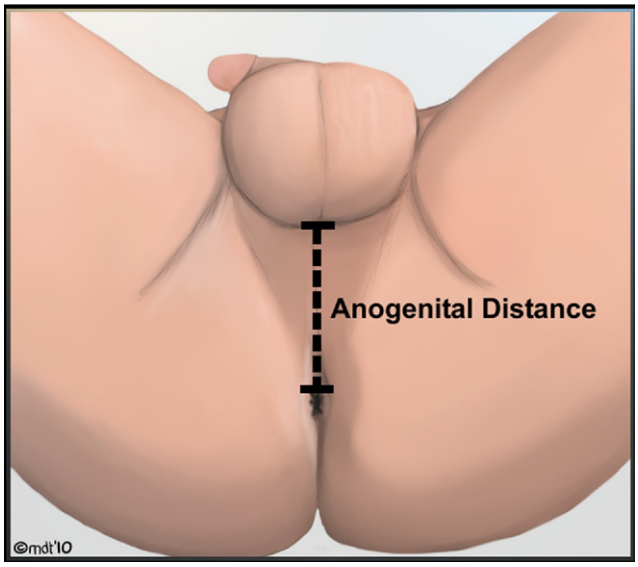

Figure 1. Anogenital distance as measured with men in supine, frog-leg position.

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