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Relationships between circulating and intraprostatic sex steroid hormone concentrations

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Abstract

Background—Sex hormones have been implicated in prostate carcinogenesis, yet epidemiological studies have not provided substantiating evidence. We tested the hypothesis that circulating concentrations of sex steroid hormones reflect intraprostatic concentrations using serum and adjacent microscopically-verified benign prostate tissue from prostate cancer cases.

Methods—Incident localized prostate cancer cases scheduled for surgery were invited to participate. Consented participants completed surveys, and provided resected tissues and blood. Histologic assessment of the ends of fresh frozen tissue confirmed adjacent microscopically-verified benign pathology. Sex steroid hormones in sera and tissues were extracted, chromatographically separated, and then quantitated by radioimmunoassays. Linear regression was used to account for variations in intraprostatic hormone concentrations by age, body mass index, race and study site, and subsequently to assess relationships with serum hormone concentrations. Gleason score (from adjacent tumor tissue), race and age were assessed as potential effect modifiers.

Results—Circulating sex steroid hormone concentrations had low-to-moderate correlations with —and explained small proportions of variations in—intraprostatic sex steroid hormone concentrations. Androstane-3α,17β-diol glucuronide (3α-diol G) explained the highest variance of tissue concentrations of 3α-diol G (linear regression r^2=0.21), followed by serum testosterone and tissue dihydrotestosterone (r^2=0.10), and then serum estrone and tissue estrone (r^2=0.09). There was no effect modification by Gleason score, race or age.

Conclusions—Circulating concentrations of sex steroid hormones are poor surrogate measures of the intraprostatic hormonal milieu.

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Impact—The high exposure misclassification provided by circulating sex steroid hormone concentrations for intraprostatic levels may partly explain the lack of any consistent association of circulating hormones with prostate cancer risk.

Keywords

Gonadal Steroid Hormones; Prostate; Prostate Neoplasms; Prostatectomy; Serum; Sex Hormone-Binding Globulin; Tissues

Introduction

Prostate cancer has long been hypothesized to have a hormonal pathogenesis. Endogenous sex steroid hormones—particularly androgens—are undoubtedly essential for normal physiological development, maintenance and function of the prostate gland. Pre-pubertally castrated men and male pseudo-hermaphrodites with deficient 5α-reductase type II have a mal-developed male phenotype including a small and immature prostate gland (1, 2). The Nobel Prize studies by Huggins and Hodges in 1941 reported that castration and injection of estrogen cause temporary regression of metastatic prostate cancer, implicating androgenic action in prostate cancer progression (3). This led to development of androgen deprivation therapy (ADT) which remains the mainstay therapy for men with advanced prostate cancer. Androgen signaling also functions in cell proliferation, differentiation, and apoptosis, and evidence from basic science indicates that androgens—and possibly estrogens—are critically important for prostate carcinogenesis (4–6).

Despite this evidence that implicates sex steroid hormones in prostate cancer pathogenesis, epidemiologic studies that have assessed prediagnostic circulating hormone concentrations have not found any consistent association with subsequent prostate cancer risk (7–9). There are various explanations for why a true association may have been missed, including inter-assay variability, lack of assay standardization, use of a single peripheral blood measurement typically at middle age or later, and case heterogeneity with inclusion of a variable proportion of indolent disease. Regardless of the true explanation, an inherent assumption of the prior observational studies is that circulating concentrations are proxies of the intraprostatic environment. Testosterone (T) and the more potent metabolite—dihydrotestosterone (DHT)—bind the androgen receptor within the prostate eliciting gene expression profiles and biological effects that maintain prostate function. T is predominantly produced by the testes and released into the circulation. DHT, however, is primarily produced within the prostate gland, thus circulating DHT precursors (T, androstenedione [A]) and metabolites (5α-androstane-3α,17β-diol glucuronide [3α-diol G]) have traditionally been assessed as proxies. The validity of these proxies has not been tested. Therefore, we set out to test the hypothesis that circulating sex steroid hormone concentrations are valid proxies of intraprostatic concentrations using a large set of blood samples paired with microscopically-verified benign tissue samples adjacent to prostate cancers.

Materials and Methods

Study Population

Patients were enrolled in the study between January 2000 and April 2004 at five locations: George Washington University Medical Center (Washington DC), University of California at San Francisco (San Francisco, CA), Doctor's Community Hospital (Lanham-Seabrook, MD), Washington Hospital Center (Washington DC), and INOVA Fairfax Hospital (Falls Church, VA), the latter three of which were primarily coordinated by the staff at George Washington University Medical Center. Study subject eligibility included: 18 years of age or older; scheduled for radical prostatectomy; and newly diagnosed with localized prostate cancer. Patients provided written informed consent to be part of the study. Prior to surgery, study patients had standard anthropometric measures taken and were administered a questionnaire to confirm that they were fasting and had not taken any hormones (e.g., DHEA) or substances that could potentially affect hormone concentrations (e.g., finasteride) in the preceding 24 hours. Study subjects also provided 30 ml of blood, which were processed within 4 hours into aliquots of serum, plasma, buffy coat and red cells, and subsequently stored for long-term storage at −70°C.

During surgery and immediately after the prostate had been resected, the pathologist conducted a sterile dissection of macroscopically benign tissue to obtain a maximum of three peripheral and three periurethral tissue samples, each weighing 200–400 mg. Each macroscopically benign tissue sample had the ends trimmed and placed in formalin for H&E slide preparation for morphologic and histologic evaluation (by IAS) to ensure that these trimmed ends of the tissue sample for hormone analysis were free of cancer. The main central piece of the tissue sample was placed in a pre-labeled cryovial, flash frozen in liquid nitrogen and stored at −70°C. All collected samples remained available for diagnosis until the surgical pathology report was deemed to be complete.

After surgery and histologic assessment of the remainder of the prostate, medical records and pathology review forms were abstracted. A 30-minute telephone questionnaire was administered to study subjects approximately six weeks after surgery to elicit information on personal characteristics, medical history, family history of cancer, medication use, and lifestyle exposures. This study was conducted in accordance with recognized ethical guidelines (e.g., Declaration of Helsinki, CIOMS, Belmont Report, U.S. Common Rule) and was pre-approved by the required institutional review boards.

Serum Hormone Quantitation

Serum levels of androstenedione (A), testosterone (T), dihydrotestosterone (DHT), 5α-androstane-3α,17β-diol glucuronide (3α-diol G), estrone (E_1), and estradiol (E_2) were measured at the University of Southern California (under the direction of FZS) by immunoassay methods during 2007. A, T, DHT, E_1, and E_2 were measured by RIA with preceding purification steps, including extraction of steroids with ethyl acetate:hexane (3:2) followed by Celite column partition chromatography of individual hormones using ethylene glycol as the stationary phase (10–13). For optimum chromatographic steroid separation, it was necessary to process A, T, E_1 and E_2 in one assay (hormone set 1 tubes) and DHT in

another assay (hormone set 2 tubes); 0.8 ml serum aliquots were used in each assay. In the first assay, chromatographic elution of A, T, E_1 and E_2 was achieved with isooctane, 40% toluene in isooctane, 25% toluene in ethyl acetate, and 40% toluene in isooctane, respectively. In the second assay, DHT was eluted with 10% toluene in isooctane. In each RIA, a highly specific antiserum was used in conjunction with an iodinated radioligand, and an appropriate 8-point standard curve was constructed. After an appropriate incubation period, antibody-bound and unbound hormones were separated by use of a second antibody. The resulting raw values were corrected for dilution factors and procedural losses.

3α-diol G was measured using a commercial 3α-diol G RIA kit (Diagnostics Systems Laboratories, Webster TX, presently Beckman-Coulter, Minneapolis, MD). The assay measured both isomers of 3α-diol G (14). It required no preceding purification steps and was validated extensively in the laboratory.

The assay sensitivities were as follows: 3, 0.5, 1.5, 50, 0.4 and 0.2 ng/dL for A, DHT, T, 3α-diol G, E_1 and E_2, respectively. Coefficients of variation (CVs)—calculated on the logarithmic scale using a mixed model which included an average of four blinded technical replicates from each of seven different individuals assayed at the same time as the sera for the main analysis across the four analytic batches—were all less than 15% (mean=10%, Supplementary Table 1).

Tissue Processing and Hormone Quantitation

Tissue processing and hormone quantitation were also conducted at the University of Southern California (by FZS) during 2008–2009. Each tissue sample was weighed prior to processing. For each man, we aimed to quantify hormones using two distinct peripheral and two distinct periurethral prostate tissue samples. For each tissue sample, approximately 100–400 mg of tissue was used for analysis of both sets of hormones (set 1: A, T, E_1 and E_2; set 2: DHT and 3α-diol G). The actual weight of tissue used for the assays depended on the amount of tissue that was available. After being weighed, each sample of tissue was immediately cut into small pieces and transferred to a vial to which 0.5 ml of 0.1 M phosphate-buffered saline (PBS), pH 7.4, was added. The tissue was then minced thoroughly and finely until a homogenate-like material was obtained before addition of a further 1.5 ml of PBS. One ml aliquots of the diluted homogenates were transferred to separate extraction tubes to be used for hormone quantitation. In order to monitor procedural losses, approximately 500 cpm (counts per minute of radioactivity) of each of the appropriate tritiated internal standards were added to each of the extraction tubes (^{3}H-A, ^{3}H-T, ^{3}H-E_1 and ^{3}H-E_2 were added to hormone set 1 tubes, and ^{3}H-DHT was added to hormone set 2 tubes). The internal standards had a high specific activity to ensure that no significant mass was being added. The contents in each tube were mixed and incubated at 37°C for 30 minutes. Hormones were extracted twice with 10 ml of hexane:ethyl acetate (3:2), and each time the organic layer was transferred to the same new tube. The remaining aqueous layer was saved for subsequent analysis of 3α-diol G. The organic solvents were evaporated under nitrogen at 40°C and the extracts re-dissolved in 1 ml of isooctane. Each re-dissolved extract was then applied on a Celite partition chromatography column and the individual hormones were eluted and quantified by RIA, as described in the previous section on serum hormone

quantitation. 3α-diol G was measured by direct RIA (Diagnostic Systems Laboratories, Webster, TX) in the aqueous fractions by use of a commercial kit, as described for 3α-diol G in serum. The tissue hormone concentrations are expressed as picograms per gram of wet weight of tissue. Tissue hormone assays had small technical variability—CVs which included an average of six blinded technical replicates from each of five different individuals across four of the 15 analytic batches and calculated on the logarithmic scale using a mixed model were all below 20% (mean=13.8%) with the exception of A which was 21% (Supplementary Table 1).

Statistical Analysis

For each man, we calculated mean tissue hormone concentrations using all peripheral and periurethral prostate tissue pieces assayed. Overall mean values for each subject were used for statistical analysis because a pilot study of 30 men recruited at George Washington University Medical Center that included a total of 171 prostate biopsies provided lower CV and higher ICC values compared with peripheral-specific mean values and periurethral-specific mean values (Supplementary Table 2).

To compare individual-level average intraprostatic hormone concentrations with serum hormone concentrations, we computed Spearman rank correlations with non-detectable values set to zero. Percent agreement between quartiles of serum hormone and quartiles of tissue hormones were calculated to determine accuracy of exposure in epidemiological studies that use quartile categorization as the main exposure. We regressed continuous natural log-transformed intraprostatic hormone concentrations on age (continuous), body mass index (kg/m^2; BMI, continuous), race (categorical: white/black/other or missing) and study site (categorical), and estimated the residuals of the outcome (“adjusted” continuous natural log-transformed intraprostatic hormone concentrations). We then regressed these residuals on categorical (quartile) and continuous (per quartile) serum hormone concentrations. Gleason score (from adjacent malignant tissue), race (white vs. black) and age were assessed as potential effect modifiers by conducting stratified analyses and p values for interaction were obtained using the likelihood ratio test to assess the difference between strata by inclusion of an interaction term in the linear regression model. Given that not all subjects had four tissue samples and that the resultant inequality in errors of mean tissue hormone concentrations among subjects may affect the results, we conducted a sensitivity analysis in which mean tissue hormone concentrations were estimated from two tissue samples per subject (for subjects with more than two tissue samples, we randomly selected two; subjects with one tissue sample were excluded).

Results

Table 1 shows the study population demographics. There were a total of 251 prostate cancer cases that had sufficient adjacent microscopically-verified benign tissue for hormone quantitation. The mean age at diagnosis was 60.1 years and the mean BMI was 27.2. Forty-two percent of cases had a prostate cancer Gleason score of 7 or greater. The average number of tissue samples assayed per patient was 3.4 after exclusions for evidence of

malignancy on slides of trimmed edges, and unsatisfactory slides for assessment of evidence of malignancy.

Table 2 shows the medians and interquartile ranges of the serum and tissue hormones. The serum values were within the reference ranges established for men (15). In serum, T had the highest median concentration, which was about 10 times higher than the median serum DHT concentration. In contrast, in tissue, DHT had the highest concentration and the median tissue DHT concentration was about 32 times higher than tissue T concentration. The median tissue DHT value was also higher (2.5 times) than the tissue 3α-diol G value, whereas in serum the median 3α-diol G value was about 9 times higher than DHT. In serum, the median A level was about one-seventh of median serum T, but in the tissue A was 2.7 times higher.

The unadjusted Spearman rank (r) correlations between serum and tissue hormones were low to occasionally moderate (Table 3). The strongest correlation was observed between serum 3α-diol G and tissue 3α-diol G (r=0.54), followed by serum and tissue E1 (r=0.40), and then serum T and tissue DHT (r=0.35). Unadjusted percent agreements between quartile categorizations of serum and intraprostatic hormones were often not greater than 25%, which is what would be expected by chance alone (Table 4).

The results of the multivariable linear regressions are shown in Table 5. After taking into account tissue hormone concentration variations attributable to age, BMI, race and study site, the correlations of, and variations explained by, serum hormone concentrations were generally low ranging. As per the Spearman rank and quartile-quartile correlations, circulating concentrations of 3α-diol G explained the highest variance of tissue concentrations of 3α-diol G in the regression analysis with an r^2 of 0.21 for the categorical model. This was followed by serum T and tissue DHT (r^2=0.10), and then serum E_1 and tissue estrone (r^2=0.09). Despite prior evidence of mild associations of tobacco smoking and alcohol consumption with circulating sex steroid hormone concentrations (16), our ability to adjust for these factors was limited given the large amount of missing data (Table 1). However, amongst those with smoking or alcohol information, adjustment for each of these factors did not affect associations between circulating and intraprostatic hormone concentrations. There was little evidence for effect modification by Gleason score, race or age upon stratified analysis (Supplementary Tables 3–5), with the exception that the serum estrone:tissue estrone relationship appeared to be stronger in black men compared with white men (p for effect modification by race=0.009, Supplementary Table 4). The sensitivity analysis in which mean tissue hormone concentrations were calculated from exactly two tissue samples per subject—and which included 237 of the 251 cases that had two or more tissue samples—did not materially affect the results from the main analysis.

Discussion

This study provides evidence that circulating sex steroid hormone concentrations have low-to-moderate correlations with—and explain small proportions of variations in—intraprostatic sex steroid hormone concentrations. Thus, circulating concentrations of sex steroid hormones likely have poor accuracy as surrogate measures of the intraprostatic

milieu. This high exposure misclassification of circulating sex steroid hormone concentrations may partly explain the consistent lack of any association of these metrics with prostate cancer risk.

Three prior smaller studies have also assessed the relationship of specific sex steroid hormones in the circulation and prostate tissue. The first included 121 prostate cancer cases and 57 benign prostatic hyperplasia (BPH) cases who underwent prostatectomy (17). Tissue-serum Spearman rank correlations were poor for T (PCa r=−0.18; BPH r=0.24) and for DHT (PCa r=−0.12; BPH r=−0.16). Although these correlations did not take into account variations due to covariates such as age, BMI and race, adjustment for such in our study did not increase predictive accuracy. The second prior tissue-serum comparison included just 16 prostate cancer cases undergoing radical prostatectomy (18). No correlation was found between intraprostatic DHT concentrations and circulating concentrations of T and DHT (neither local prostatic vein nor peripheral sera). The third prior study used LC-MS/MS to quantitate T and DHT concentrations from a single peripheral needle core biopsy for each of 196 Japanese men diagnosed with prostate cancer (19). The only strong correlation observed was that of serum T and serum DHT (r=0.77). Although these studies are smaller, did not conduct pathological review to exclude prostate cancer tissue from hormone analysis, and used a single tissue sample per subject, the findings are supportive of the inferences drawn from our study; namely, that circulating measures of sex steroid hormones are poor indicators of the intraprostatic hormonal environment.

Historically, researchers have focused their attention on circulating DHT precursors (predominantly T) and metabolites (3α-diol G) as proxies of intraprostatic androgen exposure, given the rapid intraprostatic enzymatic conversion of T to the highly-potent DHT by 5α-reductase type II. This is the reason why we observe high T:low DHT in the circulation yet low T:high DHT within the prostate. DHT can also be formed via a pathway that does not require T; androstenedione (A) is converted into 5α-androstanedione which can then be intraconverted with DHT via 17β-hydroxysteroid dehydrogenase (20). Although we didn’t measure 5α-androstanedione in this study, we did quantify A which is the parent precursor of both T and 5α-androstanedione. Once formed, DHT provides the androgenic action that is essential for normal prostate gland growth and function (1, 2). DHT has a relative binding affinity for androgen receptor three times that of T (21) and a 2–10 times greater potency (22). Thus, the elicited effect of DHT provides a stronger molecular cascade resulting in an androgen-regulated gene expression profile and associated biological effects. Although circulating precursors and metabolites of DHT are readily quantifiable in blood, our study provides evidence that they are poor surrogates of intraprostatic DHT concentrations.

Strengths of this study include macroscopic selection of benign tissues coupled with pathology assessment of trimmed edges to reduce the likelihood of hormone quantitation of malignant tissues, the use of multiple tissue samples to calculate mean tissue hormone concentrations for each individual, the use of a relatively large sample size to assess correlations with stratification by potential effect modifiers, and the use of well-validated RIAs with preceding purification steps to measure the serum and tissue androgens and estrogens and use of internal standards to follow procedural losses in the assays. The main

limitation of this study is the use of prostate cancer patients for analysis which may alter tissue-serum hormone correlations even if measured in benign tissue. In addition, tissue hormone concentrations appear to be variable within the prostate, a phenomenon which does not appear to be explained by differences between peripheral and periurethral tissue zones and which requires further study.

In conclusion, this study finds that circulating concentrations of sex steroid hormones are poor surrogate measures of the intraprostatic milieu. Future studies aiming to elucidate whether sex steroid hormones are implicated in prostate carcinogenesis should quantitate precancerous intraprostatic hormone concentrations using new technologies that only require small tissue samples, such as that available from needle biopsy (19, 23, 24).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Descriptive statistics of prostate cancer cases included in the study (n=251)

Demographic Variable	n
Age (years; mean [SD])	60.1 [7.1]
Body mass index (kg/m2; mean [SD])	27.2 [3.6]
Site (%)	
George Washington University	91 (36.3)
INOVA Fairfax Hospital	30 (12.0)
UC San Francisco	58 (23.1)
Washington Hospital Center	33 (13.2)
Doctor's Community Hospital	39 (15.5)
Race (%)	
White	135 (53.8)
Black	108 (43.0)
Other	6 (2.4)
Missing	2 (0.8)
Smoking Status (%)	
Never	10 (4.0)
Former	94 (37.5)
Current	20 (8.0)
Missing	127 (50.6)
Alcohol Use (%)	
Never/Former	113 (45.0)
Current	98 (39.0)
Missing	40 (15.9)
Gleason (%)	
<7	139 (55.4)
>=7	106 (42.2)
Missing	6 (2.4)

Table 2

Hormone medians and interquartile ranges

Serum (ng/dL) (n=251)	Overall (n=251) Median (IQR)	White (n=135) Median (IQR)	Black (n=108) Median (IQR)
Testosterone	468 (340, 621)	423 (333, 551)	518 (386, 662)
DHT	50.0 (38.3, 67.3)	45.2 (36.7, 59.1)	58.0 (46.6, 75.8)
3α-diol G	498 (366, 714)	470 (315, 735)	501 (404, 693)
Androstenedione	69.4 (51.7, 90.8)	68.2 (51.7, 91.2)	70.8 (54.8, 88.1)
Estrone	5.5 (4.3, 6.8)	5.0 (3.9, 6.1)	6.3 (4.7, 7.7)
Estradiol	3.2 (2.4, 4.1)	2.9 (2.1, 3.7)	3.8 (3.0, 4.5)
Tissue (pg/g W)			
Testosterone (n=248)	215 (165, 292)	215 (165, 284)	204 (165, 281)
DHT (n=251)	6811 (5300, 8058)	6465 (5045, 8066)	6915 (6006, 8048)
3α-diol G (n=247)	2696 (1899, 3557)	2725 (1861, 3572)	2686 (1966, 3503)
Androstenedione (n=232)	583 (419, 819)	566 (418, 837)	600 (425, 766)
Estrone (n=235)	72.6 (52.5, 99.1)	67.8 (50.3, 87.5)	80.1 (58.0, 107.5)
Estradiol (n=235)	47.3 (34.7, 65.4)	45.0 (34.1, 58.5)	53.2 (38.3, 68.5)

Table 3

Spearman rank correlations (r) between tissue hormones and serum hormones (continuous)

Tissue	Serum					
	Testosterone	**DHT**	**3α-diol G**	**Androstenedione**	**Estrone**	**Estradiol**
Testosterone	**0.18**	0.10	0.09	**0.19**	0.11	0.10
DHT	**0.35**	**0.30**	**0.12**	0.10	0.03	**0.16**
3α-diol G	**0.16**	**0.14**	**0.54**	**0.13**	0.02	0.11
Androstenedione	−0.02	0.00	0.12	**0.29**	**0.17**	0.02
Estrone	0.08	0.04	**0.18**	0.05	**0.40**	**0.32**
Estradiol	**0.14**	0.07	0.09	0.06	**0.22**	**0.30**

Missing values set to zero

Spearman Rank

Bolded coefficients significant at the 0.05 level

Table 4

Percent agreements between quartile categorizations of tissue and serum hormones

Tissue	Serum					
	Testosterone	DHT	3α-diol G	Androstenedione	Estrone	Estradiol
Testosterone	26	24	27	33	26	27
DHT	32	33	31	29	26	31
3α-diol G	29	24	45	24	25	29
Androstenedione	25	21	31	31	28	21
Estrone	26	23	27	24	35	32
Estradiol	26	21	24	25	25	28

Table 5

Multivariable Linear Regressions between Log Serum Hormone and Log Continuous Tissue Hormone

Hormone	β (95% CI)	p value	r	r^2
Serum Testosterone (ng/dL): Tissue Testosterone (pg/g W)				
<343.8				
343.8-<470.6	0.14 (−0.01, 0.30)	0.07		
470.6-<622.2	0.13 (−0.02, 0.29)	0.09		
>622.2	0.22 (0.07, 0.37)	0.005	0.18*	0.03*
continuous (per quartile)	0.06 (0.02, 0.11)	0.009	0.17¥	0.03¥
Serum DHT (ng/dL): Tissue DHT (pg/g W)				
<38.4				
38.4-<50.3	0.01 (−0.09, 0.11)	0.87		
50.3-<67.2	0.08 (−0.02, 0.18)	0.12		
>67.2	0.17 (0.07, 0.26)	0.001	0.24*	0.06*
continuous (per quartile)	0.06 (0.03, 0.09)	0.000	0.23¥	0.05¥
Serum 3α-diol G (ng/dL): Tissue 3α-diol G (pg/g W)				
<369				
369-<501	0.19 (0.07, 0.32)	0.002		
501-<716	0.25 (0.13, 0.38)	0.000		
>716	0.51 (0.38, 0.64)	0.000	0.46*	0.21*
continuous (per quartile)	0.16 (0.12, 0.20)	0.000	0.45¥	0.20¥
Serum Androstenedione (ng/dL): Tissue Androstenedione (pg/g W)				
<52.6				
52.6-<69.5	0.09 (−0.08, 0.25)	0.30		
69.5-<90.8	0.15 (−0.01, 0.31)	0.07		
>90.8	0.34 (0.18, 0.51)	0.000	0.27*	0.07*
continuous (per quartile)	0.11 (0.06, 0.16)	0.000	0.26¥	0.07¥
Serum Estrone (ng/dL): Tissue Estrone (pg/g W)				
<4.3				
4.3-<5.5	0.17 (0.03, 0.32)	0.022		
5.5-<6.9	0.24 (0.09, 0.39)	0.001		
>6.9	0.34 (0.19, 0.49)	0.000	0.30*	0.09*
continuous (per quartile)	0.11 (0.06, 0.15)	0.000	0.29¥	0.08¥
Serum Estradiol (ng/dL): Tissue Estradiol (pg/g W)				
<2.4				
2.4-<3.2	−0.01 (−0.18, 0.16)	0.88		
3.2-<4.1	0.06 (−0.11, 0.23)	0.48		
>4.1	0.23 (0.06, 0.40)	0.007	0.21*	0.04*
continuous (per quartile)	0.08 (0.02, 0.13)	0.005	0.18¥	0.03¥

Hormone	β (95% CI)	p value	r	r^2
Serum Testosterone (ng/dL): Tissue DHT (pg/g W)				
<343.8				
343.8-<470.6	0.16 (0.07, 0.26)	0.001		
470.6-<622.2	0.14 (0.05, 0.24)	0.004		
>622.2	0.25 (0.16, 0.35)	0.000	0.32 *	0.10 *
continuous (per quartile)	0.07 (0.04, 0.10)	0.000	0.29 ¥	0.08 ¥
Serum 3α-diol G (ng/dL): Tissue DHT (pg/g W)				
<369				
369-<501	0.04 (−0.06, 0.14)	0.40		
501-<716	0.00 (−0.10, 0.10)	0.99		
>716	0.08 (−0.02, 0.18)	0.14	0.11 *	0.01 *
continuous (per quartile)	0.02 (−0.01, 0.05)	0.26	0.07 ¥	0.01 ¥

Adjusted for age continuous, BMI continuous, race, and site

* r and r^2 for quartile model

¥ r and r^2 for continuous model (per quartile)