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Persistent Intraprostatic Androgen Concentrations after Medical Castration in Healthy Men

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Context: The impact of serum androgen manipulation on prostate tissue hormone levels in normal men is unknown. Studies of men with prostate cancer have suggested that prostatic androgens are preserved in the setting of castration. Tissue androgens might stimulate prostate growth, producing adverse clinical consequences.

Objective: The objective of the study was to determine the effect of serum androgen manipulation on intraprostatic androgens in normal men.

Design: Thirteen male volunteers ages 35–55 yr (prostate-specific antigen < 2.0 ng/ml; normal transrectal ultrasound) were randomly assigned to: 1) a long-acting GnRH-antagonist, acyline, every 2 wk; 2) acyline plus testosterone (T) gel (10 mg/d); or 3) placebo for 28 d. Serum hormones were assessed weekly. Prostate biopsies were obtained on d 28. Extracted androgens were measured by RIA, and immunohistochemistry for androgen-regulated proteins was performed.

Results: The mean decrease in serum T was 94%, whereas prostatic T and dihydrotestosterone levels were 70 and 80% lower, respectively, in subjects receiving acyline alone compared with controls ($P < 0.05$). Despite this decrease in prostate androgens, there were no detectable differences in prostate epithelial proliferation, apoptosis, prostate-specific antigen, and androgen receptor expression.

Conclusion: In this small study of healthy subjects, despite a 94% decrease in serum T with medical castration, intraprostatic T and dihydrotestosterone levels remained 20–30% of control values, and prostate cell proliferation, apoptosis, and androgen-regulated protein expression were unaffected. Our data highlight the importance of assessing tissue hormone levels. The source of persistent prostate androgens associated with medical castration and their potential role in supporting prostate metabolism deserves further study. **(*J Clin Endocrinol Metab* 91: 3850–3856, 2006)**

THE PROSTATE IS an androgen-sensitive organ. Normal prostate development is androgen-dependent, requiring not only testosterone (T) but also the activity of the 5α-reductase enzymes, which convert T to the more potent androgen dihydrotestosterone (DHT). In hypogonadal men, androgen replacement that raises serum T levels increases prostate size to age-appropriate levels. The prostate expresses high levels of type II 5α-reductase (1, 2) and enzymes involved in steroid metabolism (3, 4); therefore the endocrine milieu within the prostate may substantially differ from that of serum. For example, treatment with a 5α-reductase inhibitor minimally impacts serum T, yet results in substantial reduction in intraprostatic DHT with concomitant increases in intraprostatic T (5–7). Data regarding the relationship between serum and prostate tissue hormone levels in normal men are lacking.

Previous analyses of prostate tissue hormone levels obtained at the time of autopsy or organ donation have demonstrated that DHT is the dominant androgen in the human prostate, consistent with the high levels of intraprostatic 5α-reductase (3, 8, 9). In these studies, however, serum steroid levels were not available for comparison and may have been significantly affected by end-of-life physiological stresses, as they are with surgery or prostate or other disease states (10, 11). Reports of prostate hormone levels in surgical specimens from patients with prostate cancer or benign prostatic hypertrophy (BPH) have been conflicting regarding the effect of serum hormone manipulation on intraprostatic androgen concentrations (12–16). GnRH agonist treatment associated with castrate serum T levels results in a 75–80% decrease in prostatic T and DHT levels in BPH patients (16). In contrast, Mohler *et al*. (15) reported that despite prolonged castration treatments, intraprostatic T levels in hormone refractory prostate cancer were normal, whereas intraprostatic DHT levels were reduced by 75%. In addition, inhibition of 5α-reductase results in a significant decrease in intraprostatic DHT and increased prostate T, without impacting serum T levels (7, 17). Together, these results demonstrate that serum androgen concentrations do not reflect those in the prostate gland in disease states or postmortem and suggest that manipulation of serum androgens may not alter target tissue androgens in an equivalent manner. It is unclear from these

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Abbreviations: AR, Androgen receptor; BPH, benign prostatic hypertrophy; CV, coefficient of variation; DHEA, dehydroepiandrosterone; DHEA-S, DHEA sulfate; DHT, dihydrotestosterone; PSA, prostate-specific antigen; T, testosterone.

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reports, however, whether this difference is due to prostate diseases or whether these differences are present in healthy men.

We hypothesized that experimentally induced decreases in serum androgens in normal, middle-aged men would result in decreases in intraprostatic androgens and that this change in the androgenic milieu would alter prostate epithelial cell apoptosis and expression of androgen-regulated proteins. We performed a randomized, single-blind study of 12 healthy men to determine the effect of serum androgen deprivation and replacement on tissue androgen levels within the normal prostate. Tissue hormone levels were measured in prostate core biopsies obtained using a standard office prostate biopsy procedure to minimize the possible impact of operative medications and surgical stress on hormone measurements.

Subjects and Methods

Acyline

Acyline is a 10-amino acid peptide that acts as a GnRH antagonist (18). Acyline lyophilized powder synthesized by NeoMDS (San Diego, CA) was suspended in bacteriostatic water at 2 mg/ml and administered by sc injection in the abdomen. This dose has been previously demonstrated to render subjects medically castrate ($T < 1.7$ nmol/liter) within 24 h, with maintenance of castrate T levels for 2 wk (19).

Subjects

All procedures involving human subjects were approved by the Institutional Review Board at the University of Washington and performed in accordance with the guidelines in the Declaration of Helsinki. Of 14 men (ages 35–55 yr) recruited by advertisement, 13 met study criteria, including: normal medical histories; taking no medications; normal baseline physical examinations including prostate size by digital rectal exam and transrectal ultrasound; normal serum chemistries, complete blood count, gonadotropins, and T; and a prostate-specific antigen (PSA) of less than 2.0 ng/ml. One man was lost to follow-up after the first study visit and was not included in the analyses.

Protocol

After screening, subjects were randomly assigned to one of three treatment groups (n = 4 per group): 1) placebo vehicle injections sc plus placebo gel daily; 2) sc acyline 300 μg/kg d 0 and 14 plus placebo gel daily (acyline only); or 3) sc acyline 300 μg/kg d 0 and 14 plus T gel, 100 mg topically daily (Testim 1%, Auxilium Pharmaceuticals, Norristown, PA) (acyline+T). Blood was collected at baseline (d 0), weekly during treatment, and 1 month after drug exposure (recovery, d 56) for hormone analyses as well as biweekly for PSA. Transrectal ultrasound to measure prostate size was performed on d 0, 28, and 56 by the same operator to minimize operator-dependent error. On d 28, prostate tissue was obtained by laterally directed, transrectal ultrasound-guided biopsies of the peripheral zone after administration of local anesthesia (1% lidocaine). Two biopsies were obtained from each sextant for a total of 12 cores, each weighing 5–10 mg. For tissue hormone measurements, individual biopsies were immediately snap-frozen in liquid nitrogen. For immunohistochemistry, individual biopsies were immediately embedded in Optimal Cutting Temperature compound (Tissue-Tek, Pelco International, Redding, CA) and snap-frozen in isopentane precooled in liquid nitrogen. All samples were stored at −80 C. A study pathologist (L.D.T.) reviewed at least two biopsies from each subject. No evidence of malignancy or prostatic epithelial neoplasia was found in any case.

Measurements

Serum and tissue T and DHT. Prostate and serum androgens T and DHT were measured as described previously (20). In brief, thawed tissues were individually homogenized with an 8-mm probe at 4 C. Each homogenate was decanted to an extraction tube, the homogenization tube and probe were washed three times with diethyl ether, and the washes were transferred to the extraction tube. All samples were extracted by repeated inversion of the tubes, and the organic and aqueous phases were separated by centrifugation at 2500 rpm; the aqueous phase was frozen in a dry ice/ethanol bath, the ether was decanted and dried, and the extract was stored in ethanol until chromatography. Serum samples were similarly extracted in diethyl ether at 4 C. All extracted samples were subjected to chromatography on Sephadex LH-20 microcolumns to isolate T and DHT (and androstenedione for serum). The ether extract from each sample was applied to individual 1.0 g Sephadex LH-20 columns, and the estradiol and neutral fractions were collected using hexane:benzene:methanol (62:20:13) for elution as previously described (20). The neutral fractions were chromatographed on individual 2.5 g Sephadex LH-20 columns, and individual androgen fractions were collected using hexane:benzene:methanol (85:15:5) as the application and eluting solvent. Steroid concentrations of the appropriate fractions were estimated by RIA (21). Estimation of steroid losses during extraction and chromatography were monitored by recovery of added H^3T, C^{14} DHT (and H^3 androstenedione for serum, approximately 8000 cpm each) to equivalent weights of prostate tissue obtained from healthy individuals undergoing transurethral resection of the prostate or to equivalent volumes of control serum. The average percentage recovery for T, DHT, and androstenedione for both serum and tissue was 73.5, 69.7, and 75.2%, respectively. Six prostate core biopsies, one from each sextant, were analyzed per subject, and reported measurements average all values per tissue weight after correction for recovery and blank values. The lower limit of detection for each hormone in tissue was 0.38 ng/g. The intraassay coefficients of variation (CVs) were 7.9, 12.9, and 14%, respectively. All samples were measured in a single assay for each hormone.

Serum dehydroepiandrosterone (DHEA), DHEA sulfate (DHEA-S), and estradiol

Serum DHEA and DHEA-S were measured by RIA (Diagnostic Systems Laboratories, Webster, TX). Intraassay CVs for mid- and high-range values were 7.8 and 6.3% for DHEA and 10.6 and 7.3% for DHEA-S. Interassay CVs were 10.0% for DHEA and 7.8% for DHEA-S. Serum estradiol was assessed using a Roche Diagnostics Elecsys 2010 clinical assay instrument. Sensitivity was 18.3 pmol/liter, and intraassay CVs were 3.7 and 2.8% for mid- and high-range values, whereas the interassay CVs were 4.7%. The normal range for serum estradiol in this assay in men was 40–220 pmol/liter. All samples were run in the same assay.

Serum gonadotropins. FSH and LH levels were measured by immunofluorometric assay (Delfia, Turku, Finland). Samples from a given individual were measured in a single assay. The sensitivity of the assay for FSH and LH was 0.016 and 0.019 IU/liter, respectively. For low-, mid-, and high-pooled values of 0.054, 1.04, and 20.8 IU/liter of FSH, the intraassay CVs were 12, 1.9, and 2.9%, and the interassay CVs were 18, 6.1, and 4.1%, respectively. For low-, mid-, and high-pooled values of 0.056, 0.95, and 15.6 IU/liter of LH, the intraassay CVs were 6.5, 3.9, and 5.4%, and the interassay CVs were 21, 8, and 6.6%.

Clinical chemistries and PSA. Screening and monitoring labs for PSA, complete blood count, electrolytes and glucose (chemistry 7), calcium, and liver function tests were measured by the Department of Laboratory Medicine, University of Washington. PSA was assessed using a Hybridtech Enzyme Immunoassay. Inter- and intraassay CVs for this assay are 2–4%.

Immunohistochemistry

Five-micron frozen tissue sections were stained for the following antigens using a three-step indirect avidin-biotin immunoperoxidase method: Ki-67 (monoclonal MIB-1, Dako Inc., Carpinteria, CA), a marker of cell proliferation; PSA (polyclonal, Dako Inc.); androgen receptor (AR) (monoclonal F36.4.1, BioGenex, San Ramon, CA); and caspase-3 (monoclonal 9661, Cell Signaling Technology, Inc., Danvers, MA), a marker of apoptosis. Specificity of labeling was confirmed by omission of the primary antibody. All sections for the entire study were prepared in a single batch for each stain. Ki-67 index was calculated as the number of

positively staining nuclei divided by the total gland area for that cross-section; at least five glands were tabulated for each subject. For PSA, AR, and caspase-3, analyses were performed using Image Pro-Plus version 4.2 software (Media Cybernetics, Silver Spring, MD). All images for analyses were made at 40× with the same light intensity and color thresholding for all sections for each stain. The percentage of total epithelial (for PSA and caspase-3) or nuclear (for AR) area positively stained in a minimum of five glands and/or 500 epithelial cells was assessed. Successive tabulations were accumulated until the marginal change in the coefficient of variation of the running mean value (for the percentage area positively stained) was less than 5% (22).

Statistical analysis

Hormone measurement data were log-transformed before analysis. For differences between groups, data were compared by ANOVA with a Scheffé *post hoc* correction for multiple comparisons. For within-group comparisons to baseline, paired *t* tests were performed with a Bonferroni correction for multiple comparisons. Pearson correlations were performed to examine the relationship between serum and intraprostatic hormone concentrations. P values < 0.05 were considered statistically significant. Statistical analyses were performed using STATA version 8.0 (Stata Corp., College Park, TX).

Results

Study population

The baseline characteristics of the study subjects are given in Table 1. The subjects randomized to the placebo group were older, with greater average prostate volumes at baseline. There were no significant differences between baseline hormone values between groups, with the exception of slightly lower LH levels in the acyline+T group.

There were no serious adverse events during the study. The subjects receiving acyline had minor erythema and pruritis at the injection site that resolved within 24 h, as has been reported previously (19). Two men in the acyline group noted hot flashes that resolved during the first week of recovery. One subject in the acyline+T group noted hot flashes during recovery that resolved by d 56.

Serum gonadotropins

LH and FSH declined in both groups receiving the GnRH antagonist, acyline, during treatment and were unchanged in the placebo group (Table 2). In both groups treated with acyline, there was a significant decrease in LH compared with baseline and placebo with treatment ($P < 0.05$ for both comparisons). Similarly, FSH was significantly lower in both the acyline-treated groups compared with both placebo and baseline and was lower in the acyline+T group compared with acyline alone ($P < 0.05$).

TABLE 1. Baseline characteristics of study subjects

	Placebo	Acyline	Acyline+T
No. per group	4	4	4
Age (yr)	46.5 ± 3.5	39.8 ± 2.6	41.8 ± 1.6
BMI (kg/m^2)	28.6 ± 2.9	24.1 ± 0.5	28.1 ± 1.5
T (nmol/liter)	12.2 ± 3.9	23.7±2.4	12.6 ± 2.2
LH (IU/liter)	5.9 ± 0.9	6.5 ± 0.7	3.5 ± 0.2
FSH (IU/liter)	4.5 ± 1.2	3.3 ± 0.3	2.3 ± 0.1
PSA (ng/ml)	0.5 ± 0.2	0.8 ± 0.1	0.8 ± 0.1
Prostate volume (cm^3)	27.0 ± 2.0	18.7 ± 1.4	22.5 ± 2.4

Data represent mean ± SEM.

Serum sex steroid levels

In the subjects receiving acyline alone, serum T and DHT declined significantly, compared with both baseline and the placebo and acyline+T groups, at all time points during the drug exposure period ($P < 0.05$) (Table 2 and Ref. 23). In the acyline-only group, serum T concentrations during treatment were near or below castrate levels (T < 1.7 nmol/liter) throughout the 28 d of treatment (23). There were no significant differences in serum T between the placebo and acyline+T groups at any time point, nor within these groups compared with baseline; therefore T supplementation maintained physiological T levels in men receiving acyline+T. Serum DHT levels were slightly increased in subjects receiving acyline+T compared with the placebo group on d 7 and 21 (23), but this difference was not significant on d 28 or at recovery.

Serum estradiol declined by approximately 50% in the subjects receiving acyline compared with baseline and with the acyline+T group throughout the drug exposure period ($P < 0.05$) (Table 2). Serum SHBG, androstenedione, DHEA, and DHEA-S were unchanged in all three groups throughout the treatment and recovery periods, and no differences were noted between the groups.

Prostate tissue androgens

Prostate tissue T and DHT measured on d 28 of treatment were significantly lower in subjects receiving acyline alone compared with the other two groups ($P < 0.05$) (Fig. 1). Subjects receiving acyline alone had prostate T levels approximately 70% lower than the placebo group and 60% lower than subjects treated with acyline+T (Fig. 1A). Prostate DHT levels were similarly lower in subjects receiving acyline alone, reduced by approximately 80 and 70% compared with levels in subjects receiving placebo or acyline+T, respectively (Fig. 1B). There were no significant differences in prostate tissue androgens between the two control groups (placebo and acyline+T). The ratio of intraprostatic DHT:T was 5.0, 3.5, and 4.9 for placebo, acyline, and acyline+T, respectively.

Comparison of prostate and serum androgen levels

In the placebo group, the ratio of prostate to serum T levels was 0.5 (Fig. 1A), whereas DHT was the dominant androgen in the prostate, 22-fold more concentrated in tissue than serum in subjects in the placebo group (Fig. 1B). With acyline treatment, both serum and prostate T and DHT were lower, but the relative ratio of T in the prostate *vs.* serum increased to 2, whereas the ratio of DHT in prostate to serum was comparable to the placebo group (19 compared with 22). The acyline+T group maintained tissue:serum T levels in the same range as the placebo group, and, due to an increase in serum DHT, had a relative decrease in the ratio of tissue: serum DHT levels to 5.

We examined the association between serum and prostate tissue androgens in all three groups. When all three groups were considered, there was a significant correlation between serum T and prostate androgens (r = 0.79 for serum T *vs.* prostate T, $P = 0.002$; and r = 0.81 for serum T *vs.* prostate

TABLE 2. Serum hormone levels and prostate size

	Placebo			Acyline			Acyline+T		
	Baseline	Day 28	Recovery	Baseline	Day 28	Recovery	Baseline	Day 28	Recovery
T (nmol/liter)	12.2 ± 3.9	12.4 ± 3.0	10.8 ± 1.1	23.7 ± 2.4	0.9 ± 0.3[b]	25.9 ± 1.7	12.6 ± 2.2	16.7 ± 5.8	13.4 ± 2.0
DHT (nmol/liter)	2.0 ± 0.7	1.4 ± 0.5	1.7 ± 0.6	3.7 ± 0.5	0.3 ± 0.1[b]	3.0 ± 0.3	3.5 ± 1.9	4.8 ± 2.3	2.5 ± 0.7
DHT:T	0.16 ± 0.02	0.11 ± 0.04	0.16 ± 0.04	0.16 ± 0.01	0.33 ± 0.08	0.12 ± 0.01	0.28 ± 0.09	0.29 ± 0.06	0.19 ± 0.03
LH (IU/liter)	5.9 ± 0.9	4.6 ± 1.1	4.9 ± 1.2	6.5 ± 0.7	0.8 ± 0.4[b]	10.3 ± 2.3	3.5 ± 0.2	0.1 ± 0.1[b]	4.0 ± 1.0
FSH (IU/liter)	4.5 ± 1.2	4.7 ± 1.3	4.5 ± 1.2	3.3 ± 0.3	0.4 ± 0.1[b]	2.8 ± 0.4	2.3 ± 0.1	0.1±0.02[b]	2.0 ± 0.1
Androstenedione (nmol/liter)	1.4 ± .4	1.7 ± 0.8	1.4 ± 0.4	1.0 ± 0.4	2.4 ± 0.4	2.1 ± 0.7	1.7 ± 1.0	1.4 ± 0.4	1.7 ± 0.8
DHEA (nmol/liter)	15.8 ± 2.0	19.3 ± 5.7	16.3 ± 2.0	19.8 ± 2.3	19.8 ± 2.9	28.8 ± 3.0	14.6 ± 3.0	15.0 ± 3.9	21.3 ± 6.4
DHEA-S (pmol/liter)	3.7 ± 1.0	4.7 ± 1.4	3.3 ± 9.8	5.9 ± 1.8	5.5 ± 2.2	5.3 ± 2.5	4.4 ± 1.2	4.4 ± 1.2	3.8 ± 0.8
Estradiol (pmol/liter)	118 ± 13	112 ± 13	128 ± 20	148 ± 13	61 ± 10[a]	181 ± 13[a]	128 ± 11	143 ± 29	159 ± 13
SHBG (nmol/liter)	30 ± 9	27 ± 8	32 ± 9	51 ± 13	50 ± 12	44 ± 14	28 ± 6	22 ± 3	24 ± 7
PSA (ng/ml)	0.5 ± 0.2	0.5 ± 0.2	0.5 ± 0.2	0.8 ± 0.1	0.3 ± 0.1[a]	0.8 ± 0.4	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.5
Prostate volume (cm^3)	27.0 ± 2.0	24.2 ± 1.5	20.7 ± 1.8	18.7 ± 1.4	17.2 ± 0.8	18.5 ± 1.4	22.5 ± 2.4	24.8 ± 2.5	24.3 ± 1.8

Baseline, d 28 (the day of the prostate biopsy), and recovery measures are shown. Values are expressed as mean ± SEM. To convert from SI to metric units, divide by 3.67 for estradiol in picograms per milliliter; by 34.7 for SHBG in micrograms per deciliter; by 0.0347 for T in nanograms per deciliter; and by 0.0344 for DHT in nanograms per deciliter.

[a] $P < 0.05$ *vs.* baseline.

[b] $P < 0.05$ *vs.* baseline and placebo.

DHT, $P = 0.001$). In contrast, there was no significant correlation between serum DHT (r = 0.5 for serum DHT *vs.* prostate T, $P = 0.1$; r = 0.5 for serum DHT *vs.* prostate DHT, $P = 0.08$), DHEA, DHEA-S, or androstenedione (data not shown) and prostate tissue androgens.

Limiting the analyses to the medically castrate group (acyline alone), there was no correlation between serum T and DHT and prostate tissue androgen levels (data not shown).

However, when we examined the relationship between adrenal androgens in the medically castrate subjects, there was a strong positive correlation between serum DHEA and prostate androgen levels (r = 0.94 for prostate T, $P = 0.06$; and r = 0.99 for prostate DHT, $P = 0.006$).

Serum PSA and prostate volume

Serum PSA was significantly reduced by d 28 of treatment compared with baseline only in those subjects receiving acyline alone (Table 2), and serum PSA recovered to baseline levels 1 month after cessation of drug. The PSA differences between groups were not significant. There were no significant differences in prostate volume between the groups, and prostate volume was maintained in all groups throughout the study.

Prostate immunohistochemistry

Because androgens play a role in prostate growth, we evaluated prostate epithelial cell turnover in our study biopsies. Neither Ki-67 index, a measure of prostate epithelial cell division that is hormone sensitive (24), nor caspase-3 expression (as a measure of apoptosis) (25–27), was significantly different between subjects receiving acyline and the control groups (Table 3). We found no detectable difference in the cellular area of PSA or AR immunostaining [two genes

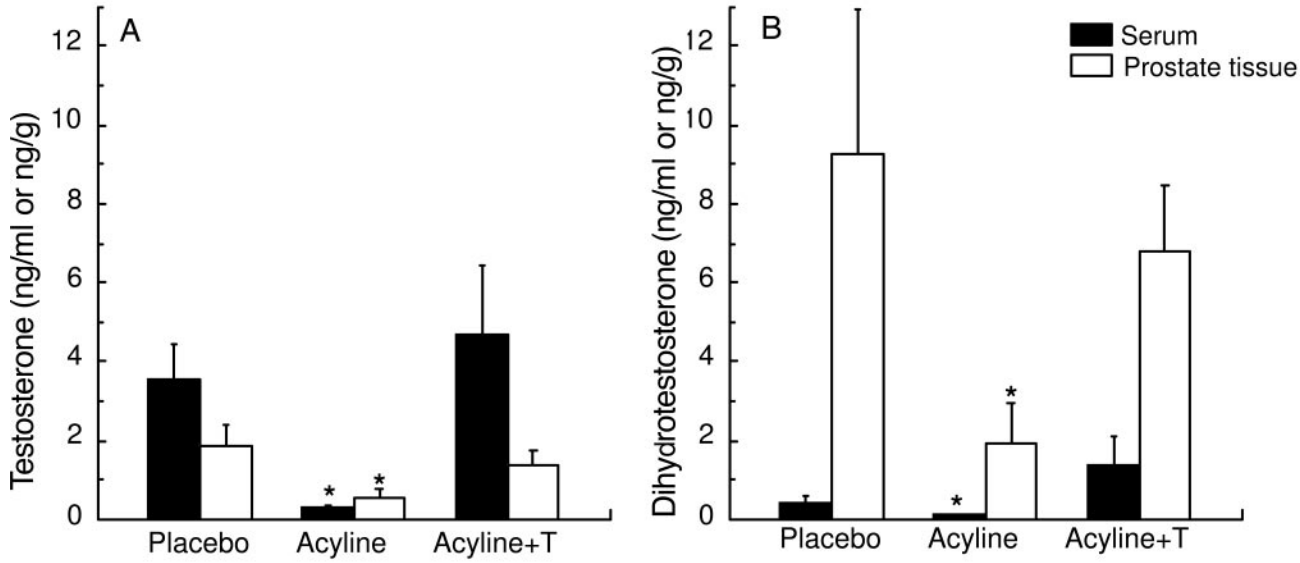

FIG. 1. Comparison of prostate and serum androgen levels. Serum androgens (nanograms per milliliter in *black*) on d 28 and prostate androgens (nanograms per gram in *white*) are compared. *, $P < 0.05$ *vs.* baseline. Error bars are ± SEM.

TABLE 3. Quantitative immunohistochemistry

	Placebo	Acyline	Acyline+T
Ki-67 labeling index (no. of Ki-67 positive nuclei/gland area × 10,000)	3.3 ± 0.9	3.5 ± 1.3	3.4 ± 1.6
AR (% positive nuclei)	57 ± 5	36 ± 2.6	41.8 ± 1.6
PSA (% epithelial area positively stained)	47 ± 8	39 ± 12	42 ± 53
Caspase-3 (% epithelial area positively stained)	35 ± 10	36 ± 3	49 ± 5

Data are expressed as mean ± SEM.

known to be androgen-regulated (28, 29)] between the medically castrate group and the control subjects (Table 3).

Discussion

To our knowledge, this is the first study to measure prostate tissue hormone levels in normal, unanesthetized men without prostate disease. Using prostate tissue cores from these healthy volunteers, we demonstrate that whereas T is the principal androgen in serum, DHT is the predominant androgen within the prostate gland, where it is present in concentrations roughly 6-fold greater than T (Fig. 1). This ratio is comparable to previous reports examining prostate androgen concentrations in the setting of BPH (12, 30) and prostate cancer (15). Furthermore, we found that in response to 1 month of GnRH antagonist treatment, which decreases serum T and DHT to castrate levels, prostate tissue androgens decreased by approximately 70 and 80%, respectively, compared with controls (Fig. 1). This effect resulted from the decrease in circulating T, because it was not observed in subjects treated with acyline+T (Fig. 1).

Despite a markedly lower level of prostate androgens in subjects treated with acyline for 1 month compared with placebo (Fig. 1), tissue DHT and T were still easily detected in the acyline-alone group. In fact, the absolute level of prostate DHT in the medically castrate group remained remarkably high (1.9 ng/g), approximately 20-fold higher than serum DHT (0.1 ng/ml, Fig. 1B) and comparable to levels of serum T in intact men (3.6 ng/ml, Fig. 1A). Similar levels of DHT have been found in other studies of longer-term medical castration for prostate cancer (14, 31). Because DHT is thought to be 10-fold more potent than T (32), this level of prostate DHT may have significant biological activity. Our immunohistochemistry results support this hypothesis because there were no detectable changes in prostate epithelial cell proliferation (Ki-67 labeling index), PSA and AR expression, or apoptosis after 1 month of androgen deprivation in normal men. These findings suggest that the level of prostate tissue androgens preserved, albeit significantly lower levels than controls, in these medically castrate men might be sufficient to support several biological processes within the normal prostate. Our data demonstrate that serum and tissue hormone manipulations may not be equivalent in healthy subjects, and suggest that examination of tissue hormone levels may be an important endpoint in understanding human physiology.

Our tissue hormone results are similar to those measured after prostatectomy in men with BPH treated for 3 months with a GnRH agonist (12). However, they are in contrast to recent observations by Mohler *et al*. (15) who found a preservation of prostate T concentrations in hormone-refractory prostate cancer despite a minimum of 6 months of medical or surgical castration. The reason for the discrepancy between the effects of castration on intraprostatic T concentrations reported here and those of Mohler *et al*. (15) is not known but may be a function of evaluating different tissue types (normal *vs.* androgen independent prostate cancer) or the duration of treatment.

The source of prostate androgens in the setting of medical castration is not clear from our study. Although it is possible that intraprostatic androgens arise from residual testicular-derived serum androgens, the prostate has been shown to express, at least at the transcript level, all the enzymatic machinery required to convert adrenal precursors such as androstenedione, DHEA, and DHEA-S to DHT (3, 4), and increased expression of enzymes involved in androgen metabolism has recently been demonstrated in androgen-independent prostate cancer (29). The hypothesis that adrenal androgens become an important source of prostate androgen precursors in the setting of low serum T associated with medical castration is supported by our observation that there is a strong correlation between serum DHEA (but not T or DHT) and prostate tissue hormone levels among subjects receiving acyline alone (albeit in a small number of subjects). The effectiveness of "androgen ablation" therapy in the treatment of hormone-sensitive disease relies upon the achievement of low serum T levels as a clinical endpoint. If non-gonadotropin-dependent androgens can arise from the conversion from adrenal precursors within the prostate gland itself, the achievement of castrate serum androgen levels may be insufficient for true androgen ablation within the prostate and may contribute to the emergence of recurrent disease. The possible relationship between adrenal and intraprostatic androgens in the setting of medical castration should be further examined in a greater number of subjects to confirm these suggestive findings. Determining the source and clinical impact of remaining tissue androgens after longer-term medical castration will require further study.

The absolute levels of intraprostatic androgens measured in our study are somewhat higher than those reported by others in prostate cancer and BPH (5, 7, 16, 30), but are consistent with early results in normal prostate from Geller *et al.* (33) and may be related to our sampling of younger men who may have higher intraprostatic androgens (34). This may be attributable to our use of flash-frozen needle biopsy material. The needle biopsy procedure, unlike autopsy studies and specimens collected during surgery, does not entail warm ischemia time, which has been shown to result in significant decrements in intraprostatic DHT (30). The same is likely true for T, given the high concentration of intraprostatic 5α-reductase. Nor does our method require the use of general anesthesia or occur after significant stress associated with end of life, both of which have been associated with significant reductions in serum androgens (10). In addition, the average age of our subjects was lower than those enrolled in previous studies, and it has been suggested that tissue androgens, like those in serum, may fall with age (34).

Our study has some limitations. The number of subjects per group is small. Other studies with more subjects have

examined men who were undergoing prostate biopsy for cause (*i.e.* symptoms, rising PSA); thus our data are unique in studying normal, healthy volunteers. However, due to the small sample size, we may have missed small differences between groups in epithelial cell turnover and androgen-regulated protein expression. Our results may be impacted by the length of treatment. Although the half-life of serum T is in the order of hours (35), the half-life of tissue androgens is not known, and it is possible that longer periods of androgen ablation are required to see changes in prostate epithelial cell proliferation or apoptosis. Very small but detectable changes in total and transitional zone volume have been observed with treatment using a potent 5α-reductase inhibitor as soon as 1 month in a very large cohort (36), although a previous small study of a GnRH agonist failed to detect changes in prostate size at 1 month (37). Interestingly, we observed a decrease in serum PSA (Table 2) in the acyline-treated subjects, whereas there was no difference in prostate tissue PSA staining (Table 3). We are not the first to observe a lack of correlation between serum and tissue PSA levels (24, 38). It will be important in future studies to determine whether these factors change with a longer treatment period. On the other hand, our period of observation may have been too long, because prostate cells may be able to adapt to changes in androgen concentration by regulation of downstream effectors. For example, Bozec *et al.* (39) reported that caspase-3 expression increased after 3 d of finasteride treatment, however it returned to baseline after 30 d of treatment. Similarly, Ohlson *et al.* (40) recently demonstrated that in both neoplastic and adjacent normal prostate epithelium, maximum increases in apoptosis and suppression of proliferation occurred 3 d after castration, yet returned to baseline by 8–10 d after castration. Future studies examining the impact of serum androgen manipulation on tissue hormone concentration over time are warranted. It is also possible that although we achieved castrate serum T levels, the degree of gonadotropin and testicular suppression was not adequate to reduce maximally prostate androgens. Finally, in an effort to limit the morbidity associated with the study procedures, biopsies were limited to the prostate peripheral zone, the area where the majority of prostate cancers arise. Some investigators have found differences in prostate androgen concentrations among different zones (peripheral *vs.* transitional zones) of the prostate in subjects with BPH (41); it is possible that we may have missed regional differences in prostatic androgen concentrations.

In conclusion, in this randomized, controlled trial we determined the level of prostate T and DHT in the normal, human prostate as well as the effect of GnRH antagonist treatment for 1 month on intraprostatic androgens. The ratio of DHT:T within the prostate is preserved in the setting of medical castration. Despite more than 90% suppression of serum androgens, intraprostatic T and, to a lesser extent, DHT are not suppressed to the same degree after 1 month of acyline treatment. Levels of the potent androgen DHT within the prostate gland after 1 month of medical castration may continue to support androgen-dependent protein expression because we observed no differences in prostate epithelial cell proliferation, apoptosis, PSA, or AR expression after 1 month of acyline treatment. These findings highlight the importance of assessing tissue hormone levels in understanding prostate endocrine physiology.

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