Serum Androgens and Prostate Cancer

Abraham M. Y. Nomura, Grant N. Stemmermann, Po-Huang Chyou, Brian E. Henderson, and Frank Z. Stanczyk

Japan-Hawaii Cancer Study, Kuakini Medical Center, Honolulu, Hawaii 96817 [A. M. Y. N.]; Department of Pathology, University of Cincinnati Medical Center, Cincinnati, Ohio 45267 [G. N. S.]; Department of Epidemiology and Biostatistics, Marshfield Medical Research Foundation, Marshfield, Wisconsin 54449 [P. H. C.]; and Departments of Preventive Medicine [B. E. H.] and Obstetrics and Gynecology [F. Z. S.]; University of Southern California, Los Angeles, California 90033

Abstract

It is suspected that male hormones are associated with the risk of prostate cancer. To test this hypothesis, we conducted a nested case-control study in a cohort of 6860 Japanese-American men examined from 1971 to 1975. At the time of examination, a single blood specimen was obtained, and the serum was frozen. After a surveillance period of more than 20 years, 141 tissue-confirmed incident cases of prostate cancer were identified, and their stored sera and those of 141 matched controls were assayed for total testosterone, free testosterone, dihydrotestosterone, 3α-androstanediol glucuronide, androstenedione glucuronide, and androstenedione. Odds ratios for prostate cancer, based on quartiles of serum hormone levels, were determined using conditional logistic regression methods. The odds ratios for the highest quartiles were 1.37 (95% confidence interval, 0.73–2.55) for 3α-androstanediol glucuronide and 1.24 (95% confidence interval, 0.62–2.47) for androstenedione, but none of the differences was statistically significant. The results were unremarkable for the other four hormonal measurements. In addition, the patients and controls were compared by hormonal ratios (i.e., total testosterone:dihydrotestosterone), but the results were also unremarkable. The findings of this study indicate that none of these androgens is strongly associated with prostate cancer risk.

Introduction

Prostate cancer is now the most frequently diagnosed cancer among men in the United States (1). It is estimated for 1996 that 317,100 new cases will be diagnosed in the country, representing 41% of all new cancer cases among men. There is a high index of suspicion that male hormones have a role in the development of prostate carcinoma. Androgens are required for the growth, maintenance, and functional activity of the prostate gland. Eunuchs whose testes have been removed or never developed have not been clinically observed to develop prostate carcinoma (2, 3). The enzyme 5α-reductase converts testosterone to dihydrotestosterone (4), which is the principal intracellular androgenic hormone, binding to androgen receptors in the nuclei of prostate cells (5). A recent study (6) suggested that 5α-reductase activity is related to prostate cancer risk. Finasteride, which is a competitive inhibitor of 5α-reductase, is currently being administered in a large 7-year clinical trial, which will enroll 18,000 men, to determine whether finasteride decreases the incidence of prostate cancer (7, 8). Because of the importance of this issue, we conducted a prospective study to determine whether 5α-reductase activity is associated with the risk of developing prostate cancer.

This study is focused on American men of Japanese ancestry who have recently experienced a marked change in prostate cancer risk. Their annual prostate cancer incidence rate in Hawaii has increased from 24.6 per 100,000 men in 1968–1972 (9) to 34.4 per 100,000 men in 1983–1987 (10), which resulted in a 40% increase. In comparison, Japanese men in Osaka Prefecture had an annual prostate cancer incidence rate of 6.6 per 100,000 men in 1983–1987 (10). As a result, studying Japanese men in Hawaii provides us with an opportunity to identify hormonal factors related to prostate cancer risk in a transition group for this disease.

Materials and Methods

Study Population. From 1965 to 1968, 8006 Japanese-American men were examined by the Honolulu Heart Program on the Hawaiian island of Oahu. They were born from 1900 to 1919 and were 45 to 68 years of age at the time of examination. Approximately 6 years later, 6860 of these men returned for another round of examinations from 1971 to 1975. At that time, a nonfasting venous blood sample was obtained. The sera were stored at −75°C. The data collected on these men included birthplace, marital status, religion, education, history of alcohol use, cigarette smoking history, blood pressure, and body mass index, based on weight (kg)/height (m²). Serum cholesterol values were determined by the AutoAnalyzer N-2A4 method, and serum glucose values were determined 1 h after a 50-g glucose load had been given by the AutoAnalyzer N-2B method (11). Twenty-eight of the 6860 men had been diagnosed with prostate cancer at the time of examination and were thus excluded from the study.

To identify cases of prostate cancer occurring in the cohort during the study, discharge records of all general hospitals on Oahu were monitored. To reduce the possibility of missing diagnosed cases, a computer linkage file was established with the Hawaii Tumor Registry, a member of the Surveillance, Epidemiology and End Results Program of the National Cancer Institute. The data should be nearly complete, because only 2.5% of the 6832 men could not be located on Oahu during a survey completed in 1993.

There were 281 cases of prostate carcinoma diagnosed

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from 1971 to 1993 and confirmed by examination of tissue obtained surgically or by biopsy. An additional 23 cases were diagnosed clinically but were not confirmed histologically and were excluded from the study. Resources were not available to measure serum hormone levels in all 281 cases and their controls. Consequently, alternating cases based on date of diagnosis were removed from the study, leaving 141 tissue-confirmed incident cases for the investigation.

Each case patient was matched with one control subject from the study. Of the 6528 subjects in the control pool, 1067 (16.3%) were excluded because they were diagnosed with cancer of another site after the serum collection. This was done because serum samples from these men were being used for other studies. Of the remaining 5461 men, 141 (2.6%) were matched to patients with prostate cancer. The controls were selected so that each case-control pair had the same h of examination, the same age, except four pairs (median difference, 1.1 year), and the same month and year of examination, except 20 pairs (median difference, 1.8 months). The control subjects were alive and did not have any cancer diagnoses at the time of the diagnoses of the matched cases. Therefore, death was not a competing risk in this study.

The frozen sera, which had never been thawed before, were sent in dry ice to Los Angeles for analysis. The laboratory technician could not distinguish sera of cases from those of controls and treated them identically in the analysis.

**Laboratory Analysis.** Serum levels of testosterone (total), dihydrotestosterone, androstenedione, 3-α-androstanediol glucuronide, and androsterone glucuronide were quantified by validated specific RIAs in the Reproductive Endocrine Research Laboratory of one of the authors (F. Z. S.; University of Southern California, School of Medicine). Testosterone, dihydrotestosterone, and androsterone were first extracted from serum with hexane:ethyl acetate (1:1) and subjected to Celite column partition chromatography prior to RIA (12-14). 3-α-Androstanediol glucuronide was measured directly in serum (15). Androsterone glucuronide was first hydrolyzed using a specific β-glucuronidase (Sigma Chemical Co., St. Louis, MO), and the product, androsterone, was then extracted with diethyl ether and chromatographed on Celite prior to RIA (16). The appropriate factor was used to correct for molecular weight differences due to the hydrolysis step.

Free testosterone (fraction not bound to sex hormone-binding globulin) was quantified by determining its percentage of total testosterone present in serum, using ammonium sulfate precipitation to remove the globulins, as described previously (17). The concentration of free testosterone was then calculated by multiplying the percentage of free testosterone times the total testosterone concentration. Free testosterone levels were determined for just 105 prostate cancer cases and matched controls because of insufficient amounts of sera.

The intra-assay and interassay coefficients of variation for the six hormonal assays ranged between 5-10 and 10-15%, respectively.

**Data Analysis.** The χ² test was used for the comparison of the distribution of various characteristics (e.g., born in United States), and the ANOVA was used for the comparison of means (e.g., serum cholesterol) between prostate cancer patients and controls. Due to the skewed frequency distributions of several hormones, Spearman’s correlation coefficients were used to examine the interrelationship between serum hormone levels.

Because of the skewed distributions, we did a transformation using natural logarithm that resulted in a more (approximately) normal distribution for statistical tests. Means of serum hormones were calculated for patients and controls, with adjustment for age at examination, date, and time of phlebotomy, because of some differences in the matching criteria between cases and controls. Adjustment for these covariates was done using one-way unbalanced analyses of covariance (18).

**Odds ratios for prostate cancer.** Based on the quartile levels of specific serum hormones, were determined using conditional logistic regression methods (19). The cut points for the quartiles were based on their distribution among the controls. Tests for linear trend in the logit of risk were derived from conditional logistic regression models through the use of grouped serum hormone test results (coded 1-4). All models of conditional logistic regression were fitted by using the iterative maximum likelihood method and a special application of the proportional hazards regression model (20). Power calculations indicate that an odds ratio of two or greater could be detected with 80% or higher power at the 5% level of significance, depending on the proportion exposed using 141 case-control pairs, which were included in this study (21).

**Results.** The mean age of the 141 prostate cancer patients and their matched controls at time of their examination was 62 (range, 53–75) years. As shown in Table 1, the two groups of men were similar with regard to their demographic characteristics and laboratory values, except the patients had a higher mean body mass index than controls.

**Table 1** Characteristics of prostate cancer patients and controls

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patients (n = 141)</th>
<th>Controls (n = 141)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Born in United States (%)</td>
<td>87</td>
<td>86</td>
<td>0.72</td>
</tr>
<tr>
<td>Buddhist/Shinto religion (%)</td>
<td>74</td>
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<td>0.98</td>
</tr>
<tr>
<td>High school education (%)</td>
<td>46</td>
<td>46</td>
<td>0.46</td>
</tr>
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<td>Married once (%)</td>
<td>87</td>
<td>87</td>
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<td>0.26</td>
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<tr>
<td>Ever smoked cigarettes (%)</td>
<td>59</td>
<td>59</td>
<td>0.81</td>
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<tr>
<td>Mean systolic blood pressure (mm Hg)</td>
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<td>137.9</td>
<td>0.80</td>
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<tr>
<td>Mean serum cholesterol (mg/dl)</td>
<td>215.7</td>
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<td>0.73</td>
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<td>Mean serum glucose (mg/dl)</td>
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Spearman’s correlation coefficients for the six hormone measurements among controls are given in Table 2. Total testosterone was moderately correlated (r = 0.4-0.7) with free testosterone and dihydrotestosterone.

Table 3 compares patients and controls according to their serum hormone measurements. The prostate cancer cases had higher mean levels of 3-α-androstanediol glucuronide, androstenedione, dihydrotestosterone, and total free testosterone, but none of the differences was statistically significant.

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The odds ratios and 95% confidence intervals for each quartile of serum hormonal levels are presented in Table 4. Although the odds ratios for the highest quartile were 1.37 for 3-α-androstanediol glucuronide and 1.24 for androstenedione, none of the observed trends was statistically significant. Further adjustment for body mass index changed the odds ratio of the highest quartile to 1.16 (95% confidence interval, 0.61–2.23) for 3-α-androstanediol glucuronide and 1.41 (95% confidence...
Cut points for the quartiles are: androstenedione (ng/ml) <1.20, 1.20-1.44, 1.45-1.79, and 1.80+; total testosterone (ng/ml) <5.00, 5.00-5.39, 5.40-5.89, and 5.90+; free testosterone (ng/ml) <0.45, 0.45-0.68, 0.69-0.89, and 0.90+; dihydrotestosterone (ng/ml) <0.17, 0.17-0.32, 0.33-0.54, and 0.55+; 3-α-androstanediol glucuronide (ng/ml) <0.02, 0.02-0.13, 0.14-0.21, and 0.22+; total testosterone/androsterone (ng/ml) <10.17, 10.17-12.50, 12.51-15.72, and 15.73+; total testosterone/dihydrotestosterone (ng/ml) <5.90, 5.90-6.59, 6.60-7.29, and 7.30+; androstenedione/dihydrotestosterone (ng/ml) <0.16, 0.16-0.21, 0.22-0.28, and 0.29+; androstenedione/androsterone (ng/ml) <1.80, 1.80-2.13, 2.14-2.47, and 2.48+; androstenedione/3-α androstanediol glucuronide (ng/ml) <0.08, 0.08-0.12, 0.13-0.18, and 0.19+; androsterone glucuronide (ng/ml) <0.37, 0.37-0.41, 0.42-0.51, and 0.52+; 3-α-androstanediol glucuronide (ng/ml) <0.12, 0.12-0.17, 0.18-0.21, and 0.22+; total testosterone/androsterone glucuronide (ng/ml) <1.69, 1.69-1.80, 1.81-2.07, and 2.08+; total testosterone/3-α-androstanediol glucuronide (ng/ml) <10.00, 10.00-10.17, 10.18-10.34, and 10.35+; androstenedione glucuronide (ng/ml) <1.00, 1.00-1.21, 1.22-1.41, and 1.42+. The distributions in controls were similar and not statistically significant. The analyses were repeated excluding the 17 cases diagnosed within 5 years of their examination. The results were again similar.

**Discussion**

Although there is suggestive evidence that increased 5-α-reductase activity, as reflected by elevated serum levels of 3-α-androstanediol glucuronide and androsterone glucuronide, enhances the risk of prostate carcinoma (6), there has not been any case-control study investigating this association. It was found in this study that 3-α-androstanediol glucuronide was higher, and that the ratio of testosterone:3-α-androstanediol glucuronide was lower in patients compared with controls, but the differences were not statistically significant. There was essentially no difference in androsterone glucuronide levels between patients and controls.

It is possible that a single specimen measuring serum levels of 3-α-androstanediol glucuronide, androsterone glucuronide, and other androgens is not sufficiently reliable to characterize an individual’s typical hormonal profile and, consequently, not sensitive enough to detect meaningful differences in prostate cancer risk. This suggests that hormonal measurements over time would be more informative, especially among Japanese men in Hawaii, who have experienced a significant increase in prostate cancer risk over a recent 15-year period.

Although the plasma concentration of testosterone is about 0.69-2.91 for androstenedione, but the trends were still not statistically significant. The patients and controls were also compared by the ratio of a hormone to its metabolic product. For example, testoster-

one metabolizes to dihydrotestosterone, 3-α-androstanediol glucuronide, and androsterone glucuronide. The ratio of testoster-

one:3-α-androstanediol glucuronide was lower in prostate cancer patients than controls, but the difference was not statistically significant (P = 0.16), as shown in Table 5. None of the other differences in hormonal ratios was significant.

When the ratio of testosterone:3-α-androstanediol glucur-

onide was separated into quartile groupings based on their distributions in controls, the odds ratios were 1.4, 1.0, and 0.6 for the second, third, and fourth quartiles, respectively (P for trend = 0.12).

The analyses in Tables 3–5 were repeated excluding the 37 prostate cancer patients who were diagnosed with nonaggressive lesions by transurethral resection procedures. The results were similar and not statistically significant. The analyses were also repeated excluding the 17 cases diagnosed within 5 years of their examination. The results were again similar.

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Although the plasma concentration of testosterone is about 10 times greater than that of dihydrotestosterone (5), dihy-

drotestosterone is more potent in bioassay systems and is the major intracellular androgenic hormone, regulating growth and function of the prostate. This would indicate that a more direct
Androgens and prostate cancer.

A method of measuring intracellular androgenic activity would be desirable in assessing prostate cancer risk. There is a circadian rhythm of several hormones in the blood. For example, maximum levels of testosterone are usually present in the early morning, with a progressive decrease during the day until low levels are present in the early evening (22). Androstenedione levels are low at midnight and high in the morning (23). Because of this, we matched the controls to the cases not only on age and date of examination, but also on h of day of blood collection.

Three previous studies used prediagnostic sera and found no association with either testosterone or dihydrotestosterone and prostate cancer risk (24–26). There was a weak suggestion of day of blood collection. A similar pattern was not found in this study.

In other case-control studies that have measured serum levels of testosterone or dihydrotestosterone, the blood samples were obtained after the diagnosis of prostate carcinoma (27–32). The presence of the tumor could affect the results. These studies found that the testosterone or dihydrotestosterone levels in patients were either higher, lower, or similar to those of controls.

An earlier study using prediagnostic sera found that plasma androstenedione levels were higher in prostate cancer cases than controls (24). The study was limited to 57 cases, 26 of whom were identified by death certificates. This result has not been replicated by others. Although we found that serum androstenedione levels were higher in patients than controls, the difference was minimal. Androstenedione, dehydroepiandrosterone, and dehydroepiandrosterone sulfate are weak androgens that are secreted primarily by the adrenal gland.

The 141 prostate carcinoma patients in this study could be divided into two clinical groups: patients with clinically overt cancer and patients with latent prostate cancer diagnosed in tissue specimens usually obtained by means of transurethral resection for benign prostate hyperplasia. Due to the concern that the latent cases might have differences from the clinical cases, the analyses were repeated, excluding the 37 latent cases. The results were very similar to those with all patients included.

The lack of a significant association between serum androgens and prostate cancer is surprising, given the favorable responses to androgen ablation in patients with this cancer and the observation that metastatic disease is directly associated with the presence of androgen receptors (33, 34). The distribution of allele frequencies of the microsatellites at the androgen receptor locus differs significantly among healthy African-American, white, and Asian men (35). African-Americans, who are at high risk of prostate cancer, have a high prevalence of short CAG microsatellite alleles and a low frequency of 16 GGC repeats compared with whites and Asians. It has been suggested that shorter CAG microsatellite alleles of the androgen receptor gene cause a more active growth in prostate cells, which could result in a higher prostate cancer risk.

This study had sufficient statistical power to detect a positive association, with an odds ratio of two or more between any one of the measured hormones and prostate carcinoma. As a result, our data indicate that these androgens are not strongly related to the risk of prostate cancer. However, it may still be worthwhile to conduct larger studies to determine whether 3-α-androstanediol glucuronide and other androgens are markers for identical individuals at increased risk for prostate cancer. Such studies should include more than one serum sample on each subject and other hormonal parameters, which would better reflect intracellular androgen activity or androgen receptor function.

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References


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