Serum and Urinary Prostate-specific Antigen and Urinary Human Glandular Kallikrein Concentrations Are Significantly Increased after Testosterone Administration in Female-to-Male Transsexuals

Chrisitna V. Obiezu,[1] Erik J. Giltay,[2] Angeliki Magklara,[1] Andreas Scorilas,[1] Louis J.G. Gooren,[2] He Yu,[3] David J.C. Howarth,[1] and Eleftherios P. Diamandis[1*]

Background: The genes that encode prostate-specific antigen (PSA) and human glandular kallikrein (hK2) are up-regulated by androgens and progestins in cultured cells, but no published studies have described the effect of androgen administration in women on serum and urinary PSA or hK2.

Methods: We measured serum and urinary PSA and hK2 before, and 4 and 12 months post testosterone treatment by immunofluorometric methods in 32 female-to-male transsexuals.

Results: Mean serum PSA increased from 1.1 ng/L to 11.1 ng/L and then to 22 ng/L by 4 and 12 months post treatment, respectively; the corresponding mean values in urine were 17, 1420, and 18 130 ng/L, respectively. Serum hK2, another kallikrein closely related to PSA, remained undetectable at the three time points. However, urinary hK2 concentration rose from below the detection limit (<6 ng/L) before treatment to 18 and 179 ng/L by the 4th and the 12th month of treatment, respectively. All changes were statistically significant (*P* <0.001) at 4 months.

Conclusions: Testosterone administration increases serum and urinary PSA and urinary hK2 in women. These measurements may be useful as indicators of androgenic stimulation in women.
© 2000 American Association for Clinical Chemistry

Prostate-specific antigen (PSA)[4] is a 33-kDa serine protease produced not only by prostatic epithelial cells (*1, 2*), but also by healthy and malignant female breast tissue and by several other tissues (*3–6*). Serum PSA is increased in women with hyperandrogenic syndromes (*7, 8*). In addition, PSA is also present in female urine, possibly originating from the Skene gland, which has a common embryological origin with the male prostate (*9–11*). A closely related protease, human glandular kallikrein (hK2), is expressed in the prostate (*12*) as well as in female breast cancer (*13*), the breast carcinoma cell line T-47D (*14*), and elsewhere (*15*). PSA and hK2 increase in breast cancer cells cultured in androgens or progestins (*14, 16*), presumably by receptor-mediated transactivation (*17, 18*).

Androgens down-regulate androgen receptor gene expression in vitro (*17, 19*). Long-term administration of androgens in vivo produces self-limited androgen receptor-dependent transactivation, without significant changes in androgen receptor concentrations. This most probably reflects posttranslational control of androgen receptor activity (*18*).

Testosterone is present in females, although at only 5–10% of the concentrations found in males (*20*). In

[1] Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, 600 University Ave., Toronto, Ontario M5G 1X5, Canada, and Department of Laboratory Medicine and Pathobiology, University of Toronto, 100 College St., Toronto, Ontario M5G 1L5, Canada.

[2] Department of Endocrinology, Division of Andrology, Hospital Vrije Universiteit, PO Box 7057, 1007 MB Amsterdam, The Netherlands.

[3] Louisiana State University Medical Center, Department of Medicine, 1501 Kings Hwy., Shreveport, LA 71130-393.

*Address correspondence to this author at: Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, 600 University Ave., Toronto, Ontario M5G 1X5, Canada. Fax 416-586-8628; e-mail ediamandis@mtsinai.on.ca.

[4] Nonstandard abbreviations: PSA, prostate-specific antigen; hK2, human glandular kallikrein; DHT, 5α-dihydrotestosterone; LH, luteinizing hormone; FSH, follicle-stimulating hormone; and GH, growth hormone.

women, androgens affect sexual as well as cognitive function, mood, and general well-being (21), and they are used for female-to-male transsexuals to help the assumption of male secondary sexual characteristics.

In this study, we monitored PSA responses in urine and serum, as well as hK2 response in urine, obtained from female-to-male transsexuals undergoing long-term androgenizing treatment with testosterone. We also attempted to correlate PSA and hK2 concentrations to those of testosterone and other hormones and biomarkers directly or indirectly affected by the administered androgen.

Materials and Methods

DRUG TREATMENT AND SAMPLE COLLECTION

Thirty-two female-to-male transsexuals (mean age, 25 years; range, 18–43 years) were treated intramuscularly with 250 mg of testosterone esters (SustamonR; Organon Oss) every 2 weeks. Venous blood samples were collected between 0900 and 1100, after an overnight fast, at baseline and after 4 and 12 months of hormonal administration. Urine samples (24-h) were collected at the same times. Serum and urine samples were stored at −20 °C until analyzed.

IMMUNOLOGICAL ASSAYS

PSA was measured in undiluted serum and in urine initially diluted 1:10 in a 60 g/L bovine serum albumin solution, pH 7.40, using a one-step time-resolved fluorometric immunoassay (22). Urine samples with results below the detection limit (1 ng/L) were reanalyzed without prior dilution.

hK2 was measured in undiluted serum and urine using a two-step time-resolved immunofluorometric assay with a detection limit of 6 ng/L (23).

A competitive chemiluminescence immunoassay was used to determine serum free thyroxine (in pmol/L; ACS:180 System; Chiron Diagnostics). RIAs were used to measure serum testosterone (Coat-A-Count; DPC), serum 5α-dihydrotestosterone (DHT, in nmol/L) after oxidation and extraction (Intertech), and serum thyrotropin (in mIU/L; ACS:180). Immunochemiluminogenic assays were used to assess serum luteinizing hormone (LH) and serum follicle-stimulating hormone (FSH), both in IU/L (Amerlite; Amersham). IRMAs were used to assess serum sex hormone-binding globulin (in nmol/L; Orion Diagnostica), and serum growth hormone (GH, in μg/L; GH color; Sorin Biomedica).

STATISTICAL ANALYSIS

Because the distribution of all measured variables was nongaussian (Z-score), statistical analyses were performed using nonparametric tests. Associations were examined at pretreatment, 4 month post treatment, and where applicable, at 12 months post treatment using Spearman correlation and Wilcoxon signed-ranks test.

Results

Testosterone increased PSA in serum and urine, and hK2 in urine. As seen in Fig. 1A, mean PSA in serum increased from almost undetectable concentrations to 11 ng/L by the 4th month of steroidal treatment ($P < 0.001$) and to 22 ng/L by the 12th month of treatment. Similarly, urinary PSA increased dramatically in response to testosterone treatment, from 17 ng/L to 1420 ng/L by the end of the 4th month of therapy, and to 18 130 ng/L by the end of
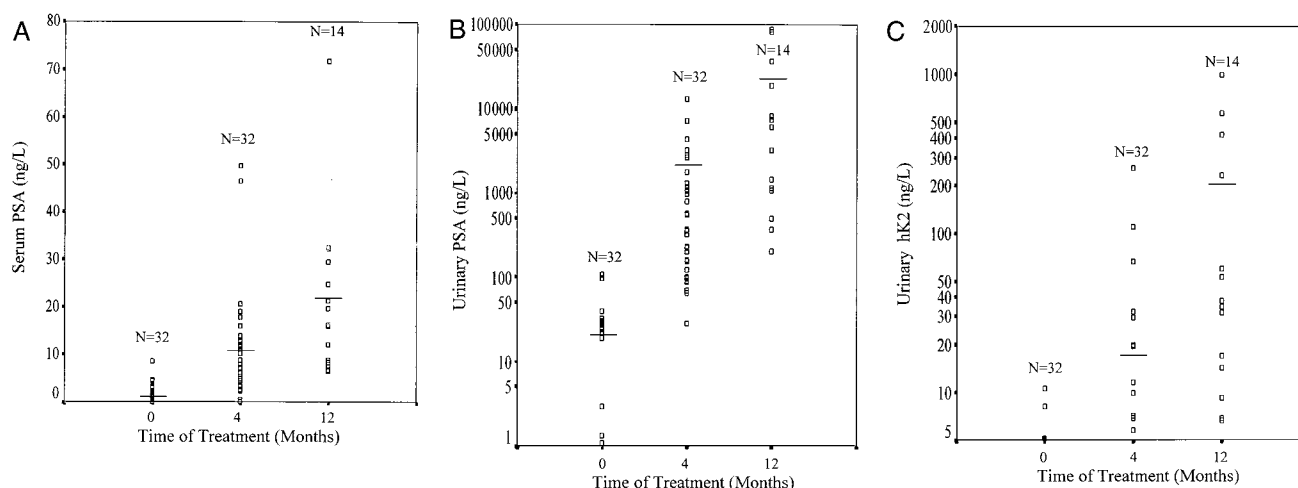

Fig. 1. Concentrations of serum PSA (A), urinary PSA (B), and urinary hK2 (C) in females undergoing testosterone therapy at 0 months (pretreatment) and at 4 and 12 months after treatment.

Solid horizontal lines indicate mean values. (A), mean PSA concentrations: 1.1 ng/L before treatment, 11.1 ng/L at 4 months of treatment, and 22 ng/L at 12 months of treatment. The differences between the PSA concentrations were determined by Wilcoxon signed-ranks test ($P < 0.001$ between 0 and 4 months; nonsignificant difference between 4 and 12 months). (B), mean PSA concentrations: 17 ng/L before treatment, 1420 ng/L at 4 months of treatment, and 18 130 ng/L at 12 months of treatment. The differences between the PSA concentrations at 0 and 4 months as well as 4 and 12 months were determined by Wilcoxon signed-ranks test ($P < 0.001$ in both cases). (C), mean hK2 concentrations: <6 ng/L before treatment, 18 ng/L at 4 months of treatment, and 179 ng/L at 12 months of treatment. The differences between the hK2 concentrations were determined by Wilcoxon signed-ranks test ($P < 0.001$ between 0 and 4 months; $P = 0.035$ between 4 and 12 months).

the treatment period ($P <0.001$; Fig. 1B). hK2 could not be detected in female serum but was readily detectable in urine. Urinary hK2 increased from a mean of <6 ng/L prior to treatment to 18 ng/L at 4 months of treatment ($P <0.001$) and 179 ng/L at 12 months ($P <0.001$; Fig. 1C). Mean serum DHT increased from 0.7 nmol/L to 2.3 nmol/L at 4 months, and to 3.5 nmol/L at 12 months.

Before treatment, no correlation of FSH and/or LH with either PSA or hK2 was apparent. At 4 months of treatment, however, urinary PSA correlated weakly with LH and FSH. FSH correlated with LH ($r = 0.639$; $P <0.0001$) at 4 months of treatment, as well as at 12 months post treatment ($r = 0.858$; $P = 0.001$). After 4 months of testosterone therapy, serum PSA was found to correlate negatively with serum testosterone and positively with insulin, whereas urinary PSA correlated with urinary hK2 (Table 1). By the 12th month of treatment, serum PSA had no noteworthy correlations with any of the variables tested. However, at 12 months of treatment, the correlation between urinary hK2 and PSA was better ($r = 0.723$; $P = 0.003$) compared with the correlation at 4 months post treatment for the same set of subjects (Table 1). Other associations relevant to our study included the expected correlation between testosterone and DHT at 4 months ($r = 0.719$; $P = 0.004$) and at 12 months of treatment ($r = 0.802$; $P = 0.001$). As expected, testosterone and DHT significantly increased after testosterone administration; LH, FSH, sex hormone-binding globulin, and thyroxine decreased, whereas GH and thyrotropin showed no significant change (data not shown). We also noted a negative correlation of urinary hK2 with body mass index ($r = -0.692$; $P = 0.006$) and with serum insulin ($r = -0.681$; $P = 0.007$).

Discussion

Although in vitro and in vivo studies have provided evidence of androgenic regulation of PSA (16), previous efforts could not confirm either the presence or the up-regulation of PSA in serum from healthy females after testosterone administration (9). The present study demonstrates a 20-fold increase of PSA in serum in response to testosterone therapy and confirms the previously de-scribed increase of PSA in urine in response to testosterone treatment (9).

Increased urinary PSA could not have originated by clearance from plasma alone, based on calculations of fractional clearances (data not shown). Thus, the source of PSA in urine may be different from that of PSA in serum. Interestingly, in a previous case study of a woman with carcinoma of the Skene gland, serum PSA was 5900 ng/L before surgery; after surgery, serum PSA became undetectable (24). This case report points out that under normal circumstances, the Skene gland does not contribute significantly to serum PSA concentrations as was also confirmed in males (25). Relying on our previous studies of PSA and hK2 in breast tumor tissues and nipple aspirate fluid (3, 4, 26, 27), we suggest that the source of PSA in serum may be the female breast. We propose that PSA production is under androgenic control in breast tissue as well as in the Skene gland. The latter is the most likely source of both PSA and hK2 in urine.

The increased urinary hK2 in response testosterone administration suggests that hK2 is under androgenic control. The good correlation between urinary PSA and hK2 supports the notion that hK2 arises from the same source as PSA. Furthermore, the fact that PSA itself is known to be activated by hK2 provides a physiological rationale for their coexpression (12, 13).

Serum LH concentrations are known to be lower in testosterone-treated female-to-male transsexuals than in controls (28). According to our data, LH and FSH decreased, and this decrease can be accounted for by testosterone-induced negative feedback at the hypothalamo-pituitary level (29, 30). Interestingly, hK2 in urine correlated negatively with body mass index and with serum insulin. The expected increase in DHT concentration was also appreciable, considering the fact that male serum DHT values usually are ~2 nmol/L. Testosterone also had a good correlation with GH, which may be explained by the fact that, at least in males, androgen affects GH release into the circulation (20).

It should be noted that androgen is not the sole physiological up-regulator of the PSA and hK2 genes. In vitro studies (14, 16) and in vivo data (6, 31, 32) clearly demonstrate up-regulation of these genes by progestins. Our study demonstrates, for the first time in vivo, that testosterone can up-regulate quite significantly the PSA and hK2 genes in target tissues and mediate serum and urinary PSA increases post treatment. This finding gives credence to previous reports of increased serum PSA in patients with hyperandrogenic syndromes (7, 8). The target tissues have not yet been identified. Likely, both urinary PSA and hK2 are produced by the periurethral and paraurethral glands and then secreted into the urine. It will be worthwhile to examine whether urinary PSA and hK2 have any value as biochemical indicators of hyperandrogenism in women. This possibility is now under investigation.

Table 1. Spearman correlation values for variables measured in serum 4 months post treatment with serum and urinary PSA, and with urinary hK2.[a]

	Serum PSA	Urinary PSA
Testosterone	−0.411 ($P = 0.02$)	NS[b]
LH	NS	0.356 ($P = 0.04$)
FSH	NS	0.379 ($P = 0.03$)
Urine hK2	NS	0.477 ($P = 0.006$)
Insulin	0.662 ($P = 0.01$)	NS

[a] n = 32 in all cases except with insulin, where n = 14. Only statistically significant correlations are presented.
[b] NS, not significant.

References

1. Oesterling JE. Prostate specific antigen: a critical assessment of the most useful tumor marker for adenocarcinoma of the prostate. J Urol 1991;145:907–23.
2. McCormack RT, Rittenhouse HG, Finlay JA, Sokoloff RL, Wang TJ, Wolfert RL, et al. Molecular forms of prostate-specific antigen and the human kallikrein gene family: a new era. Urology 1995;45:729–44.
3. Diamandis EP, Yu H. New biological functions of prostate specific antigen? J Clin Endocrinol Metab 1995;80:1515–7.
4. Diamandis EP, Yu H. Nonprostatic sources of prostate-specific antigen. Urol Clin N Am 1997;24:275–82.
5. Parish DC. Prostate-specific antigen in the breast. Endocr Relat Cancer 1998;5:223–9.
6. Yu H, Diamandis EP, Levesque M, Giai M, Roagna R, Ponzone R, et al. Prostate specific antigen in breast cancer, benign breast disease and normal breast tissue. Breast Cancer Res Treat 1996;40:171–8.
7. Melegos DN, Yu H, Ashok M, Wang C, Stanczyk F, Diamandis EP. Prostate-specific antigen in female serum, a potential new marker of androgen excess. J Clin Endocrinol Metab 1997;82:777–80.
8. Escobar-Morreale HF, Serrano-Gotarredona J, Avila S, Villar-Palasi J, Varela C, Sancho J. The increased circulating prostate-specific antigen concentrations in women with hirsutism do not respond to acute changes in adrenal or ovarian function. J Clin Endocrinol Metab 1998;83:2580–4.
9. Breul J, Pickl U, Schaff J. Extraprostatic production of prostate specific antigen is under hormonal control. J Urol 1997;157:212–3.
10. Wernert N, Albrech M, Sesterhenn I, Goebbels R, Bonkhoff H, Seitz G, et al. The "female prostate". Location, morphology, immunohistochemical characteristics and significance. Eur Urol 1992;22:64–9.
11. Zaviacic M, Ablin RJ. The female prostate. J Natl Cancer Inst 1998;90:713–4.
12. Rittenhouse HG, Finlay JA, Mikolajczyk SD, Partin AW. Human kallikrein 2 (hK2) and prostate specific antigen (PSA): two closely related, but distinct, kallikreins in the prostate [Review]. Crit Rev Lab Sci 1998;35:275–368.
13. Black MH, Magklara A, Obiezu CV, Levesque MA, Sutherland DJA, Tindall DJ, et al. Expression of a prostate-associated protein, human glandular kallikrein (hK2), in breast tumors and in normal breast secretions. Br J Cancer 2000;82:361–7.
14. Hsieh M, Charlesworth MC, Goodmanson M, Zhang S, Seay T, Klee GG, et al. Expression of human prostate-specific glandular kallikrein protein (hK2) in the breast cancer cell line T47-D. Cancer Res 1997;57:2651–6.
15. Clements JA, Mukhtar A, Verity K, Pullar M, McNeill P, Cummins J, Fuller PJ. Kallikrein gene expression in human pituitary tissues. Clin Endocrinol 1996;44:223–31.
16. Zagrami N, Grass L, Diamandis EP. Steroid hormone regulation of prostate specific antigen gene expression in breast cancer. Br J Cancer 1997;43:415–21.
17. Rundlett SE, Wu XP, Miesfeld RL. Functional characterization of the androgen receptor confirm that the molecular basis of androgen action is transcriptional regulation. Mol Endocrinol 1990;4:708–14.
18. Wolf DA, Herzinger T, Hermeking H, Blaschke D, Horz W. Transcriptional and posttranscriptional regulation of human androgen receptor expression by androgen. Mol Endocrinol 1993;7:924–36.
19. Quarmby VE, Kemppainen JA, Sar M, Lubahn DB, French FS, Wilson EM. Expression of recombinant androgen receptor in cultured mammalian cells. Mol Endocrinol 1990;4:1399–407.
20. Burtis CA, Ashwood ER, eds. Tietz textbook of clinical chemistry, 2nd ed. Philadelphia: WB Saunders, 1994:2326pp.
21. Buckler HM, Robertson WR, Wu FC. Which androgen replacement therapy for women? J Clin Endocrinol Metab 1998;83:3920–4.
22. Ferguson RA, Yu H, Kalyvas M, Zammit S, Diamandis EP. Ultra-sensitive detection of prostate-specific antigen by a time-resolved immunofluorometric assay and the Immulite® immunochemiluminescent third generation assay: potential applications in prostate and breast cancers. Clin Chem 1996;42:675–84.
23. Black MH, Magklara A, Obiezu CV, Melegos DN, Diamandis EP. Development of an ultrasensitive immunoassay for human glandular kallikrein with no cross-reactivity with prostate-specific antigen. Clin Chem 1999;45:790–9.
24. Tepper SL, Jagirdar J, Heath D. Homology between the female paraurethral Skene's gland and the prostate: immunohistochemical demonstration. Arch Pathol Lab Med 1984;108:423–5.
25. Oesterling JE, Tekchandani AH, Martin SK, Bergstrahl EJ, Reichstein ER, Diamandis, EP, et al. The periurethral glands do not significantly influence the serum prostate specific antigen concentration. J Urol 1996;155:1658–60.
26. Sauter ER, Daly M, Linahan K, Ehya H, Engstrom PF, Bonney G, et al. Prostate specific antigen levels in nipple aspirate fluid correlate with breast cancer risk. Cancer Epidemiol Biomark Prevent 1996;5:967–70.
27. Sauter ER, Babb J, Daly M, Engstrom PF, Ehya H, Malik J, Diamandis EP. Prostate specific antigen production in the female breast: Association with progesterone. Cancer Epidemiol Biomark Prevent 1998;7:315–20.
28. Pache TD, Hop WC, de Jong FH, Leerentveld RA, van Geldorp H, Van de Kamp TM, et al. 17 β-Oestradiol, androstenedione and inhibin levels in fluid from individual follicles of normal and polycystic ovaries, and in ovaries from androgen treated female to male transsexuals. Clin Endocrinol 1992;36:565–71.
29. Foresta C, Bordon P, Rossato M, Mioni R, Veldhuis JD. Specific linkages among luteinizing hormone, follicle-stimulating hormone, and testosterone release in the peripheral blood and human spermatic vein: evidence for both positive (feed-forward) and negative (feedback) within-axis regulation. J Clin Endocrinol Metab 1997;82:3040–6.
30. Gooren LJG. Androgens and estrogens in their negative feedback action in the hypothalamo-pituitary-testis axis: site of action and evidence of their interaction. J Steroid Biochem 1989;33:757–61.
31. Katsaros D, Melegos DN, Diamandis EP. Prostate specific antigen production by breast tumors after induction with oral contraceptives. Clin Biochem 1998;31:285–8.
32. Diamandis EP, Helle SJ, Yu H, Melegos DN, Lundgren S, Lonning PE. Prognostic value of plasma prostate specific antigen after megestrol acetate treatment in patients with metastatic breast carcinoma. Cancer 1999;85:891–8.