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Effect of Testosterone Administration on Serum and Urine Kallikrein Concentrations in Female-to-Male Transsexuals

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Background: **Concentrations of human tissue kallikreins (hKs), a group of 15 secreted serine proteases found in many tissues, are modulated by steroid hormones in cancer cell lines. To gain insight into in vivo kallikrein regulation we measured kallikrein concentrations in serum and urinary tissue in female-to-male transsexuals before and after testosterone administration.**

Methods: **We collected blood and urine samples before treatment and after 4 and 12 months from 28 female-to-male transsexuals who received 250 mg of testosterone esters intramuscularly every 2 weeks. We used ELISA assays to measure multiple kallikreins in serum and urine.**

Results: **After testosterone administration, serum testosterone concentrations increased by ~15-fold. Serum kallikrein concentrations increased dramatically for hK3 (prostate-specific antigen) and increased moderately for hK2, hK5, hK6, hK7, hK8, hK10, and hK11. In urine, we noted major increases for hK3 and hK2 only. For all other kallikrein concentrations, we observed no considerable changes.**

Conclusions: **We conclude that, in serum and urine of female-to-male transsexuals after testosterone administration, hK3 (prostate-specific antigen) and to a lesser extent hK2 concentrations increase dramatically, but concentration of other kallikreins increase either moderately in serum (hK5, hK6, hK7, hK8, hK10, and hK11) or not at all in either serum (hK4, hK13, hK14) or urine (hK4, hK5, hK6, hK7, hK8, hK10, hK11, hK13, hK14).**

Human tissue kallikreins (hKs)[6] comprise a subgroup of 15 homologous secreted serine proteases encoded by a tightly clustered multigene family on the long arm of chromosome 19 (KLK1-KLK15)[7]. hKs are involved in a wide range of physiological functions and are primarily expressed within the glandular epithelia in many tissues, including the skin, the central and peripheral nervous system, and also in endocrine glands and hormone-dependent tissues such as breast, endometrium, and prostate *(1)*.

In addition to prostate-specific antigen (PSA, *hK3*), which has important applications as a marker for prostate cancer diagnosis and follow-up, and hK2, an emerging prostate cancer biomarker, many other members of the kallikrein family are also implicated in endocrine-dependent malignancies. All kallikreins studied to date are known to be up- or down-regulated at the mRNA and/or protein level in breast, prostate, ovarian, and testicular cancers *(2)*.

Transcription of the kallikrein genes is modulated by a large number of stimulatory and inhibitory substances, among which sex steroid hormones are the best characterized. Sex steroid hormones affect the initiation and/or progression of endocrine-dependent malignancies, and the role of hKs as cancer biomarkers is only beginning to be understood *(3, 4)*. Studies on the regulation of the

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[6] Nonstandard abbreviations: hK, kallikrein protein; PSA, prostate-specific antigen; LH, luteinizing hormone; FSH, follicle-stimulating hormone
[7] Human gene: *KLK*, kallikrein gene.

kallikrein genes have been performed in vitro mainly with cancer cell lines *(5)*.

Information on the in vivo regulation of hKs by steroid hormones would be useful, but opportunities for in vivo study of the effects of sex steroids on the expression of hKs are rather limited. Transsexuals undergoing cross-sex hormone treatment with high doses of sex steroids provide a relatively unambiguous model, allowing us to study the effects of sex steroids in healthy persons.

In an earlier in vivo study we demonstrated that, in female-to-male transsexuals, concentrations of hK2 and hK3 (PSA) were highly increased in both serum and urine after androgen administration *(6)*. Conversely, antiandrogen plus estrogen administration in male-to-female transsexuals (reducing plasma testosterone values to almost zero) down-regulated concentrations of hK2 and hK3 in serum and urine *(7)*.

Recently developed specific ELISA-type assays of most other kallikreins provided the opportunity to extend our observations on the in vivo hormonal regulation of hK2 and hK3 to other kallikreins. In the present study, we monitored the effects of hormone treatment on serum and urine concentrations of hK2, hK3, hK4, hK5, hK6, hK7, hK8, hK10, hK11, hK13, and hK14 in female-to-male transsexuals receiving high-dose testosterone treatment for 4–12 months.

Materials and Methods

PARTICIPANTS

This single-center, open-label study was approved by the Medical Ethics Committee of the Free University Medical Centre (Amsterdam, The Netherlands). Transsexuals, after careful psychological evaluation, received cross-sex hormone treatment according to the standards of the Harry Benjamin International Gender Dysphoria Association (www.hbigda.org). Participants were included if they were willing to sign informed consent, were able and willing to visit the study center, and had not used exogenous sex hormones before the start of the treatment. These participant data were assessed by questioning the participants and by evaluation of pretreatment hormone concentrations, specifically gonadotropin concentrations, which are suppressed by exogenous sex hormones.

A total of 28 female-to-male transsexuals (mean age 22 years, range 16–37 years) were included in this study. All were treated with 250 mg of testosterone esters (Sustanon®; Organon Oss) injected intramuscularly every 2 weeks. Venous blood samples were collected before cross-sex treatment and after 4 and 12 months of testosterone administration. Serum samples were stored at −80 °C immediately after collection and until analyzed. At the same time, urine was collected and stored similarly.

HORMONE MEASUREMENTS

For serum 17β-estradiol and testosterone, we performed measurements on commercially available RIAs, and for luteinizing hormone (LH) and follicle-stimulating hormone (FSH) on commercially available immunometric luminescence assays. If values were below the lower limit of detection, the value of this lower limit was used for statistical analysis (for testosterone, 1.0 nmol/L; for LH, 0.3 IU/L; and for FSH, 0.5 IU/L). The body mass index (weight/height^2) was also determined (Table 1).

IMMUNOLOGICAL ASSAYS

For all kallikrein measurements in serum and urine, we used ELISA-type immunofluorometric procedures developed in house. Most of these methods have been de-

Table 1. Age, body mass index, and hormonal data before and after 4 months of testosterone administration in 25 female-to-male transsexuals

Variable	Mean (SE)	Median	Range	*P* value[a]
Age, years	26.0 (1.2)	24.0	16.4–37.0	
Body mass index, kg/m^2				
Before treatment	22.6 (0.8)	22.2	16.6–32.4	
After treatment	24.0 (0.8)	23.0	18.6–35.1	<0.001
Testosterone, nmol/L				
Before treatment	1.9 (0.2)	1.8	1.0–3.7	
After treatment	30.9 (2.0)	30.0	13.0–49.0	<0.001
17β-estradiol, pmol/L				
Before treatment	177 (16)	162	83–399	
After treatment	124 (6)	124	67–201	0.003
Luteinizing hormone, IU/L				
Before treatment	5.3 (0.6)	4.5	1.9–16.0	
After treatment	2.5 (0.5)	1.8	0.30–9.1	<0.001
Follicle-stimulating hormone, IU/L				
Before treatment	4.4 (0.2)	4.2	2.6–6.3	
After treatment	3.0 (0.3)	3.0	0.50–5.0	0.001

If hormone concentrations declined below the lower limit of detection, the value of that lower limit was used in the analysis (for testosterone, 1.0 nmol/L; for LH, 0.3 IU/L; and for FSH, 0.5 IU/L).

[a] Calculated by Wilcoxon signed rank test.

scribed and validated in previous publications *(8–18)*. Information on these methods is provided in Table 2. We have tested the cross-reactivity of these ELISA assays against all other kallikreins and have found no cross-reactions. The precision of all assays within the dynamic range cited in Table 2 was <10%. These arrays were standardized with recombinant proteins produced in yeast or mammalian expression systems, as previously described *(8–18)*.

STATISTICAL ANALYSIS

Because the distributions of kallikrein concentrations were not gaussian, the nonparametric Wilcoxon signed rank test was used to determine the differences of kallikrein concentrations before and after treatment. For all analyses, a P value of <0.05 was considered statistically significant.

Results

Serum concentrations of 17β-estradiol, testosterone, FSH, and LH, and the body mass index, before and after treatment in the 28 female-to-male transsexuals who were treated with 250 mg of testosterone esters every 2 weeks are summarized in Table 1. After testosterone administration, serum 17β-estradiol concentrations [mean (SD)] decreased only modestly compared with pretreatment measures [from 177 (16) to 124 (6) pmol/L; $P = 0.003$]. These estrogens are derived mainly from peripheral aromatization of testosterone rather than from ovarian production and show a correlation with circulating plasma testosterone concentrations (unpublished observation). Serum testosterone concentrations profoundly increased [from 1.9 (0.2) to 30.9 (2.0) nmol/L; $P <0.001$]. Concentrations of LH and FSH decreased from 5.3 (0.6) to 2.5 (0.5) ($P <0.001$) and 4.4 (0.2) to 3.0 (0.3) ($P = 0.001$), respectively. Body mass index increased from 22.6 (0.8) to 24.0 (0.8) ($P <0.001$). In general, the major endocrine change in these patients was the dramatic increase of serum testosterone.

Serum kallikrein concentrations, before and after 4 months of testosterone treatment, are presented in Table 3. During testosterone administration, serum kallikrein concentrations measured at 4 months were not substantially different from those measured at 12 months. Serum hK2, hK3, hK5, hK6, hK7, hK8, hK10, and hK11 concentrations were significantly ($P <0.05$) up-regulated by testosterone treatment, with the most profound impact on hK3 and hK2. No statistically significant differences between pre- and posttreatment concentrations were noted for the other serum kallikreins (hK4, hK13, and hK14). We obtained similar data after we used logarithmic transformation to normalize the distributions of each kallikrein concentration.

Urine kallikrein concentrations before and after 4 months of testosterone treatment are presented in Table 4. Urine kallikrein concentrations measured at 4 months were not significantly different from those measured at 12 months. Urine hK2 and hK3 concentrations were significantly up-regulated after testosterone treatment ($P <0.001$). No statistically significant pre- or posttreatment differences were noted for the other 9 hKs (Table 4). Similar data were obtained after normalization of the distributions of each kallikrein concentration by logarithmic transformation.

Discussion

By measuring changes in serum and urine kallikrein concentrations, we determined the impact that androgens exert on kallikrein gene expression in healthy women of reproductive age. In humans, limited opportunities are available to monitor the effects of profound changes in hormonal milieus on biological variables. Cross-sex hormone treatment of transsexuals provides such an opportunity. Our study may be clinically relevant to women who suffer from hyperandrogenic syndromes, including polycystic ovary syndrome and hirsutism. The participants in this study were female-to-male transsexuals who received testosterone therapy. This treatment led to only minor suppression of plasma estrogen concentrations but profound increases of androgen concentrations. Thus, we attributed the changes in serum and urine hK concentrations mainly to the androgenic stimulation. This hormonal intervention produced a significant up-regulation

Table 2. ELISA assays used in the present study

Kallikrein	Coating/Detection Antibody	Dynamic Range, ng/L	Detection Limit, ng/L	Reference
hK2	mono/mono	2000	6	*(8)*
hK3	mono/mono	2000	1	*(9)*
hK4	mono/poly	20 000	100	*(10)*
hK5	mono/mono	25 000	100	*(11)*
hK6	mono/mono	50 000	100	*(12)*
hK7	mono/mono	20 000	200	*(13)*
hK8	mono/mono	20 000	200	*(14)*
hK10	mono/mono	20 000	50	*(15)*
hK11	mono/mono	50 000	100	*(16)*
hK13	mono/mono	20 000	50	*(17)*
hK14	mono/poly	20 000	100	*(18)*

Mono, monoclonal mouse antibody; poly, polyclonal rabbit antibody.

Table 3. Serum kallikrein concentrations before and after testosterone administration for 4 months in female-to-male transsexuals

Kallikrein, ng/L	Mean (SE), ng/L	Median, ng/L	Range	*P* value[a]
hK2				
Before treatment, n = 25	0.7 (0.2)	0	0–4	0.013
After treatment, n = 23	1.5 (0.5)	1	0–10	
hK3				
Before treatment, n = 25	2.3 (0.7)	1	0–16	<0.001
After treatment, n = 23	20 (4)	16	4–83	
hK4[b]				
Before treatment, n = 25	0	0	<100	
After treatment, n = 23	0	0	<100	
hK5	171 (14)			
Before treatment, n = 25	207 (14)	153	80–369	<0.001
After treatment, n = 23		204	107–366	
hK6				
Before treatment, n = 25	3,160 (129)	2998	1882–4498	0.001
After treatment, n = 22	3,686 (143)	3635	2668–5200	
hK7				
Before treatment, n = 25	4,209 (729)	3360	16–18 964	<0.001
After treatment, n = 23	5,444 (913)	4548	702–23 796	
hK8				
Before treatment, n = 25	985 (276)	614	186–7164	<0.001
After treatment, n = 23	1,334 (408)	906	306–9956	
hK10				
Before treatment, n = 25	870 (80)	754	448–2460	0.001
After treatment, n = 23	1,094 (129)	1052	368–3568	
hK11				
Before treatment, n = 24	322 (32)	300	103–773	0.003
After treatment, n = 23	376 (35)	337	89–545	
hK13				
Before treatment, n = 25	10 (6)	0	0–165	0.28
After treatment, n = 23	15 (11)	1	0–270	
hK14				
Before treatment, n = 25	412 (39)	376	126–838	0.078
After treatment, n = 23	434 (37)	449	00–773	

[a] Calculated by Wilcoxon signed rank test.
[b] No detectable concentrations in any sample.

of serum hK2, hK3, hK5, hK6, hK7, and hK10 concentrations, with the most profound impact on hK2 and hK3. Conversely, in urine, we observed significant up-regulation only of hK2 and hK3, but not of any other hKs.

We previously reported up-regulation of hK2 and hK3 by exogenous androgens in a similar group *(6)*. The up-regulation of hK3 (PSA) by androgens has also been confirmed in other studies. Most of these studies investigated serum and urine PSA concentrations in women with hirsutism or polycystic ovary syndrome, both conditions accompanied in most cases by increased circulating androgen concentrations *(19–22)*. Highly sensitive PSA assays are used to diagnose hyperandrogenic hirsutism *(23–24)*.

It is known that the expression of PSA is mainly regulated by the androgen receptor at the transcriptional level *(25)*. Another study of female-to-male transsexuals found high concentrations of testosterone–up-regulated PSA production, in agreement with our findings *(26)*. PSA concentrations drop significantly after mastectomy, suggesting that breast tissue in women is likely to be the source of androgen up-regulated production of PSA *(27, 28)*. However, PSA concentrations after mastectomy remained higher than the PSA concentrations of nonandrogenized women, suggesting other PSA sources in females. PSA has also been detected in endometrial tissues, as well as in several body fluids (breast milk, breast cyst fluid, nipple aspirate fluid, and amniotic fluid) *(29–31)*.

The increased serum concentration of hK2 in response to testosterone administration suggests that hK2 is also under androgenic control, supporting the finding that KLK2 is up-regulated by androgens in prostate and breast cancer cell lines *(30, 32)*. The breast also appears to be the main source of hK2 production in females *(33)*. hK2 is secreted in seminal plasma, amniotic fluid, breast milk, and saliva *(34)*. It should be noted that androgens are not

Table 4. Urinary kallikrein concentrations before and after testosterone administration for 4 months in female-to-male transsexuals

Kallikrein, ng/L	Mean ± SE[a]	Median	Range	*P* value[a]
hK2				
Before treatment, n = 28	0.14 (0.0)	0	0–2	<0.0001
After treatment, n = 23	4.2 (1.0)	3	0–17	
hK3				
Before treatment, n = 28	5.4 (2.6)	1	0–64	<0.0001
After treatment, n = 23	1604 (453)	539	22–7734	
hK4				
Before treatment, n = 28	2405 (160)	2302	552–4637	0.51
After treatment, n = 23	2273.9 (203)	2429	627–3616	
hK5				
Before treatment, n = 28	254 (60)	130	20–1436	0.79
After treatment, n = 23	435 (180)	89	0.00–3374	
hK6				
Before treatment, n = 28	1205 (424)	352	35–8788	0.67
After treatment, n = 23	894 (315)	243	28–6172.0	
hK7				
Before treatment, n = 25	3239 (743)	1376	229–16 262	0.62
After treatment, n = 21	3440 (1,016)	1772	214–17 738	
hK8				
Before treatment, n = 28	523 (132)	224	31–3122	0.10
After treatment, n = 23	615 (146)	265	53–2439	
hK10				
Before treatment, n = 28	920 (429)	377	0–12 231	0.73
After treatment, n = 23	617 (208)	232	0–4182	
hK11				
Before treatment, n = 28	2008 (493)	705	76–12 159	0.24
After treatment, n = 23	1334 (418)	748	73–9160	
hK13				
Before treatment, n = 28	3460 (1,024)	1305	82–22 561	0.49
After treatment, n = 23	2458 (804)	991	25–16 246	
hK14				
Before treatment, n = 28	482 (73)	380	84–2170	0.84
After treatment, n = 23	585 (144)	371	73–3302	

[a] Calculated by Wilcoxon signed rank test.

the only steroids that could up-regulate PSA and hK2 genes. In vivo and in vitro studies demonstrate up-regulation of these genes by progestins *(35–37)*.

Results of tissue-culture studies suggest that hK1-hK4 and hK13-hK15 are mainly up-regulated by androgens, and hK5-hK12 are mainly up-regulated by estrogens *(1, 5)*. In vivo, up-regulation by androgens occurs for hK2 and hK3 and also for hK5, hK6, hK7, hK8, hK10, and hK11.

We observed major changes in urine only for kallikreins hK2 and hK3. Previously, we reported the highly increased expression of hK2 and hK3 in urine of female-to-male transsexuals receiving testosterone treatment *(6)*. We attributed these changes to production of hK2 and hK3 by the periurethral glands. Apparently, the other studied kallikreins do not seem to be up-regulated by androgens in this tissue.

The serum changes of hK2 and, especially, hK3 in our studied population were much more dramatic than the changes of the other kallikreins (hK5, hK6, hK7, hK8, hK10, and hK11). We attribute this finding to 2 reasons: hK2 and PSA transcription is under direct androgenic control; and these 2 kallikreins are produced only by a small number of tissues, mainly breast tissue, in females. Other kallikreins are produced in a variety of tissues *(1)*. These tissues may contribute significantly to the serum concentration, and they are likely not as sensitive to androgenic stimulation as is female breast tissue. These findings are supported by results of tissue culture studies with breast cancer cell lines in which the androgenic effect on hK2 and hK3 expression is much more pronounced than for the other kallikreins *(35–37)*.

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