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Serum and Urine Tissue Kallikrein Concentrations in Male-to-Female Transsexuals Treated with Antiandrogens and Estrogens

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Background: **The expression of human tissue kallikrein genes is regulated by steroid hormones, but most studies have been conducted with cancer cell lines. Our purpose was to examine serum and urinary tissue kallikrein concentration changes in male-to-female transsexuals before and after treatment with antiandrogens and estrogens.**

Methods: **Thirty-five male-to-female transsexuals receiving cyproterone acetate and estrogens (orally or transdermally) were included in this study. Serum and urine samples were collected before initiation of therapy and 4 and 12 months post therapy. ELISAs were used to measure multiple kallikreins in serum and urine.**

Results: **After antiandrogen and estrogen therapy, serum testosterone concentrations decreased dramatically, as did serum and urinary concentrations of human glandular kallikrein (hK2) and prostate-specific antigen (PSA; hK3). Statistically significant but relatively small changes in serum and urinary concentrations of many other kallikreins were also seen. Kallikreins in serum and urine were correlated before and after treatment.**

Conclusions: **The concentrations of hK2 and hK3, but not of any other kallikreins, decrease dramatically after combined antiandrogen and estrogen treatment in male-to-female transsexuals. The smaller responses of the other kallikreins presumably reflect their expression in multiple tissues.**

The human tissue kallikrein family includes 15 genes (*KLK1* to *KLK15*),[6] clustered in tandem on chromosome 19q13.4. These genes encode for 15 kallikrein proteins (hK1 to hK15),[7] which are all secreted serine proteases. Groups of kallikreins are expressed in many tissues, including the salivary glands, the central nervous system, the skin, endocrine glands such as the testis and ovaries, and hormone-dependent tissues such as the breast, endometrium, and prostate *(1)*. Among the 15 kallikreins, pancreatic/renal kallikrein (hK1), glandular kallikrein (hK2), and prostate-specific antigen (hK3; PSA) have been studied the most, and specific biological functions have been assigned to them. The primary function of hK1 is the release of lysyl-bradykinin, a vasoactive peptide *(2)*, hK2 activates the proform of hK3, and hK3 is involved in semen liquefaction, increasing the motility of spermatozoa *(3)*. The remaining kallikreins are less well characterized, but they are implicated in a wide range of physiologic functions, from regulation of cell growth to tissue remodeling *(1, 4–6)*.

hK3, better known as PSA, is a valuable tumor marker

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[6] Human genes: *KLK1*, kallikrein 1, renal/pancreas/salivary; *KLK2*, kallikrein 2, prostatic; *KLK3*, kallikrein 3 (prostate-specific antigen); and *KLK4*, kallikrein 4 (prostase, enamel matrix, prostate).
[7] Nonstandard abbreviations: hK1, pancreatic/renal kallikrein protein; hK2, glandular kallikrein; hK3 (PSA), prostate-specific antigen; hK4–hK15, human kallikreins 4–15; HR, hormone receptor; HRE, hormone response element; ARE, androgen response element; and DHT, dihydroxytestosterone.

of prostate cancer *(7)*. The potential significance of the other kallikreins in tumor biology has been reviewed *(1, 4–6, 8)*. Accumulating evidence indicates that, in addition to hK2 and hK3, many other members of the human kallikrein gene family are also implicated in carcinogenesis, in particular, in endocrine-dependent malignancies. Recent reports suggest a possible role for kallikreins in controlling vital elements of tumor biology, such as apoptosis, angiogenesis, and tumor metastasis, by cleavage of critical substrates such as growth factors, hormones, receptors, or extracellular matrix proteins *(5, 9, 10)*. Furthermore, many kallikreins are known to be up- or down-regulated by steroid hormones in breast and prostate cancer cell lines and may represent important new tumor markers for the diagnosis, monitoring, and treatment of endocrine-dependent malignancies *(4, 5, 9, 11, 12)*.

Studies on the regulation of the kallikrein genes have been performed mainly in vitro in prostate, endometrial, and breast cancer cell lines *(13–16)*. Most genes appear to be up- or down-regulated by estrogens, androgens, and progestins but with different potencies. One notable observation is the tissue-specific pattern of regulation of some genes (e.g., the prostate-specific regulation of *KLK3* and *KLK2*) and the different patterns of hormonal regulation in different tissues; e.g., *KLK4* is up-regulated by androgens in prostate cancer cell lines *(17)* and by estrogens in endometrial cancer cell lines *(16)*. The control of kallikrein gene transcription may involve integration of many transcription factors and pathways, which provide diversity in regulation mechanisms and create opportunities for cell- and tissue-specific interactions *(14)*.

Sex steroid hormones impact on the initiation and/or progression of endocrine-dependent malignancies *(18)*. As outlined above, most kallikreins appear to be under sex steroid hormonal regulation. Taken together, kallikreins may belong to the targets by which hormones affect the initiation or progression of such tumors *(19)*. Therefore, a better understanding of the hormonal regulation of kallikreins could increase our insights into tumor biology. To date, mainly cancer cell lines have been studied. The role of sex steroids on the expression of kallikreins has been studied in vivo only for hK2 and hK3 *(20–22)*. These studies, mainly performed in female-to-male and male-to-female transsexuals receiving cross-sex hormones, have shown that hK2 and hK3 are up-regulated in serum and urine by testosterone *(21)* and down-regulated by antiandrogens and estrogens, respectively *(20)*. Recently, specific ELISAs for most other kallikreins have been developed. This allowed us to study the hormonal regulation of other kallikreins in vivo.

In this study, we examined the effect of hormone treatment on serum and urine concentrations of hK2 to hK8, hK10, hK11, hK13, and hK14, obtained from male-to-female transsexuals who were receiving combined estrogen and antiandrogen therapy.

Materials and Methods

PARTICIPANTS

This single-center, open-label study was approved by the Medical Ethics Committee of the Free University Medical Centre. Transsexuals, after careful psychological evaluation, received cross-sex hormone treatment following the standards of the Harry Benjamin International Gender Dysphoria Association (www.hbigda.com). Inclusion criteria included signing of informed consent, willingness to visit the study center, and no use of exogenous (sex) hormones before the start of treatment. This was assessed by a questionnaire and by evaluation of pretreatment gonadotropin concentrations, which are suppressed by prior use of exogenous sex hormones. Thirty-five male-to-female transsexuals (mean age, 30 years; range, 19–45 years) were included in this study. All received treatment with 100 mg/day of the antiandrogen cyproterone acetate (Androcur; Schering; this antiandrogen also has some progestational properties), combined with estrogen therapy. Estrogen treatment consisted of 100 μg of oral ethinyl estradiol (Organon) daily (oral group; n = 20) or transdermal 17β-estradiol (Estraderm TTS 100; Ciba-Geigy) twice weekly with a patch releasing, on average, 100 μg of estradiol per day (transdermal group; n = 15). Urine and venous blood samples were collected before cross-sex treatment and 4 and 12 months after the start of the hormonal therapy. Serum and urine samples were stored immediately at −80 °C until the analyses were performed.

HORMONE MEASUREMENTS

Serum measurements were performed for 17β-estradiol (RIA; Sorin Biomedica), testosterone (Coat-A-Count RIA; Diagnostic Products Corporation), luteinizing hormone (Amerlite immunometric luminescence assay; Amersham Pharmacia Biotech), follicle-stimulating hormone (immunometric luminescence assay; Amerlite), and prolactin (IRMA; Biosource Technologies, Inc.).

KALLIKREIN ASSAYS

For all kallikrein measurements in serum and urine, we used ELISA-type immunofluorometric procedures developed in-house. Most of these methods have been previously described and validated (Table 1). The cross-reactivity of these ELISA assays against all other kallikreins was negligible *(23–33)*.

STATISTICAL ANALYSIS

For data analysis, patients were subdivided into groups based on different types of treatment. Because the distributions of kallikrein concentrations were not gaussian, we used the nonparametric Wilcoxon signed-rank test to determine the differences between kallikrein concentrations before and after treatment. Correlations between kallikreins before and after treatment were assessed by the Spearman correlation coefficient. These tests treated each kallikrein concentration as a continuous variable. For

Table 1. ELISAs used in the present study.

Kallikrein	Coating/Detection antibody[a]	Upper limit of dynamic range, ng/L	Detection limit, ng/L	Reference
hK2	Mono/Mono	2000	6	*(23)*
hK3	Mono/Mono	2000	1	*(24)*
hK4	Mono/Poly	20 000	100	*(25)*
hK5	Mono/Mono	25 000	100	*(25)*
hK6	Mono/Mono	50 000	100	*(27)*
hK7	Mono/Mono	20 000	200	*(28)*
hK8	Mono/Mono	20 000	200	*(29)*
hK10	Mono/Mono	20 000	50	*(30)*
hK11	Mono/Mono	50 000	100	*(31)*
hK13	Mono/Mono	20 000	50	*(32)*
hK14	Mono/Poly	20 000	100	*(33)*

[a] Mono, monoclonal mouse antibody; Poly, polyclonal rabbit antibody.

all analyses, a P value <0.05 was considered statistically significant.

Results

Thirty-five male-to-female transsexuals were treated with oral ethinyl estradiol plus cyproterone acetate for 4 or 12 months (n = 20) or with transdermal 17β-estradiol plus cyproterone acetate for 4 or 12 months (n = 15). After estrogen and antiandrogen administration, circulating sex hormone and prolactin concentrations were profoundly altered (Table 2). Serum concentrations of testosterone were markedly suppressed, in most participants below the lower limit of detection of the assay used.

Serum kallikrein concentrations before and after administration of antiandrogens plus estrogens, specified for the type of estrogen used, oral or transdermal, are presented in Table 3. We found no difference between 4 months and 12 months of hormone administration (data not shown). The combined administration of antiandrogens and estrogens had a profound impact on serum concentrations of some, but not all, kallikreins. The effects of oral and transdermal estrogens were sometimes discrepant. Serum hK2 and hK3 concentrations were dramatically suppressed by both types of treatment. Serum hK6 and hK10 concentrations were also suppressed by both treatments but to a much lesser degree. Antiandrogens

Table 2. Hormonal data before and after 4 months of administration of cross-sex hormones in 35 male-to-female transsexuals treated with estrogens in combination with cyproterone acetate.

Variable	Mean (SE)[a]	Median	Range	P[b]
Testosterone, nmol/L				
Before treatment (n = 35)	22.1 (1.1)	20.0	9.9–34.0	
After treatment with EE+CA[c] (n = 20)	1.0 (0.0)	1.0	1.0–1.4	<0.001
After treatment with TTS+CA[d] (n = 15)	1.1 (0.1)	1.0	1.0–1.6	0.001
17β -Estradiol, pmol/L				
Before treatment (n = 35)	89 (5)	82	61–149	
After treatment with EE+CA[e] (n = 20)	23 (1)	24	18–33	0.001
After treatment with TTS+CA (n = 15)	183 (34)	151	18–508	0.009
Luteinizing hormone, IU/L				
Before treatment (n = 35)	2.81 (0.27)	2.70	0.30–6.80	
After treatment with EE+CA (n = 20)	0.40 (0.10)	0.30	0.30–2.30	<0.001
After treatment with TTS+CA (n = 15)	0.47 (0.12)	0.30	0.30–1.90	0.001
Follicle-stimulating hormone, IU/L				
Before treatment (n = 35)	3.44 (0.50)	2.55	0.60–15.00	
After treatment with EE+CA (n = 20)	0.50 (0.00)	0.50	0.50–0.50	<0.001
After treatment with TTS+CA (n = 15)	0.51 (0.01)	0.50	0.50–0.70	0.001
Prolactin, IU/L				
Before treatment (n = 35)	0.15 (0.01)	0.14	0.06–0.33	
After treatment with EE+CA (n = 20)	0.44 (0.04)	0.42	0.16–0.79	0.001
After treatment with TTS+CA (n = 15)	0.54 (0.05)	0.54	0.20–0.85	0.001

[a] If hormone concentrations decreased below the assay lower limit of detection, the value of that lower limit was used in the analyses.
[b] Calculated by Wilcoxon signed-rank test.
[c] EE+CA, 100 μg of oral ethinyl estradiol daily + 100 mg of cyproterone acetate daily for 4 months.
[d] TTS+CA, transdermal 17β-estradiol administered twice weekly + 100 mg of cyproterone acetate daily for 4 months.
[e] Ethinyl estradiol does not cross-react and could not be detected by the assay used.

Table 3. Serum kallikrein concentrations before and after treatment in male-to-female transsexuals.

Variable	Mean (SE)	Median	Range	*P*[a]
hK2, ng/L				
Before treatment (n = 35)	39.7 (4.4)	36.0	1.0–98	
After treatment with EE+CA[b] (n = 20)	1.2 (0.2)	1.0	1.0–4.0	<0.001
After treatment with TTS+CA[c] (n = 15)	2.3 (0.6)	1.0	1.0–7.0	0.001
hK3, ng/L				
Before treatment (n = 35)	525 (44)	437	156–1174	
After treatment with EE+CA (n = 20)	32 (6)	26	0.5–121	<0.001
After treatment with TTS+CA (n = 15)	177 (72)	63	11–995	0.001
hK4, ng/L				
Before treatment (n = 35)	28.4 (6.7)	10[d]	10–155	
After treatment with EE+CA (n = 20)	18.1 (5.1)	10	10–89	0.46
After treatment with TTS+CA (n = 15)	10.0 (0.0)	10	10–10	0.042
hK5, ng/L				
Before treatment (n = 35)	171 (8)	173	25–261	
After treatment with EE+CA (n = 20)	135 (7)	137	76–188	0.002
After treatment with TTS+CA (n = 15)	164 (15)	163	18–296	0.39
hK6, ng/L				
Before treatment (n = 35)	3035 (114)	2980	1554–4300	
After treatment with EE+CA (n = 20)	1942 (101)	2054	1022–2604	<0.001
After treatment with TTS+CA (n = 15)	2710 (96)	2789	2154–3128	0.002
hK7, ng/L				
Before treatment (n = 35)	3339 (398)	2930	50–8738	
After treatment with EE+CA (n = 20)	3067 (735)	1715	50–1382	0.39
After treatment with TTS+CA (n = 15)	2793 (521)	2408	50–7518	0.021
hK8, ng/L				
Before treatment (n = 35)	532 (88)	346	90–2538	
After treatment with EE+CA (n = 20)	728 (163)	435	46–3266	0.76
After treatment with TTS+CA (n = 15)	512 (164)	204	44–2018	0.91
hK10, ng/L				
Before treatment (n = 35)	745 (40)	680	430–1306	
After treatment with EE+CA (n = 20)	562 (54)	522	334–1434	0.002
After treatment with TTS+CA (n = 15)	609 (37)	614	228–822	0.047
hK11, ng/L				
Before treatment (n = 35)	348 (27)	346	71–684	
After treatment with EE+CA (n = 20)	240 (34)	272	10–472	0.006
After treatment with TTS+CA (n = 15)	374 (29)	366	203–589	0.31
hK13, ng/L				
Before treatment (n = 35)	11.5 (3.5)	5.0	5.0–106	
After treatment with EE+CA (n = 20)	14.9 (8.1)	5.0	5.0–168	0.22
After treatment with TTS+CA (n = 15)	7.6 (2.0)	5.0	5.0–34	0.14
hK14, ng/L				
Before treatment (n = 35)	266 (29)	192	36–651	
After treatment with EE+CA (n = 20)	262 (44)	155	39–710	0.42
After treatment with TTS+CA (n = 15)	234 (33)	224	75–476	0.53

[a] Calculated by Wilcoxon signed-ranks test; comparison with pretreatment values.
[b] EE+CA, 100 μg of oral ethinyl estradiol daily + 100 mg of cyproterone acetate daily for 4 months.
[c] TTS+CA, transdermal 17β-estradiol administered twice weekly + 100 mg of cyproterone acetate daily for 4 months.
[d] Undetectable concentrations were set at 10 ng/L for statistical analysis.

plus oral estrogens suppressed serum hK5 and hK11 concentrations, but these kallikreins were not suppressed by antiandrogens plus transdermal estrogens. Conversely, antiandrogens plus transdermal estrogens suppressed serum hK4 and hK7 concentrations, but these kallikreins were not suppressed by antiandrogens plus oral estrogens. No suppression by antiandrogens and either type of estrogen was noted for serum hK8, hK13, and hK14.

In general, the most consistent and dramatic changes in serum kallikrein concentrations after estrogen and antiandrogen treatment were seen only with hK2 and hK3.

The correlations between serum concentrations of kallikreins before hormone administration are shown in

Table 1 of the Data Supplement that accompanies the online version of this article at http://www.clinchem.org/content/vol52/issue7/. We found significant correlations between serum concentrations of hK2 and hK3 (positive) or hK10 (negative), between hK4 and hK13 or hK14 (both positive), between hK5 and hK6 (positive), and between hK7 and hK8, hK13, or hK14 (all positive). The strongest correlations (positive) were between hK2 and hK3 and between hK7 and hK8.

The correlations between serum kallikreins after 1 year of administration of 100 mg cyproterone acetate plus oral ethinyl estradiol are presented in Table 2 of the online Data Supplement. After 1 year of treatment, serum concentrations of hK2, hK4, and hK10 no longer showed any correlations with any of the other kallikreins. Serum hK3 no longer showed a correlation with hK2 but still showed positive correlations with hK6 and hK11. Serum hK5 was positively correlated with hK6, as before hormone treatment. Serum hK6 was correlated with hK13. Serum hK7 was positively correlated, as before hormone administration, with hK8, hK13, and hK14.

The correlations between serum kallikreins after 1 year of administration of 100 mg of cyproterone acetate plus transdermal 17β-estradiol are shown in Table 3 of the online Data Supplement. Similar to the changes induced by cyproterone acetate plus oral ethinyl estradiol, serum concentrations of hK2, hK4, and hK10 no longer showed correlations with any of the other kallikreins. This was also the case for hK5, hK11, and hK13. Before hormone administration, serum hK6 correlated with hK5, but this correlation was lost; a negative correlation with hK13 became apparent, which was also observed with oral estrogens but in the opposite direction. Serum hK7 continued to correlate with hK8, but its correlations with hK13 and hK14, observed both before treatment and with oral estrogens, were lost after 1 year of treatment with cyproterone acetate plus transdermal 17β-estradiol. Serum hK8 showed a positive correlation with hK14, not present before hormone administration, which not observed with oral estrogens.

The kallikrein concentrations in urine before and after 1 year of treatment with antiandrogens plus estrogens (oral or transdermal) are presented in Table 4. No difference in effects was found between 4 months and 12 months of hormone administration (data not shown). The combined administration of antiandrogens plus estrogens had a profound impact on urine concentrations of some, but not all, kallikreins. There was a dramatic decrease in urine concentrations of hK2 and hK3 with both treatment regimens. The decrease in hK4 was much smaller and reached significance only with treatment with antiandrogens plus transdermal 17β-estradiol. For the urine concentrations of hK5–hK8, hK10, and hK13, we observed a trend toward increased concentrations, but statistical significance was only achieved for hK6, hK10, and hK13 on administration of antiandrogens plus oral ethinyl estradiol. No significant effects were observed on urine concentrations of hK5, hK7, hK8, hK11, and hK14.

The correlations between urinary kallikrein concentrations before hormone administration are shown in Table 4 of the online Data Supplement. We found significant correlations between urine concentrations of hK2 and hK3, hK10, and hK11 (all positive); between hK3 and hK4 (negative); between hK10 and hK11 and hK13 (all positive); between hK4 and hK6 and hK10 (both negative) or hK14 (positive); between hK5 and hK6, hK7, hK8, hK11, and hK13 (all positive); between hK6 and hK7, hK8, hK10, hK11, and hK13 (all positive); between hK7 and hK8 and hK13 (both positive); between hK8 and hK10 and hK13 (both positive); between hK10 and hK11 and hK13 (both positive); and between hK11 with hK13 (positive). The correlations between urinary kallikrein concentrations after 1 year of treatment with 100 mg of cyproterone acetate plus oral ethinyl estradiol are shown in Table 5 of the online Data Supplement, and the correlations after 1 year of treatment with 100 mg of cyproterone acetate plus transdermal 17β-estradiol are shown in Table 6 of the online Data Supplement. In Tables 7–9 of the online Data Supplement, we present the correlations between urinary and serum kallikrein concentrations before and after the 2 types of treatment. The statistically significant correlations between serum and urine kallikreins were rare and weak.

Discussion

The hormonal regulation of human tissue kallikreins has been studied extensively in several cancer lines in tissue culture, by both reverse transcription-PCR and quantitative immunofluorometric ELISA methods (Table 5) *(13, 15–17, 34–40)*. Although many kallikreins show hormone sensitivity, the number of cell lines that have shown kallikrein expression on hormonal stimulation are very few. Several factors can account for this, most importantly the hormone receptor (HR) status and the presence of hormone response elements (HREs), DNA *cis* elements to which the HR binds and transcriptionally activates gene expression. Breast cancer cell lines express the largest number of kallikrein genes on hormonal stimulation, whereas prostate cancer cell lines are limited to androgen-sensitive kallikrein expression.

The "classic" hormone-dependent expression model proposes that androgen activation of the androgen receptor leads to HR binding to androgen response elements (AREs), thus leading to transcriptional activation of PSA and hK2. The genes that encode PSA and hK2 contain AREs in the proximal promoter region (100 to 400 bp) and an ARE-enhancer element located ~3000 to 4000 bp upstream from the transcriptional start site. Therefore, similarities between kallikrein expression and hormone stimulation between these genes can account for their sensitivity to androgen stimulation *(41–44)*.

Norgestrel, a synthetic progestin, has also been shown to activate PSA and hK2 expression in BT-474 and T-47D

Table 4. Urinary kallikrein concentrations before and after treatment (1 year).

Variable	Mean (SE)	Median	Range	*P*[a]
hK2, ng/L				
Before treatment (n = 35)	280 (53)	173	22.0–1398	
After treatment with EE+CA[b] (n = 17)	13.6 (7.1)	6.0	1.0–123	<0.001
After treatment with TTS+CA[c] (n = 14)	15.7 (6.3)	5.5	1.0–77	0.001
hK3, ng/L				
Before treatment (n = 35)	11 240 (907)	11893	1750–19 145	
After treatment with EE+CA (n = 17)	935 (274)	420	13.0–3673	<0.001
After treatment with TTS+CA (n = 14)	3657 (1473)	1156	8.0–16 480	0.002
hK4, ng/L				
Before treatment (n = 35)	3149 (204)	3191	191–5411	
After treatment with EE+CA (n = 17)	2930 (276)	2823	477–4638	0.38
After treatment with TTS+CA (n = 14)	2550 (276)	2231	1172–5215	0.031
hK5, ng/L				
Before treatment (n = 35)	54.0 (23.2)	8.0	2.5–760	
After treatment with EE+CA (n = 17)	251 (129)	44.0	2.5–2026	0.17
After treatment with TTS+CA (n = 14)	150 (66)	31.0	2.5–860	0.21
hK6, ng/L				
Before treatment (n = 35)	79.4 (35.1)	20.0	1.0–1098	
After treatment with EE+CA (n = 17)	182 (94.9)	36.0	1.0–1580	0.036
After treatment with TTS+CA (n = 14)	413 (188)	97.0	5.0–2387	0.22
hK7, ng/L				
Before treatment (n = 35)	403 (142)	50.0	50.0–4085	
After treatment with EE+CA (n = 17)	1884 (927)	50.0	50.0–13 388	0.26
After treatment with TTS+CA (n = 14)	1768 (852)	154	50.0–10 581	0.32
hK8, ng/L				
Before treatment (n = 35)	106 (25.1)	50.0	5.0–548	
After treatment with EE+CA (n = 17)	320 (148)	97.0	5.0–2296	0.18
After treatment with TTS+CA (n = 14)	520 (257)	132	5.0–3609	0.13
hK10, ng/L				
Before treatment (n = 35)	48.5 (17.7)	10.0	10.0–600	
After treatment with EE+CA (n = 17)	458 (317)	10.0	10.0–5430	0.038
After treatment with TTS+CA (n = 14)	629 (476)	109	10.0–6792	0.11
hK11, ng/L				
Before treatment (n = 35)	1416 (329)	721	40.0–9927	
After treatment with EE+CA (n = 17)	901 (263)	373	60.0–3946	0.91
After treatment with TTS+CA (n = 14)	3493 (1327)	999	10.0–15 816	0.47
hK13, ng/L				
Before treatment (n = 35)	267 (173)	45.0	5.0–6067	
After treatment with EE+CA (n = 17)	948 (514)	82.0	5.0–7938	0.015
After treatment with TTS+CA (n = 14)	980 (452)	331	5.0–6120	0.17
hK14, ng/L				
Before treatment (n = 35)	390 (27.4)	428	73.0–765	
After treatment with EE+CA (n = 17)	386 (61.5)	379	117.0–1224	0.55
After treatment with TTS+CA (n = 14)	559 (189)	443	48.0–2958	0.16

[a] Calculated by Wilcoxon signed-ranks test.
[b] EE+CA, 100 μg of oral ethinyl estradiol daily + 100 mg of cyproterone acetate daily for 1 year.
[c] TTS+CA, transdermal 17β-estradiol administered twice weekly + 100 mg of cyproterone acetate daily for 1 year.

breast cancer cell lines along with dihydrotestosterone (DHT) *(34)*. This may explain why hirsute women continue to display high PSA with oral contraceptive treatments, as oral contraceptives usually contain a synthetic progestin ingredient *(45, 46)*.

Several other kallikreins also show single-hormone–dependent sensitivity. hK15 and hK1 are androgen sensitive in some tissue culture cells lines; however, their expression cannot be easily explained because the genes apparently lack AREs in their proximal or upstream promoter regions *(17, 40)*. The same can be said for estradiol-specific expression of hK5, hK6, and hK8, in several breast cancer cell lines, but predictive estrogen response elements have not been found in upstream promoter regions *(26, 37)*.

The hormonal regulation is further complicated by

Table 5. Summary of human tissue kallikrein hormone regulation.

Kallikrein	Cell line	Detection method	Hormone sensitivity	Ref(s).
hK2	Breast			
	BT-474	RT-PCR;[a] ELISA	Norgestrel > DHT	*(34)*
	T-47D	RT-PCR; ELISA	DHT; Norgestrel	*(15, 34)*
PSA (hK3)	Breast			
	BT-474	RT-PCR; ELISA	Norgestrel > DHT	*(13, 34)*
	T-47D	RT-PCR; ELISA	Norgestrel > DHT	*(13, 34, 35)*
	Prostate			
	LNCaP	RT-PCR; ELISA	DHT	*(17)*
hK4	Prostate			
	LNCaP	Northern blotting	R1881[b]	*(17)*
	Endometrial			
	KLE	RT-PCR	Estradiol/Progesterone	*(16)*
hK5	Breast			
	BT-474	ELISA	Estradiol	*(26)*
	MCF-7	RT-PCR; ELISA	Estradiol	*(26)*
	Prostate			
	PC3 (AR_6)[c]	ELISA	Norgestrel > DHT	*(26)*
hK6	Breast			
	BT-474	RT-PCR; ELISA	Estradiol	*(36)*
	47D	RT-PCR; ELISA	Estradiol	Unpublished[d]
	MCF-7	RT-PCR; ELISA	Estradiol	Unpublished[d]
hK8	Breast			
	T-47D	RT-PCR	Estradiol	Unpublished[d]
hK10	Breast			
	BT-474	RT-PCR; ELISA	Estradiol	*(37)*
	T-47D	RT-PCR; ELISA	DHT	*(37)*
	MCF-7	RT-PCR; ELISA	Estradiol; DHT; Norgestrel	*(37)*; unpublished[d]
hK11	Breast			
	BT-474	RT-PCR; ELISA	Estradiol	*(38)*
	T-47D	RT-PCR; ELISA	DHT	Unpublished[d]
	MCF-7	RT-PCR; ELISA	Estradiol; DHT; Norgestrel	Unpublished[d]
hK13	Breast			
	BT-474	RT-PCR	Estradiol	Unpublished[d]
	T-47D	RT-PCR	DHT	Unpublished[d]
	MCF-7	RT-PCR; ELISA	Estradiol; DHT; Norgestrel	Unpublished[d]
hK14	Breast			
	BT-474	RT-PCR; ELISA	Estradiol	Unpublished[d]
	T-47D	RT-PCR; ELISA	DHT	*(39)*; unpublished[d]
	MCF-7	RT-PCR; ELISA	Estradiol; DHT; Norgestrel	Unpublished[d]
	ZR-75	RT-PCR	DHT	*(39)*
	Ovarian			
	BG-1	RT-PCR	DHT	*(39)*
	HTB-75	RT-PCR	DHT	*(39)*
hK15	Breast			
	BT-474	RT-PCR	DHT	*(40)*

[a] RT-PCR, reverse transcription-PCR.
[b] Synthetic androgen.
[c] PC3 prostate cancer cells stably transfected with androgen receptor.
[d] Our unpublished data.

evidence that multiple kallikreins can be activated by several different hormones. These kallikreins include hK10, -11, -13, and -14. In BT-474 cells, these kallikreins are under the regulation of estradiol, whereas in T-47D cells, they are activated by DHT stimulation *(37, 39)*. In MCF-7 breast cancer cell lines, these 4 kallikreins are stimulated by estradiol, DHT, and Norgestrel treatment (our unpublished data). Promoter-deletion constructs for hK10 failed to identify any proximal promoter region that are required for hormonal regulation in different cell lines, and scanning of upstream sequences did not identify any predictive HREs for any of these hormones *(37)*.

Although apparently lacking HREs, these kallikreins are sensitive to anti-hormone receptor treatment with Nilutamide and ICI 182,780 *(47, 48)* androgen and estrogen inhibitors, respectively, thus also making it difficult to associate their coordinated expression to a single-hormone–dependent trans-acting factor. Therefore, close examination of Table 5 shows how the kallikrein expression profile for one cell line can differ from another cell line when treated with the same steroid hormone.

Many kallikreins often have a localized tissue expression profile that is associated with different hormone-dependent endocrine organs. Therefore, hormone treatments that cause increases in kallikrein expression in serum and other biological fluids are most often associated with cancer. Studies have correlated HR status of cancer patients to the prognostic value of kallikreins. In turn, this has predictive value in selecting patients who are likely to respond to therapy. However, only prostate cancer, which is associated with increased PSA, is treated with antiandrogenic drugs because of its androgen-dependent regulation.

In humans, opportunities to monitor the effects of profound changes in hormonal milieus on biological variables are limited. Treatment of transsexuals with cross-sex hormones provides such an opportunity. The patients are usually rather young and not affected by concurrent illnesses; therefore, the alterations in biological variables can be reliably ascribed to changes in hormonal milieu after administration of cross-sex hormones.

The patients studied in this report were male-to-female transsexuals receiving a combination of the antiandrogen cyproterone acetate with an estrogen preparation, either oral ethinyl estradiol or transdermal 17β-estradiol. This combination suppresses plasma testosterone concentrations to values below the detection limit of the assay (Table 2). With this treatment, at the same time, a potent estrogen stimulus is introduced. The results of this study must therefore be viewed in the light of 2 significant alterations in hormonal milieu: the elimination of androgens and the introduction of a potent estrogen stimulus. The endocrine milieu introduced by the combination of cyproterone acetate and transdermal 17β-estradiol is similar to that in women in the late follicular phase of the cycle, whereas the administration of oral ethinyl estradiol constitutes a much more potent estrogenic stimulus.

The above hormonal intervention produced strong and significant effects on various members of the kallikrein gene family. As previously described, the most pronounced effects were the decreases in serum and urinary hK2 and hK3 after administration of antiandrogen with either oral and transdermal estrogens *(20)*. In addition, serum hK6 and hK10 decreased to a lesser, although statistically significantly, degree with both estrogen regimens. The decreases in hK2 and hK3 were observed earlier in our laboratory in a similar group of patients *(20)*. In view of the report that hK2 and hK3 are up-regulated by androgens, this effect could probably be ascribed to androgen deprivation in the persons studied rather than to an estrogenic stimulus.

Although it has been reported that estrogens up-regulate many human kallikreins (Table 5), this was not the case in our results. If anything, we observed modest decreases.

There were discrepancies between the effects on kallikreins of antiandrogens plus oral ethinyl estradiol on the one hand and antiandrogens plus transdermal 17β-estradiol on the other. Serum concentrations of hK5 and hK11 were suppressed by administration of antiandrogens plus oral ethinyl estradiol, whereas they were not significantly suppressed by antiandrogens plus transdermal 17β-estradiol. Conversely, serum concentrations of hK4 and hK7 were suppressed by administration of antiandrogens plus transdermal 17β-estradiol but not by antiandrogens plus oral ethinyl estradiol. An intriguing finding is that the type of estrogen (oral or transdermal) had different impacts on some members of the kallikrein gene family, but the explanation for these differences between the estrogen regimen is not obvious. However, a parallel may be drawn with other studies that found discrepancies between the effects of oral ethinyl estradiol vs transdermal 17β-estradiol. The "first-pass" of oral estrogens through the liver has been invoked to explain the increases in clotting factors *(49)*, C-reactive protein *(50)*, insulin-like growth factor *(51, 52)*, and triglycerides and the suppression of plasma tissue-type plasminogen activator *(50)*. Oral, but not transdermal, administration of estrogens lowers tissue-type plasminogen activator concentrations in humans without affecting endothelial synthesis. These changes are lower with the transdermal route of estradiol administration. Furthermore, oral ethinyl estradiol is more potent than 17β-estradiol *(53, 54)*.

In summary, the administration of antiandrogens plus estrogens to adult males had an impact on some, but not all, serum kallikrein concentrations. The design of the study does not allow us to determine whether these effects could be ascribed to the resulting profound androgen deprivation, to the induction of a potent estrogen stimulus, or to both. Administration of antiandrogens combined with oral ethinyl estradiol or transdermal 17β-estradiol had similar effects on serum concentrations of several kallikreins, but surprisingly, some others were affected differently, which is potentially explained by the hepatic effect of oral ethinyl estradiol, which does not occur with transdermal 17β-estradiol. The most clear effects of treatment with an antiandrogen plus estradiol were seen with hK2 and hK3 in both serum and urine (dramatic down-regulation). This is very likely attributable to the well-known up-regulation of these enzymes by androgens in both cancer cell lines and in vivo. Regarding the other kallikreins, 2 factors may complicate our interpretations: (*a*) most kallikreins other than hK2 and hK3 are not clearly regulated by the classic hormone/HR/HRE system but by more complex mechanisms; and (*b*)

our data may reflect the highly specific expression of hK2 and hK3 in the prostate and the diverse expression of most other kallikreins in many different tissues.

References

1. Yousef GM, Diamandis EP. The new human tissue kallikrein gene family: structure, function, and association to disease. Endocr Rev 2001;22:184–204.
2. Moreau ME, Garbacki N, Molinaro G, Brown NJ, Marceau F, Adam A. The kallikrein-kinin system: current and future pharmacological targets. J Pharmacol Sci 2005;99:6–38.
3. 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. Crit Rev Clin Lab Sci 1998;35:275–368.
4. Borgono CA, Michael IP, Diamandis EP. Human tissue kallikreins: physiologic roles and applications in cancer. Mol Cancer Res 2004;2:257–80.
5. Borgo[caron]no CA, Diamandis EP. The emerging roles of human tissue kallikreins in cancer. Nat Rev Cancer 2004;4:876–90.
6. Clements JA, Willemsen NM, Myers SA, Dong Y. The tissue kallikrein family of serine proteases: functional roles in human disease and potential as clinical biomarkers. Crit Rev Clin Lab Sci 2004;41:265–312.
7. Han M, Gann PH, Catalano WJ. Prostate-specific antigen and screening for prostate cancer. Med Clin North Am 2004;88:245–65.
8. Obiezu CV, Diamandis EP. Human tissue kallikrein gene family: applications in cancer. Cancer Lett 2005;224:1–22.
9. Yousef GM, Diamandis EP. Human tissue kallikreins: a family of new cancer biomarkers. Clin Chem 2002;48:1198–205.
10. Michael IP, Sotiropoulou G, Pampalakis G, Magklara A, Ghosh M, Wasney G, et al. Biochemical and enzymatic characterization of human kallikrein 5 (hK5), a novel serine protease potentially involved in cancer progression. J Biol Chem 2005;280:14628–35.
11. Diamandis EP, Yousef GM. Human tissue kallikrein gene family: a rich source of novel disease biomarkers. Expert Rev Mol Diagn 2001;1:182–90.
12. Luo LY, Katsaros D, Scorilas A, Fracchioli S, Bellino R, van Gramberen M, et al. The serum concentration of human kallikrein 10 represents a novel biomarker for ovarian cancer diagnosis and prognosis. Cancer Res 2003;63:807–11.
13. Zarghami N, Grass L, Diamandis EP. Steroid hormone regulation of prostate-specific antigen gene expression in breast cancer. Br J Cancer 1997;75:579–88.
14. Magklara A, Brown TJ, Diamandis EP. Characterization of androgen receptor and nuclear receptor co-regulator expression in human breast cancer cell lines exhibiting differential regulation of kallikreins 2 and 3. Int J Cancer 2002;100:507–14.
15. Hsieh ML, 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.
16. Myers SA, Clements JA. Kallikrein 4 (KLK4), a new member of the human kallikrein gene family is up-regulated by estrogen and progesterone in the human endometrial cancer cell line, KLE. J Clin Endocrinol Metab 2001;86:2323–6.
17. Nelson PS, Gan L, Ferguson C, Moss P, Gelinas R, Hood L, Wang K. Molecular cloning and characterization of prostase, an androgen-regulated serine protease with prostate-restricted expression. Proc Natl Acad Sci U S A 1999;96:3114–9.
18. Lopez-Otin C, Diamandis EP. Breast and prostate cancer: an analysis of common epidemiological, genetic, and biochemical features. Endocr Rev 1998;19:365–96.
19. Yousef GM, Diamandis EP. Human tissue kallikreins: a new enzymatic cascade pathway? Biol Chem 2002;383:1045–57.
20. Obiezu CV, Giltay EJ, Magklara A, Scorilas A, Gooren L, Yu H, et al. Dramatic suppression of plasma and urinary prostate specific antigen and human glandular kallikrein by anti-androgens in male-to-female transsexuals. J Urol 2000;163:802–5.
21. Obiezu CV, Giltay EJ, Magklara A, Scorilas A, Gooren LJ, Yu H, et al. Serum and urinary prostate-specific antigen and urinary human glandular kallikrein concentrations are significantly increased after testosterone administration in female-to-male transsexuals. Clin Chem 2000;46:859–62.
22. Yu H, Diamandis EP, Monne M, Croce CM. Oral contraceptive-induced expression of prostate-specific antigen in the female breast. J Biol Chem 1995;270:6615–8.
23. Black MH, Magklara A, Obiezu CV, Melegos DN, Diamandis EP. Development of ultrasensitive immunoassay for human glandular kallikrein with no cross-reactivity from prostate-specific antigen. Clin Chem 1999;45:790–9.
24. Ferguson RA, Yu H, Kalyvas M, Zammit S, Diamandis EP. Ultrasensitive 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.
25. Obiezu CV, Soosaipillai A, Jung K, Stephan C, Scorilas A, Howarth DH, et al. Detection of human kallikrein 4 in healthy and cancerous prostatic tissues by immunofluorometry and immunohistochemistry. Clin Chem 2002;48:1232–40.
26. Yousef GM, Polymeris ME, Grass L, Soosaipillai A, Chan PC, Scorilas A. et al. Human kallikrein 5: a potential novel serum biomarker for breast and ovarian cancer. Cancer Res 2003;63:3958–65.
27. Diamandis EP, Scorials A, Fracchioli S, Van Gramberen M, De Bruijn H, Henrik A, et al. Human kallikrein 6 (hK6): a new potential serum biomarker for diagnosis and prognosis of ovarian carcinoma. J Clin Oncol 2003;21:1035–43.
28. Kishi T, Soosaipillai A, Grass L, Little SP, Johnstone EM, Diamandis EP. Development of an immunofluorometric assay and quantification of human kallikrein 7 in tissue extracts and biological fluids. Clin Chem 2004;50:709–16.
29. Kishi T, Grass L, Soosaipillai A, Shimizu-Okabe C, Diamandis EP. Human kallikrein 8: immunoassay development and identification in tissue extracts and biological fluids. Clin Chem 2003;49:87–96.
30. Luo LY, Grass L, Howarth DJ, Thibault P, Ong H, Diamandis EP. Immunofluorometric assay of human kallikrein 10 and its identification in biological fluids and tissues. Clin Chem 2001;47:237–46.
31. Diamandis EP, Okui A, Mitsui S, Luo LY, Soosaipillai A, Grass L, et al. Human kallikrein 11: a new biomarker of prostate and ovarian carcinoma. Cancer Res 2002;62:295–300.
32. Kapadia C, Chang A, Sotiropoulou G, Yousef GM, Grass L, Soosaipillai A, et al. Human kallikrein 13: production and purification of recombinant protein and monoclonal and polyclonal antibodies, and development of a sensitive and specific immunofluorometric assay. Clin Chem 2003;49:77–86.
33. Borgono CA, Grass L, Soosaipillai A, Yousef GM, Petraki CD, Howarth DH, et al. Human kallikrein 14: a new potential biomarker for ovarian and breast cancer. Cancer Res 2003;63:9032–41.
34. Magklara A, Grass L, Diamandis EP. Differential steroid hormone regulation of human glandular kallikrein (hK2) and prostate-specific antigen (PSA) in breast cancer cell lines. Breast Cancer Res Treat 2000;59:263–70.
35. Monne M, Croce CM, Yu H, Diamandis EP. Molecular character-

ization of prostate-specific antigen messenger RNA expressed in breast tumors. Cancer Res 1994;54:6344–7.

36. Yousef GM, Luo LY, Scherer SW, Sotiropoulou G, Diamandis EP. Molecular characterization of zyme/protease M/neurosin (PRSS9), a hormonally regulated kallikrein-like serine protease. Genomics 1999;62:251–9.

37. Luo LY, Grass L, Diamandis EP. Steroid hormone regulation of the human kallikrein 10 (KLK10) gene in cancer cell lines and functional characterization of the KLK10 gene promoter. Clin Chim Acta 2003;337:115–26.

38. Yousef GM, Scorilas A, Diamandis EP. Genomic organization, mapping, tissue expression, and hormonal regulation of trypsin-like serine protease (TLSP PRSS20), a new member of the human kallikrein gene family. Genomics 2000;63:88–96.

39. Yousef GM, Fracchioli S, Scorilas A, Borgono CA, Iskander L, Puopolo M, et al. Steroid hormone regulation and prognostic value of the human kallikrein gene 14 in ovarian cancer. Am J Clin Pathol 2003;119:346–55.

40. Yousef GM, Scorilas A, Magklara A, Memari N, Ponzone R, Sismondi P, et al. The androgen-regulated gene human kallikrein 15 (KLK15) is an independent and favourable prognostic marker for breast cancer. Br J Cancer 2002;87:1294–300.

41. Murtha P, Tindall DJ, Young CY. Androgen induction of a human prostate-specific kallikrein, hKLK2: characterization of an androgen response element in the 5′ promoter region of the gene. Biochemistry 1993;32:6459–64.

42. Schuur ER, Henderson GA, Kmetec LA, Miller JD, Lamparski HG, Henderson DR. Prostate-specific antigen expression is regulated by an upstream enhancer. J Biol Chem 1996;271:7043–51.

43. Cleutjens KB, van der Korput HA, van Eekelen CC, van Rooij HC, Fabe PW, Trapman J. An androgen response element in a far upstream enhancer region is essential for high, androgen-regulated activity of the prostate-specific antigen promoter. Mol Endocrinol 1997;11:148–61.

44. Cleutjens KB, van Eekelen CC, van der Korput HA, Brinkmann AO, Trapman J. Two androgen response regions cooperate in steroid hormone regulated activity of the prostate-specific antigen promoter. J Biol Chem 1996;271:6379–88.

45. Guzelmeric K, Seker N, Unal O, Turan C. High serum prostate-specific antigen concentrations in hirsute women do not decrease with treatment by the combination of spironolactone and the contraceptive pill. Gynecol Endocrinol 2004;19:190–5.

46. Escobar-Morreale H, Avila S, Sancho J. Serum prostate-specific antigen concentrations are not useful for monitoring the treatment of hirsutism with oral contraceptive pills. J Clin Endocrinol Metab 2000;85:2488–92.

47. Luo LY, Grass L, Diamandis EP. The normal epithelial cell-specific 1 (NES1) gene is up-regulated by steroid hormones in the breast carcinoma cell line BT-474. Anticancer Res 2000;20:981–6.

48. Yousef GM, Borgono CA, Scorilas A, Ponzone R, Biglia N, Iskander L, et al. Quantitative analysis of human kallikrein gene 14 expression in breast tumours indicates association with poor prognosis. Br J Cancer 2002;87:1287–93.

49. Giltay EJ, Gooren LJ, Emeis JJ, Kooistra T, Stehouwer CD. Oral ethinyl estradiol, but not transdermal 17β-estradiol, increases plasma C-reactive protein levels in men. Thromb Haemost 2000; 84:359–60.

50. Toorians AW, Thomassen MC, Zweegman S, Magdeleyns EJ, Tans G, Gooren LJ, et al. Venous thrombosis and changes of hemostatic variables during cross-sex hormone treatment in transsexual people. J Clin Endocrinol Metab 2003;88:5723–9.

51. Ho KK, O'Sullivan AJ, Wolters T, Leung KC. Metabolic effects of estrogens: impact of the route of administration. Ann Endocrinol (Paris) 2003;64:170–7.

52. Leung KC, Johannsson G, Leong GM, Ho KK. Estrogen regulation of growth hormone action. Endocr Rev 2004;25:693–721.

53. Goldzieher JW. Pharmacology of contraceptive steroids: a brief review. Am J Obstet Gynecol 1989;160:1260–4.

54. Goebelsmann U, Mashchak CA, Mishell DR. Comparison of hepatic impact of oral and vaginal administration of ethinyl estradiol. Am J Obstet Gynecol 1985;151:868–77.