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# Analysis of Relations Between Serum Levels of Epitestosterone, Estradiol, Testosterone, IGF-1 and Prostatic Specific Antigen in Men with Benign Prostatic Hyperplasia and Carcinoma of the Prostate

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## Summary

Epitestosterone competes with testosterone for androgen receptors and inhibits several enzymes of steroidogenesis. Insulin-like growth factors (IGFs) stimulate the growth of prostate cells and directly activate androgen receptors in prostatic tumor cell lines. The prostate-specific antigen (PSA) decreases the affinity of IGF-binding protein-3. The samples were collected from 71 patients suffering from various diseases of the prostate (56 patients without prostate cancer but with benign prostatic hyperplasia and 15 patients with prostate cancer). Correlations between age and IGF-1 *($r = -0.281$, $p<0.05$)*, age and serum epitestosterone *($r = -0.261$, $p<0.05$)*, estradiol and testosterone *($r = 0.367$, $p<0.01$)*, and between estradiol and epitestosterone *($r = -0.414$, $p<0.001$)* were found. After age adjustment, IGF-1 correlated with epitestosterone *($r = -0.277$, $p<0.05$)*. The age correlated positively with PSA *($r = 0.286$, $p<0.05$)* and negatively with IGF-1 *($r = -0.377$, $p<0.01$)* in partial correlations. PSA levels were higher in patients with prostate cancer ($p<0.00001$). Epitestosterone, which is negatively correlating with estradiol and IGF-1, may modulate the development of prostate diseases.

**Key words**

Epitestosterone • Prostatic specific antigen • Insulin-like growth factor • Estradiol • Prostate • Carcinoma

## Introduction

Epitestosterone (17α-hydroxy-4-androsten-3-one), a naturally occurring epimer of testosterone, acts in various target tissues of mammals, principally as an antiandrogen and an inhibitor of a number of enzyme systems essential for steroid metabolism. Its antiandrogenic activity *in vivo* was first reported by Nuck and Lucky (1987) and was later confirmed as true antiandrogenic activity by Stárka *et al.* (1991), who demonstrated epitestosterone competition with testosterone in the binding to an androgen receptor. Epitestosterone also strongly inhibits steroid 5α-reductase and thus prevents the conversion of testosterone to dihydrotestosterone to some degree (Monsalve and Blaquier 1977, Stárka *et al.* 1991).



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Furthermore, other steroid metabolizing enzymes are also inhibited by epitestosterone, e.g. $C_{17,20}$-lyase (Bičíková *et al.* 1992) and 11β-hydroxysteroid dehydrogenase (Bičíková *et al.* 1997). Epitestosterone also acts non-genomically (Perusquia *et al.* 1996). Its overall antiandrogenic action in the prostate was compared with other antiandrogens by Maucher *et al.* (1994), who found its effect on prostate cell growth using an experimental model of human prostatic carcinoma, the natural cell line of prostate carcinoma (LNCaP) and Dunning's carcinoma R 3327 *in vivo*. In this model, epitestosterone acted similarly as other steroid or non-steroid antiandrogens, cyproterone acetate or flutamide, but differently from casodex.

Insulin-like growth factors (IGFs) are potent mitogens that stimulate the growth of prostate cells. In the serum, circulating IGFs are bound to IGF-binding proteins (IGFBPs) that modulate their proliferative action. IGFBPs may be involved in the growth modulation of prostate malignancy, and alteration in their serum levels may serve as a marker for prostate cancer (Kanety *et al.* 1993). In the absence of androgens, IGF-1 directly activates androgen receptors (AR) in human prostatic tumor cell lines (Culig *et al.* 1994).

Contradictory results have been published on the significance of IGF-1 in the diagnosis of prostate carcinoma. Some authors have questioned the usefulness of IGF-1 as a marker for prostate cancer, and consider the levels of prostate-specific antigen (PSA) and even age as better predictors of the presence of prostate cancer than serum IGF-1 levels (Cutting *et al.* 1999). However, other authors have found a strong positive relationship between IGF-1 levels and prostate cancer risk (Chan *et al.* 1998).

Prostate-specific antigen is a 33 kD protein synthesized in epithelial cells of the prostate gland. PSA decreases affinity of IGF-binding protein-3 (IGFBP-3) for IGF and can potentiate IGF action in the presence of inhibitory IGFBP-3. This phenomenon may contribute to normal and malignant prostate growth (Culig *et al.* 1995). Serum PSA concentrations are frequently increased in patients with prostate cancer, but this is also the case in patients with benign prostate hyperplasia. Serum PSA concentrations can successfully be combined with other methods in the diagnosis of prostatic diseases and in monitoring the success of prostate cancer treatments (Culig *et al.* 1995).

At present, a study concerning the relation of epitestosterone to PSA or IGF-1 levels is lacking. We have attempted to ascertain whether epitestosterone, as an antiandrogen and 5α-reductase inhibitor, could play a role in the hormonal regulation of prostate growth influenced by such sexual steroid hormones as estradiol and testosterone, and its relation to IGF-1. For this reason the serum levels of epitestosterone (EpiT), estradiol (E2), testosterone (T), IGF-1, prostatic specific antigen (PSA) were determined in men suffering with benign prostatic hyperplasia (BPH) and carcinoma of the prostate (CaP) and their mutual interrelationships were evaluated by means of correlation analysis.

## Materials and Methods

*Subjects, serum samples and analytical methods*

The samples were collected from 71 patients suffering from various diseases of the prostate (56 patients without prostate cancer but with benign prostatic hyperplasia, 15 patients with prostate cancer). In addition, prostatolithiasis (PL) was detected in the prostate tissue from 27 of these patients. The exclusion criteria included disorders of hepatic metabolism, previous or concurrent systemic or drug therapy of BPH and any hormonal therapy during 3 months prior to blood collection and surgery. Prostate cancer was differentiated from BPH by histological classification in the prostatic tissue after suprapubic prostatectomy. Blood was collected from the cubital vein and plasma was stored at –80 °C until analyzed. In all cases, written informed consent was obtained from the patients concerned.

Serum epitestosterone was measured by the method of Bílek *et al.* (1987). Standard RIA kits from Immunotech Praha a.s. (The Coulter Company), Czech Republic, were used for the determination of testosterone (RIA, code 119), estradiol (RIA, code 1663), IGF-1 (IRMA after extraction, code 1674) and PSA (ILMA, code 1721).

*Statistical analysis of the data*

1. One-dimensional exploratory data analyses for individual variables were performed. Diagnostic graphs (statistical software STATGRAPHICS) were used for evaluation of the data distribution, and for the detection of outliers.
2. Variables with a skewed data distribution were treated by power-transformation to obtain skewness within the interval (–0.1, 0.1).
3. Correlation analysis of variables after power transformation to minimum skewness was performed. At the same time, a robust Spearman's rank correlation matrix was computed from the non-transformed original variables.
4. For the adjustment of correlations to age, partial correlations of the second order were computed.

5. For the evaluation of inter-group differences, a robust Mann-Whitney test was used with respect to the skewed data distributions.
6. Fischer's exact two-tailed test was used for the evaluation of the relationship between frequency of benign prostate hypertrophy (BPH), prostate cancer (CaP) and PL in a 2×2 contingency table.

**Table 1.** Age and serum levels of PSA, IGF-1, E2, T and EpiT in patients without and with prostate cancer.

| | AGE [years] | PSA [ng/ml] | IGF-1 [ng/ml] | E2 [pg/ml] | T [ng/ml] | EpiT [pg/ml] |
|---|---|---|---|---|---|---|
| | | | ***Patients with BPH and without prostate cancer (n=56)*** | | | |
| *Mean* | 72.2 ± 8.3 | 10.9 ± 19.4 | 167.5 ± 59.6 | 42.2 ± 25.9 | 5.6 ± 4.4 | 111.4 ± 74.8 |
| *Median* | 71 (55-90) | 5.4 (1-129) | 160 (68-320) | 38 (1-142) | 4.5 (0.2-23) | 100 (2-293) |
| *Skewness* | 0.602 | 14.478 | 1.683 | 4.450 | 5.734 | 1.875 |
| | | | ***Patients with prostate cancer (n=15)*** | | | |
| *Mean* | 77.1 ± 6.9* | 48.1 ± 47.7** | 155.3 ± 53.0 | 32.58 ± 18.3 | 7.5 ± 8.1 | 113.6 ± 54.2 |
| *Median* | 78 (64-86) | 26 (7-148) | 156 (72-258) | 31 (9.5-71.5) | 4.0 (0.1-23.9) | 109 (10-198) |
| *Skewness* | -0.459 | 2.103 | 0.283 | 1.205 | 1.759 | -0.441 |

*Significant differences (Mann-Whitney test) between patients without and with prostate cancer: * $p<0.05$, $p<0.0001$.*

**Table 2.** Correlations between particular variables using the data from all patients (n=71).

| | | | | | | |
|---|---|---|---|---|---|---|
| *r=* | ***AGE*** | *0.181* | **-0.281** | *0.031* | *-0.105* | **-0.261** |
| *p<...* | | *0.131* | **0.018** | *0.795* | *0.385* | **0.028** |
| *r=* | *0.226* | ***PSA*** | *0.037* | *-0.059* | *0.005* | *-0.014* |
| *p<...* | *0.058* | | *0.757* | *0.623* | *0.969* | *0.910* |
| *r=* | **-0.313** | *0.048* | ***IGF-1*** | *0.097* | *0.133* | *-0.198* |
| *p<...* | **0.008** | *0.693* | | *0.420* | *0.268* | *0.098* |
| *r=* | *-0.054* | *-0.109* | *0.064* | ***E2*** | **0.367** | **-0.419** |
| *p<...* | *0.655* | *0.364* | *0.598* | | **0.002** | **0.000** |
| *r=* | *-0.046* | *-0.029* | *0.120* | **0.435** | ***T*** | *-0.119* |
| *p<...* | *0.704* | *0.813* | *0.319* | **0.000** | | *0.323* |
| *r=* | **-0.241** | *-0.023* | *-0.180* | **-0.414** | *-0.066* | ***EpiT*** |
| *p<...* | **0.043** | *0.852* | *0.133* | **0.000** | *0.586* | |

*The Pearson's correlations of the variables transformed by power transformation and the Spearman's correlations are given above and below the diagonal, respectively. The correlation coefficients and the corresponding significance levels are shown in the upper and lower parts of the cells, respectively. Bold numbers signalize the significant correlations ($p<0.05$).*

**Table 3.** Correlations between particular variables using the data from all patients adjusted to constant age.

| | | | | | | |
|---|---|---|---|---|---|---|
| *r=* | ***AGE*** | *0.000* | *0.000* | *0.000* | *0.000* | *0.000* |
| *p<...* | | *1.000* | *1.000* | *1.000* | *1.000* | *1.000* |
| *r=* | *0.000* | ***PSA*** | *0.093* | *-0.066* | *0.024* | *0.035* |
| *p<...* | *1.000* | | *0.442* | *0.587* | *0.843* | *0.771* |
| *r=* | *0.000* | *0.128* | ***IGF-1*** | *0.111* | *0.109* | **-0.293** |
| *p<...* | *1.000* | *0.292* | | *0.362* | *0.371* | **0.014** |
| *r=* | *0.000* | *-0.100* | *0.049* | ***E2*** | **0.373** | **-0.426** |
| *p<...* | *1.000* | *0.411* | *0.685* | | **0.001** | **0.000** |
| *r=* | *0.000* | *-0.019* | *0.111* | **0.434** | ***T*** | *-0.152* |
| *p<...* | *1.000* | *0.878* | *0.359* | **0.000** | | *0.208* |
| *r=* | *0.000* | *0.034* | **-0.277** | **-0.441** | *-0.079* | ***EpiT*** |
| *p<...* | *1.000* | *0.782* | **0.020** | **0.000** | *0.515* | |

*The Pearson's correlations of the variables transformed by power transformation and the Spearman's correlations are given above and below the diagonal, respectively. The correlation coefficients and the corresponding significance levels are shown in the upper and lower parts of the cells, respectively. Bold numbers signalize the significant correlations ($p<0.05$).*

## Results

Table 1 shows that significant differences between the mean values of patients with or without CaP were observed only in their age and serum levels of the prostate specific antigen (PSA). The best discriminator between the BPH patients and the patients with CaP was PSA, the difference between the group medians being highly significant ($p<0.00001$, Mann-Whitney test). The remaining variables did not differ significantly between the BPH and CaP groups. The values above 6.5 ng/ml of PSA could signalize a higher risk of prostate cancer.

Initially, a correlation analysis of the relationships between age, IGF-1, PSA, estradiol, testosterone and epitestosterone was performed in all the patients irrespective of their diagnosis. PSA, estradiol and testosterone exhibited strongly skewed data distributions (Table 1). Since a normal data distribution is a prerequisite for the application of commonly used multivariate statistical methods, the original data were transformed by power transformation to obtain a skewness within the interval (–0.1, 0.1) before they were analyzed using correlation analysis (Table 2). Concomitantly, a robust Spearman's correlation matrix was also calculated (Table 2), which yielded similar results. Significant negative correlations between age and IGF-1 ($p<0.01$), and between age and epitestosterone ($p<0.05$) were found, whereas a highly significant positive correlation was found between estradiol and testosterone ($p<0.001$). In addition, a highly significant ($p<0.001$) negative correlation between estradiol and epitestosterone was detected (Table 2, Fig. 1).

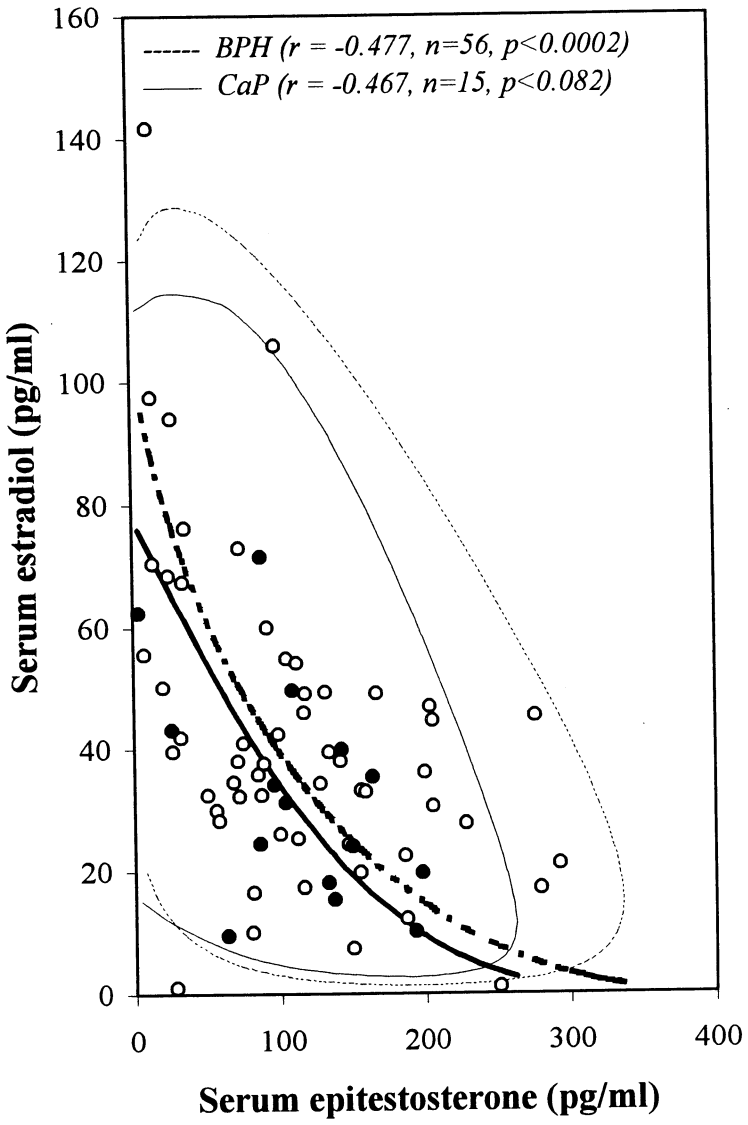


**Fig. 1.** *Correlation of serum estradiol (E2) with serum epitestosterone (EpiT). The correlation coefficients, SD lines and 95 % confidence elipsoids were computed from the variables transformed by power transformation to skewness within the interval ±0.1 separately in the group of patients with benign prostatic hyperplasia (BPH ) and in patients with prostate cancer (CaP). The values of E2 were powered to 0.48 and 0.30 in BPH and CaP patients, respectively, and the values of EpiT were powered to 0.67 and 1.1 in BPH and CaP patients, respectively. Computed S.D. lines and 95% confidence elipsoids were re-transformed to original scale to enable a graphical comparison of the relations between E2 and EpiT in BPH and in CaP patients. The bold and broken curves represent the re-transformed S.D. line of the correlation in patients with benign prostatic hyperplasia (BPH) and prostate cancer (CaP), respectively. The thinner dotted line and full line represent the re-transformed 95 % confidence elipsoids of the correlation in patients with BPH and CaP, respectively.*

To eliminate the influence of age on the mutual correlations, partial correlations of the second order with adjustment of the correlation matrix to constant age were computed for the remaining variables (Table 3). The adjustment of the correlations to constant age did not influence the significance of the correlations between the other variables studied, with the exception of IGF-1, which also negatively correlated with epitestosterone ($p<0.05$).

**Table 4.** Comparison of the correlations between particular variables using the data from patients with CaP and from patients with BPH.

| | | | | | | |
|---|---|---|---|---|---|---|
| r= | | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| n= | ***AGE*** | 15 | 15 | 15 | 15 | 15 |
| p<... | | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 |
| r= | 0.000 | | 0.230 | -0.338 | -0.445 | **0.538** |
| n= | 56 | ***PSAS*** | 15 | 15 | 15 | **15** |
| p<... | 1.000 | | 0.430 | 0.237 | 0.111 | **0.047** |
| r= | 0.000 | 0.122 | | -0.015 | -0.201 | -0.024 |
| n= | 56 | 56 | ***IGF1S*** | 15 | 15 | 15 |
| p<... | 1.000 | 0.375 | | 0.960 | 0.491 | 0.936 |
| r= | 0.000 | 0.030 | 0.043 | | **0.784** | -0.301 |
| n= | 56 | 56 | 56 | ***E2S*** | **15** | 15 |
| p<... | 1.000 | 0.831 | 0.754 | | **0.001** | 0.295 |
| r= | 0.000 | 0.062 | 0.161 | **0.332** | | 0.010 |
| n= | 56 | 56 | 56 | **56** | ***TESS*** | 15 |
| p<... | 1.000 | 0.652 | 0.241 | **0.013** | | 0.972 |
| r= | 0.000 | -0.120 | -0.259 | **-0.453** | -0.037 | |
| n= | 56 | 56 | 56 | **56** | 56 | ***ETS*** |
| p<... | 1.000 | 0.384 | 0.056 | **0.001** | 0.791 | |

*Spearman's correlations of the variables measured in CaP and BPH patients are given above and below the diagonal, respectively. The correlation coefficients, the number of patients and the corresponding significance levels are shown in individual cells. Bold numbers represent significant correlations ($p<0.05$) and gray-colored cells signalize a remarkable distinction in correlations between CaP and BPH patients.*

For a comparison of the correlations between individual variables, Spearman's correlations adjusted to constant age were used separately in the group of BPH patients and in patients with prostate cancer (Table 4). A marked difference was found in the correlation between PSA and epitestosterone, which was considerably stronger in CaP patients. A similar pattern was observed for the correlation between estradiol and testosterone. However, the correlation between estradiol and epitestosterone was stronger in BPH patients. A negative correlation between PSA and testosterone in CaP patients contrasted to that between PSA and epitestosterone. These correlations were not found in BPH patients. Some differences could also be ascertained in the correlation between IGF-1 and epitestosterone.

The frequency of prostatolithiasis was higher ($p<0.01$, Fischer's two-tailed test) in patients with benign prostatic hyperplasia (46.4 %) than in those with prostatic cancer (6.7 %).

## Discussion

Sufficient evidence has accumulated that epitestosterone elicits an antiandrogenic and 5α-reductase inhibiting activity. It is present in human blood and the prostate tissue in concentrations sufficient to influence the overall androgenic effect in the periphery. It is difficult to express the overall androgen/antiandrogen balance. However, at least in patients with prostate diseases, the significant negative correlations of epitestosterone with both estradiol and IGF-1 confirm that these two important factors in the development of both benign prostate hyperplasia and prostate cancer might be influenced by epitestosterone. The effect of epitestosterone on the cellular metabolism of estradiol could be considered as the possible mechanism of its action. In the natural cell line of prostate carcinoma (LNCaP), estradiol acts on the membrane binding sites linked to $Ca^{2+}$ channels (Audy *et al.* 1996) and mediates the response of the epithelium to androgens through IGF-1 (Audy *et al.* 1996). Estradiol significantly stimulates the growth of LNCaP, while at the same time it inhibits the proliferation of PC3 cells (Audy *et al.* 1996). It is suggested that estrogen not only induces stromal proliferation and secretion, but also conditions the response of the epithelium to androgen through IGF-1 (Farnsworth 1996). Epitestosterone *in vitro* inhibits 17β-hydroxysteroid dehydrogenase and thus regulates the local interconversion of estradiol to estrone. Growth factors also exert a potent local influence on the growth of the target organs (Audy *et al.* 1996, Farnsworth 1996). Nevertheless, the mechanism by which epitestosterone influences the production or activation of IGF-1 remains an open question. Since epitestosterone negatively correlates with estradiol and IGF-1, both known as activators of prostate growth and proliferation, and at the

same time it exhibits antiandrogenic activity, it may be concluded that this steroid may act as an anticancerogenic factor in the prostate.

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