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Female Stress Incontinence and Uterovaginal Prolapse: Collagen Turnover and Hormone Sensitivity in Urogenital Tissue

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Abstract

Background Prevalence of both stress urinary incontinence (SUI) and uterovaginal prolapse (UP) is high and rising with age. Risk factors include multiparity, obesity, chronic obstructive lung disease and previous gynecological surgery. The underlying pathology is still unknown but may include defective connective tissue.

Aims To study collagen turnover markers in urogenital tissue, in order to gain insight regarding a possible altered collagen synthesis or metabolism in SUI and UP, if circulating sex steroids have any influence on collagen turnover and if there are any differences in sex steroids between women with or without SUI.

Methods A total of 126 women were consecutively enrolled and classified according to urogenital status into three groups: SUI without UP, N=71; UP without incontinence, N=24; urologically healthy controls without UP, N=31. Urogenital tissue biopsies and serum was collected for analysis of the tissue and serum collagen turnover markers carboxy-terminal propeptide of type I procollagen (PICP), the carboxy-terminal telopeptide of type I collagen (ICTP) and the amino-terminal propeptide of procollagen III (PIIINP) and serum steroids, steroid binding proteins and IGF-I.

Result Compared to controls tissue (T-)PIIINP and T-ICTP were significantly lower in SUI and T-PICP and T-PIIINP significantly higher in UP. Tissue collagen turnover markers were positively correlated to serum estradiol-17 β , especially at physiological serum estrone levels, in the controls but not in SUI patients except to a certain degree in premenopausal subjects. Instead of being related to serum estradiol-17 β , tissue collagen turnover markers in SUI patients were negatively correlated to serum total and free testosterone. There were no significant differences between comparable subgroups of SUI patients and controls in circulating sex steroids.

Conclusions Our findings indicate a reduced collagen breakdown in SUI and an increased collagen turnover in UP, both which may negatively tissue elasticity and strength. Urogenital tissue collagen turnover may be stimulated by estrogen in urologically healthy women but not in SUI patients in general but to a certain degree in premenopausal subjects. The latter finding may indicate menopause related changes as one underlying factor behind SUI. At supraphysiological concentrations estrone may act as a partial estradiol-17 β antagonist also in vivo. Urogenital tissue collagen turnover in SUI patients may be inhibited by testosterone, perhaps by inhibition of matrix metalloprotease activity. Our findings emphasize the fact that SUI and UP have two distinctly different etiologies and shall be studied using »clean« patient materials

Key words Stress incontinence; urogenital tissue, collagen turnover markers; estradiol-17 β ; estrone; testosterone; uterovaginal prolapse; menopause

List of Publications

- I Lena Edwall, Kjell Carlström and Aino Fianu Jonasson. Markers of collagen synthesis and degradation in urogenital tissue from women with and without stress urinary incontinence. *Neurourology and Urodynamics* 24:319–324 (2005).
- II Lena Edwall, Kjell Carlström and Aino Fianu Jonasson. Endocrine Status and Markers of Collagen Synthesis and Degradation in Serum and Urogenital Tissue From Women With and Without Stress Urinary Incontinence. *Neurourology and Urodynamics* 26:410–415 (2007).
- III Lena Edwall, Kjell Carlström and Aino Fianu Jonasson. Markers of collagen synthesis and degradation in urogenital tissue and serum from women with and without uterovaginal prolapse. *Molecular Human Reproduction* Vol.14, No.3 pp. 193–197 (2008).
- IV Lena Edwall, Kjell Carlström and Aino Fianu Jonasson. Different estrogen sensitivity of urogenital tissue from women with and without stress urinary incontinence. *Neurourology and Urodynamics*, under revision (2008).

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List of abbreviations

BMI	Body Mass Index
CTR	Controls
E1	Estrone
E2	Estradiol-17 β
ER	Estrogen receptor
fT	Free testosterone
HRT	Hormone replacement therapy
ICS	International Continence Society
ICTP	The carboxy-terminal telopeptide of Type I collagen
MCF-7	Human breast cancer cells
MMP	Metallomatrix protease
MP	Menopause
OAB	Over active bladder
PICP	The carboxy-terminal propeptide of Type I procollagen
PIIINP	The amino-terminal propeptide of procollagen III
RIA	Radioimmunoassay
RNA	Ribonucleic acid
S-ICTP	Serum-ICTP
S-PICP	Serum-PICP
S-PIIINP	Serum-PIIINP
SD	Standard deviation
SHBG	Sex hormone-binding globulin
SUI	Stress urinary incontinence

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T	Testosterone
T-ICTP	Tissue-ICTP
T-PICP	Tissue-PICP
T-PIIINP	Tissue-PIIINP
TIMP-1	Tissue inhibitor of MMP-1
UP	Uterovaginal prolapse
U _{sr}	Uterosacral resilience
WHO	World Health Organization

Introduction

Background and personal reflexion

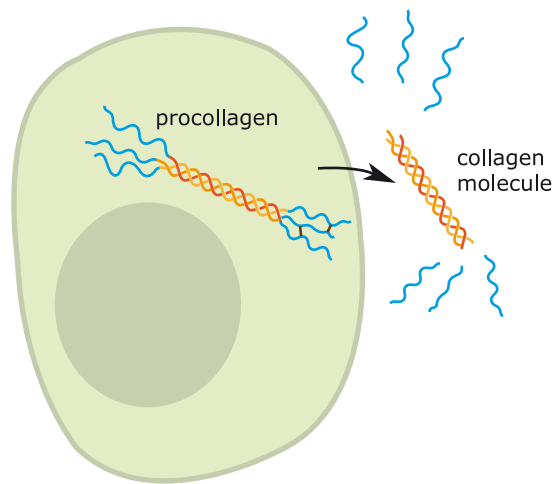
Life expectancy for women in Sweden today is 84 years and with good general health more than 20 years of active life is a reality after pension at 65. Incontinence and vaginal prolapse are disorders that hinder active life and causes embarrassment both in public and private life. Quality of life questionnaires show that sexual activity is impaired and avoided due to the risk of urinary leakage. In the case of prolapse mechanical symptoms, vaginal abrasions and pain are additional symptoms.

Female incontinence is primarily divided into stress urinary incontinence (SUI) or Over Active Bladder (OAB). In SUI involuntary leakage occurs, on effort or exertion, or when sneezing or coughing. The leakage stops once the provocation is ended. In OAB the patient has a micturition frequency of more than 8 times per day, a feeling of urge with or without urge urinary incontinence, and nocturia. Mixed incontinence is a combination of SUI and OAB.

In this thesis only SUI is adressed in the studies, and women with OAB or mixed incontinence are excluded both as patients and controls.

For SUI we have modern surgical methods, retropubic prolene slings for example, with now more than 10 years observation time and with cure rates of >80 %, with the reservation for high numbers of loss to follow up (CG Nilsson et al., 2008). However, in the case of vaginal prolapse the recurrence rates

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are high, at five years after standard surgery, 41 % (Miedel et al., 2008). Studying long term results, 12 years after prolapse surgery, Tegerstedt and co-workers reported a subjective cure rate of 46 % and an objective cure rate, with satisfactory anatomic outcome, at 56 % (Tegerstedt et al., 2004). Observation times of the many different mesh methods for correction in recurrent disorder are still short and already show relatively high numbers of complications.

With these facts at hand and the tremendous cost in personal grievance for these women and the cost for society – loss of income, medical service and all the sanitary pads – research into underlying mechanisms of incontinence and prolapse is important. We should also address these disorders separately, as not all women with prolapse are incontinent and vice versa.

Incontinence

The World Health Organization (WHO) today classifies urinary incontinence as a disease and approximately 10 % of Swedish women are handicapped in daily life by this disorder. The frequency increases linearly to 21 % at the age of 70. The

International Continence Society (ICS) definition of urinary incontinence is regularly recurring leakage, at least once per week (Abrams et al., 2002). About 50 % of the women thus defined have stress urinary incontinence (SUI). Risk factors of importance as regards SUI are obesity, pregnancy, asthma, previous gynecological surgery, and strenuous heavy work (Abrams et al., 2002). Hypermobility of the bladder neck and laxity of the vaginal wall are anatomical and structural factors proposed to explain SUI (Petros and Ulmsten, 1990). The underlying pathology and cause of SUI are still unknown, but the results of some studies suggest that the connective tissue might be defective in these women (Falconer et al., 1994, 1998).

Uterovaginal prolapse

Uterovaginal prolapse (UP) is a common pelvic support defect of low morbidity, but profoundly affecting the quality of life. In Sweden, about 6000 women will be operated on every year for this disorder. Multiparity, especially with vaginal delivery, age and genetic predisposition are suggested risk factors for UP (Timonen et al., 1968; Porges and Smilen, 1994; Gill and Hurt, 1998). Concerning biomechanical properties, the uterosacral ligament resilience (Usr) is significantly reduced in UP and reduced Usr was also associated with vaginal delivery, menopause and age. Reduced Usr may facilitate progression to symptomatic pelvic visceral prolapse (Reay Jones et al., 2003). Furthermore, the amounts of smooth muscle in vaginal tissue appear to be lowered in women with UP (Goh, 2003). In addition, a poor contractility of the genital myofibroblasts was recently reported in women with UP (Poncet et al., 2005).

Collagen

The content as well as the spatial organization of different types of collagen are the key to tissue strength and elasticity. Type I collagen is the predominant protein in mineralized bone but

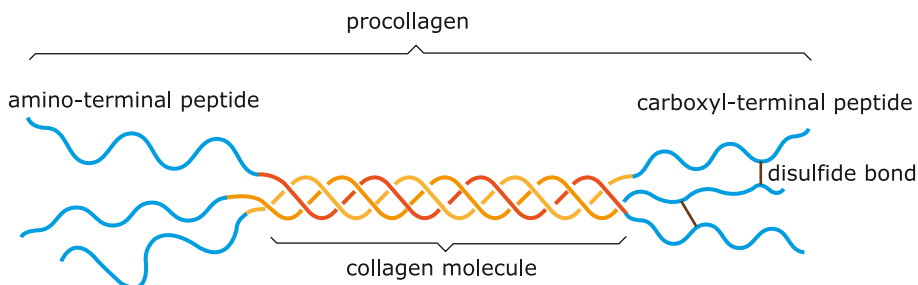
it is also abundant in soft tissues, while type III collagen only exists in soft tissue (Eriksen et al., 1993; Risteli and Risteli, 1993; Risteli et al., 1995). Several biochemical serum markers of the synthesis and degradation of collagens have been investigated in the study of bone metastasis, osteoporosis, and evaluation of estrogen treatment. In collagen synthesis, large parts of both the carboxy- and amino-terminal ends of the precursor molecule are split off and released into the extracellular fluid. Assays of circulating carboxy-terminal propeptide of type I procollagen (PICP) and of the carboxy-terminal telopeptide of type I collagen (ICTP) are widely used for studying synthesis of type I collagen and the degradation of mature, trivalently cross-linked type I collagen, respectively. The amino-terminal propeptide of procollagen III (PIIINP) is used for studying synthesis of new type III collagen as well as the degradation of existing type III collagen fibrils (Hassager et al., 1992; Risteli et al., 1995; Suvanto-Luukonen et al., 1997).

Collagen and SUI

Collagen synthesis and metabolism in urogenital tissue may certainly have direct implications as regards its function and strength and thus on its ability to control micturition and continence. There are divergent data concerning collagen in SUI. Women with SUI have a reduced total collagen content in the skin (Ulmsten et al., 1987) and urogenital tissue (Keane et al., 1997; Rechberger et al., 1998; Liapis et al., 2001), which is in accordance with decreased collagen production in skin cultures (Falconer et al., 1994). On the other hand, a higher total collagen concentration and higher levels of mRNAs for type I and type III collagen in paraurethral connective tissue from women with SUI have been reported (Falconer et al., 1998). As far as we know from the literature, markers of collagen synthesis and degradation have not been studied in urogenital tissue from women with SUI.

Collagen and UP

Studies on urogenital tissue collagen content in UP have yielded divergent results. Lack of differences in the content of collagen Type I between women with and without UP has been reported (Porges and Smilen, 1994; Ewies et al., 2003); however, Barbiero and co-workers reported that collagen Type I fibres are shorter and thinner and more sparse in tissue from women with UP (Barbiero et al., 2003). Cell cultures from vaginal fascias from women with UP are reported to have the same ability to synthesize Type I procollagen mRNA as cell cultures from healthy women (Mäkinen et al., 1987). For Type III collagen Liapis and co-workers reported similar levels in genital tissue from women with and without UP (Liapis et al., 2001), whereas Ewies and co-workers report higher collagen Type III content in tissue from women with UP (Ewies et al., 2003) and this finding was supported by some similar studies (Gabriel et al., 2005; Moalli et al., 2005) but not by others (Lin et al., 2007). For total collagen, numerous workers have reported decreased tissue levels in UP (Stanosz et al., 1995; Jackson et al., 1996; Takano et al., 2002; Wong et al., 2003; Söderberg et al., 2004). Concerning collagen turnover and structure, Jackson and co-workers also reported that there was no difference in the ratio between Types I and III collagen between women with and without UP but collagen from women with UP is considerably less acid-soluble, probably reflecting the maturity of the tissue (Jackson et al., 1996). They



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also reported a marked increase in collagen metabolism due to the increased cathepsin and metalloproteinase (MMP) levels, leading to loss of strength of the tissue. Similar findings are reported in the recent paper of Phillips and co-workers, who reported increased pro-MMP2 RNA expression in vaginal tissue from women with UP compared with tissue from healthy women (Phillips et al. 2006). They further found very close correlations between the expressions of different MMPs in vaginal mucosa and uterosacral ligaments. In addition, they discussed to what degree an increased collagen breakdown precedes the prolapse or represents a resistance to stretching within the uterosacral ligaments in UP.

Hormones

Estrogen receptors (ER) are present in urogenital tissue and estrogen treatment has been an initial treatment for incontinence in Sweden; however, estrogen treatment of SUI has become controversial (Robinson and Cardozo, 2004). Reports on effects of estrogen on urogenital tissue have been reported;(Heimer et al., 1992; Falconer et al. 1996; Jackson et al., 2002) however others have reported no such effects on total collagen levels in soft tissue (Haapasaari et al. 2002; Dundar et al., 2003). Data on estrogen levels in SUI are scarce. Bai and co-workers reported no difference in urinary estrogen excretion between healthy women and women with SUI (Bai et al., 2003). They also reported a significant negative correlation between urinary excretion of estradiol-17 β (E₂) and bladder neck descent, indicating a positive effect of estrogens on urogenital tissue in SUI. On the other hand, several recent large clinical studies have reported no effects (Hextall, 2000) or even negative effects of estrogen replacement therapy on SUI in post-menopausal women (Grady et al., 2001; Hendrix et al., 2005). It is well known that oral Hormone Replacement Therapy (HRT) including E₂, E₂ esters or conjugated equine estrogens gives rise to huge amounts of circulating estrone (E₁) as a result of the first liver pass. E₁ is

well known as an ER agonist. However, in experiments with the estrogen dependent MCF-7 breast cancer cell line, E1 has been shown to act as an E2 antagonist on cell growth (Jozan et al., 1981). There are also reports about an interference of E1 with the binding of E2 to ER, making E1 as a partial E2 antagonist (Sasson and Notides, 1983a, Sasson and Notides, 1983b).

Androgens may also play an important role for the female lower urinary tract and urogenital tissue contains androgen receptors as well as androgen metabolizing enzymes, including 5 α -reductases type I and II, which convert testosterone into the terminal biologically active androgen 5 α -dihydrotestosterone. (Berman et al., 2003; Ho et al., 2004). However, little is known about effects of androgens on collagen synthesis and degradation in urogenital tissue.

Also for androgen levels in SUI data are scarce and as far as we know from the literature, data are limited to the investigation of Bai and co-workers who reported no difference in urinary androgen excretion between healthy women and women with SUI. They also reported significant positive correlations between urinary excretion of individual androgens, including the testosterone metabolite androsterone, and bladder neck descent, which may suggest a negative effect of androgens on urogenital tissue in SUI (Bai et al., 2003).

Aims

To **assay** collagen markers in urogenital tissue in order to gain insight regarding an altered collagen synthesis or metabolism as one possible mechanism behind SUI.

To **analyze** if collagen synthesis or metabolism is altered in urogenital tissue in urologically healthy women with UP.

To **investigate** the possible differences in androgen/estrogen status between women with and without SUI.

To **study** if circulating sex steroids has any influence on markers of collagen synthesis and metabolism in the urogenital tissue of women with and without SUI.

Materials and Methods

Clinical material

A total of 126 women aged 36–88 years, referred for assessment at our department, were consecutively enrolled in the studies. Women with malignant disease, diseases needing systemic glucocorticoid treatment, bisphosphonate-treated osteoporosis and severe systemic diseases in general (cardiovascular, gastrointestinal, hepatobiliary, kidney, respiratory and neurological disease) were excluded from the study. The women were classified according to urogenital status into three groups: Women with stress urinary incontinence (SUI, N = 71), uterovaginal prolapse (UP, N = 24) and controls (N = 31). The diagnosis of SUI was verified by urodynamic investigation and controlled provocation with 300 mL saline in the bladder according to the International Continence Society (Abrams et al., 2002). The women with UP had as defined according to ICS standardization stage II or more (Bump et al., 1996). They were shown to be continent by urodynamic investigation and controlled provocation according to the International Continence Society. The controls had no history of SUI or urge incontinence when interviewed and proved urologically healthy in a simple cough provocation test with full bladder according to bladder scanning. Half of these women were scheduled for hysterectomy, in most cases a vaginal operation, owing to uterine fibroids, bleeding disorders and in one case, cervical stenosis and in another, pre-cancerous cervical epithelium. None of the patients with fibroids were on

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Table 1. Menopausal/estrogen status, age, BMI and parity in patients with stress incontinence (SUI) uterovaginal prolapse (UP) and in control patients (CTR).

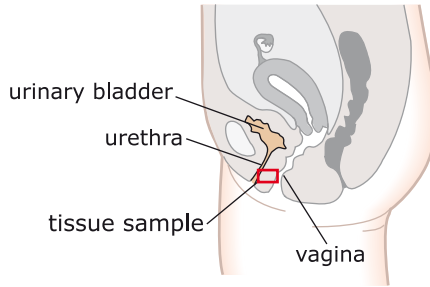
	<i>SUI total</i>	<i>SUI, matched to CTR</i>	<i>CTR, total</i>	<i>UP</i>	<i>CTR matched to UP</i>
N	71	31	31	24	24
Post-MP, no HRT	12	3	4	13	4
Post-MP, weak HRT	14	2	1	5	1
Post-MP, strong HRT	30	11	11	3	11
Pre-MP	15	15	15	3	8
Age, years	60.2+/- 13.4**	52.3+/- 10.7	51.3+/- 5.1	67.0+ 12.9***	53.3+ 5.4
BMI, kg/m ²	26.3 +/-3.7*	25.3 +/-4.0	24.6 +/-3.5	25.5 +3.5	24.8 +3.6
Parity	2.1 (0-6)	2.3 (0-6)	1,8 (0-5)	2.4 (0-7)	1,9 (0-5)

Weak HRT: Local E₂, local or oral estriol. Strong HRT: Systemic estrogen substitution. MP = menopausal. Significances of differences between patients and controls are denoted by * = $p < 0.05$, ** = $p < 0.01$ and *** = $p < 0.001$ respectively.

endocrine treatment regimens for their disease. The remainders of the controls were outpatients undergoing routine gynecological examination. All women underwent a gynecological examination and controls and SUI patients presenting with urogenital prolapse or who had undergone surgical correction of prolapse were excluded from the study. Menopausal/estrogen status, age, body mass index (BMI) and parity of the groups of women are shown in table 1. The local ethics committee of Huddinge University Hospital approved the study, and all subjects gave their informed consent before participation.

Collection of tissue and serum samples

Samples of suburethral tissue were taken from women with SUI when performing surgery for incontinence. The site of sampling



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was 10 to 20 mm beneath the external meatus of the urethra, just lateral to the midline. Samples from the controls were taken as 6 mm punch biopsies at the same site. The tissue samples were immediately placed on ice, frozen within less than 20 minutes and stored at -20°C venous blood samples were taken 1–3 days before the day of surgery between 0900 and 1200. Serum was separated after centrifugation and stored at -20°C until analysis.

Tissue homogenization

Frozen tissue (100–500 mg) was cut into small slices on a block of dry ice and transferred to a pre-chilled (liquid nitrogen) capsule containing a Teflon-coated tungsten ball. The capsule was kept in liquid nitrogen for two minutes and thereafter shaken in a dismembration apparatus (Retsch KG, Haan, Germany) at full speed for two minutes. The procedure was repeated once after intermediate freezing in liquid nitrogen. After thawing, the pulverized tissue was suspended in phosphate-buffered saline, pH 7.4, and the homogenate was kept frozen at -20°C until analysis. For assay of collagen markers in urogenital tissue the frozen homogenates were thawed, mixed thoroughly and centrifuged at $2300 \times g$ for 15 minutes in a refrigerated centrifuge. The supernatant was collected and used for assay of collagen markers and total protein.

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Table 2. Methods, manufacturers, detection limits, within and between assay coefficients of variation for the different assays of hormones and binding proteins.

Analyte	Method	Manufacturer	Detection limit	Within assay CV	Between assay CV
PICP	RIA	Orion Diagnostica AB	1.2 µg/L	3%	5%
PIIINP	RIA	Orion Diagnostica AB	0.2 µg/L	5%	6%
ICTP	RIA	Orion Diagnostica AB	0.5 µg/L	5%	6%
Testosterone	RIA	Diagnostic Products Corp.	0.1 nmol/L	6%	10%
SHBG	RIA	Eurodiagnostics AB	0.05 nmol/L	4%	8%
A-4	RIA*	In house-method	0.6 nmol/L	6%	10%
DHEA	RIA*	In house-method	1.6 nmol/L	5%	7%
DHEAS	RIA**	In house-method	200 nmol/L	8%	12%
Estrone	RIA*	In house-method	30 pmol/L	7%	10%
Total estrone [†]	RIA**	In house-method	0.3 nmol/L	7%	9%
Estradiol-17β	RIA	Orion Diagnostica AB	5 pmol/L	3%	6%
Cortisol	RIA	Diagnostic Products Corp.	11 nmol/L	5%	7%
CBG	RIA	Medgenix S: A:	0.2 mg/L	4%	6%
IGF-I	RIA***	Nichols Products Corp.	6 µg/L	5%	7%

SHBG = sex hormone-binding globulin, A-4 = 4-androstene-3,17-dione, DHEA = dehydroepiandrosterone, DHEAS = dehydroepiandrosterone sulfate, RIA = radioimmunoassay. * = RIA preceded by extraction. ** = RIA preceded by hydrolysis and extraction. *** = RIA preceded by acid ethanol extraction. † = sum of conjugated and unconjugated estrone; ≥ 85 % estrone sulfate.

Analytical methods

Assay of collagen turnover markers, steroids, steroid binding proteins and insulin-like growth factor-I IGF-I were carried out by radioimmunological methods as specified in table 2.

Apparent concentrations of free testosterone (fT) were calculated from values for total T, SHBG and a fixed albumin concentration of 40 g/L by successive approximation using a computer program based upon an equation system derived from the law of mass action (Södergård et al. 1982). Apparent concentrations of free cortisol (fC) concentrations were calculated from cortisol and CBG values using a formula based on the law of mass action as specified by the manufacturer of the CBG kit.

Total protein in the tissue homogenates was determined by the biuret method and tissue collagen markers were expressed as μg per mg of total protein.

Statistical methods

Comparison between three groups or more was performed by Kruskal-Wallis test followed by *post hoc* test by Mann-Whitney U-test or t-test for unpaired observations according to distribution. Comparison between two groups was performed by t-test for unpaired observations or Mann-Whitney U-test according to distribution. Correlations were performed by Spearman's rank correlation test and by stepwise regression. Normally distributed variables are expressed as arithmetic mean and SD, otherwise as median and range with the exception of parity, which for the sake of clarity is expressed as arithmetic mean and range. The significance level was set at $p = 0.05$.

Results and Discussion

Collagen in SUI and UP

Papers I and III

In the study comparing 71 SUI patients with 31 controls the SUI patients were significantly older, had a significantly higher BMI, and significantly lower S-PICP and T-ICTP levels than the controls. SUI patients matched for age, BMI, hormonal/menopausal status and parity had significantly lower S-PICP, T-PIIINP, and T-ICTP levels than the controls (Figure 1, paper I). When an even stricter matching of this material for parity was done, the significant differences in S-PICP, T-PIIINP and T-ICTP remained. Post-menopausal patients with strong HRT and pre-menopausal patients were significantly younger than untreated post-menopausal patients and post-menopausal patients with weak HRT. Post-menopausal patients with weak and strong HRT and pre-menopausal patients had significantly lower S-ICTP concentrations than untreated post-menopausal patients, otherwise no differences related to hormonal/menopausal status were found. The difference in T-PIIINP was influenced by BMI. To test the influence of BMI, women with the lowest BMI were removed and the significant difference of PIIINP disappeared. When removing those with highest BMI the significant difference remained. There were no correlations, whatsoever, between serum and tissue concentrations of collagen markers. Significant negative correlations to parity were found for T-PIIINP and T-PICP and to BMI for T-ICTP.

The UP patients were also significantly older than the controls. T-PICP and T-PIIINP levels in the UP patients were significantly higher and T-ICTP levels significantly lower than in the controls (Figure 1, paper II). Significant negative correlations to parity were found for T-PICP in the controls ($r_s = -0.46$, $P < 0.05$) and for T-ICTP in the UP patients ($r_s = -0.42$, $P < 0.05$); otherwise no associations between collagen turnover markers and parity were found. When the upper parity limit was reduced to four, the significance of the difference in T-ICTP disappeared but remained for T-PICP and T-PIIINP. Further reduction to three and two resulted in loss of the significance also for T-PICP but the difference in T-PIIINP remained significant. The differences in T-PICP and T-PIIINP also remained significant when the two groups were matched for menopausal/estrogen status and age, T-PICP and T-PIIINP levels were still significantly higher in the UP patients while the significance of the difference in T-ICTP disappeared. There were no significant differences in serum collagen markers. There were no significant associations, whatsoever, between age or menopausal/estrogen status and tissue collagen turnover markers, neither in the total clinical material nor in the two individual groups.

The study presented in paper I reported significantly lower levels of T-ICTP and T-PIIINP in women with SUI, together with a lack of difference in T-PICP, suggesting reduced breakdown of collagen type I and III in the presence of unchanged synthesis of type I collagen in SUI. This was supported by the suggestion of Jackson and co-workers of an impaired degradation of collagen in SUI, leading to reduced turnover, negatively affecting the strength and elasticity of urogenital tissue (Jackson et al., 2002) and may be one of the underlying mechanisms behind SUI. Our findings further confirms the previously well known role of obesity, pregnancy and multiparity as risk factors for SUI but also suggests that the nature of these risk factor may not be purely mechanical but also include changes in collagen metabolism, and perhaps act independently of each other and other defects in collagen synthesis and breakdown.

Multiparity, especially with vaginal delivery, is accepted as a risk factor for UP.

In the study on UP (paper III) there was a significant negative correlation between parity and T-ICTP this is in accordance with the conclusion in paper I that multiparity may influence a change in collagen metabolism not only constituting a mechanical challenge to the connective tissue and muscles. Furthermore the study on UP showed higher concentrations of PICP and PIIINP in the genital tissue suggesting an increased collagen synthesis following breakdown of collagen in UP. An increased breakdown of collagen in UP has been suggested based on the finding of increased MMP and cathepsin levels and increased MMP RNA expression in urogenital tissue from women with UP (Jackson et al., 1996; Phillips et al., 2006). With the reduced turnover of collagen in SUI as indicated in paper I with accumulation of aging collagen quite different patterns of collagen metabolism are demonstrated in UP and SUI. This difference could be demonstrated in using »clean« well-defined groups of patients and controls, separating SUI and UP completely and the controls in all cases free from both SUI and UP

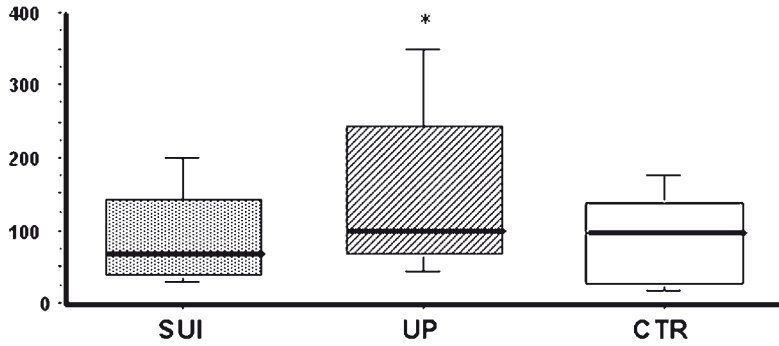
Hormones in SUI and controls

Estrogen (papers I, II and IV)

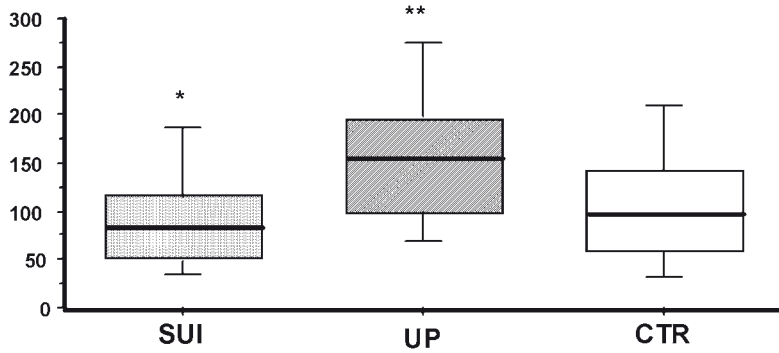
The associations between serum concentrations of collagen markers and steroid hormones were studied in paper I, II and IV. Significant positive correlations between tissue PICP and serum E₂ were found in the total control group and in controls without exogenous hormones but not in the SUI patients. Significant positive correlations between tissue ICTP and serum E₂ were found in the SUI without exogenous hormones

Figure 1. Concentrations of PICP, PIIINP and ICTP in urogenital tissue from women with stress urinary incontinence (SUI), uterovaginal prolapse (UP) and in urologically healthy controls (CTR) expressed as per cent of the median values for the control group. Horizontal lines indicate 10:th, 25:th, 50:th, 75:th and 90:th percentile. Significances of differences from controls are indicated by * = $p < 0.05$, ** = $p < 0.01$ and *** = $p < 0.001$ respectively.

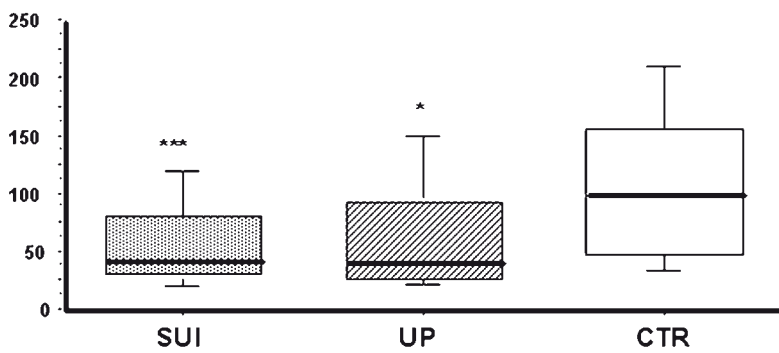
T-PICP, % of control median



T-PIIINP, % of control median



T-ICTP, % of control median



but not in the controls (paper II). The study presented in paper I reported significantly lower levels of T-ICTP and T-PIIINP in women with SUI, together with a lack of difference in T-PICP, suggesting reduced breakdown of collagen type I and III in the presence of unchanged synthesis of type I collagen in SUI. This was supported by the suggestion of Jackson et al., (2002) of an impaired degradation of collagen in SUI, leading to reduced turnover, negatively affecting the strength and elasticity of urogenital tissue. Matrix metalloproteases (MMPs) are a family of metal-ion requiring proteolytic enzymes that degrade components of the extra cellular matrix including collagens. MMPs in the female reproductive tract are estrogen dependent. Although no differences in tissue collagen markers with respect to menopausal/estrogen status in paper I, the present finding of significant positive correlations between serum E2 and tissue PICP and ICTP may suggest that estrogens cause an increased collagen type I turnover in urogenital tissue. This is also in accordance with the findings of Jackson et al. of significantly decreased total collagen content and stimulated pelvic collagen degradation by increasing proteinase activity following estrogen administration to post-menopausal women with SUI (Jackson et. al. 2002).

The positive association between E2 and collagen turnover was further supported by the findings presented in paper IV. In view of the possible role of E1 as a partial E2 antagonist (Jozan et al., 1981; Sasson and Notides, 1983a; Sasson and Notides, 1983b), lowering of the upper serum E1 limit resulted in strengthening of correlations between E2 and T-PICP and significant correlations also between E2 and T-PIIINP and finally also between E2 and T-ICTP. In general, lowering the upper serum E1 limit resulted in strengthened correlations between E2 and the collagen turnover markers. This was the case in the total control group as well as in premenopausal and postmenopausal controls and in controls without HRT.

No association between serum E2 and collagen turnover markers and no effects of lowering the upper serum E1 limit

Table 3. Correlations between urogenital tissue collagen turnover markers and serum estradiol-17 β (S-E2) at different serum estrone (S-E1) levels in subgroups of urologically healthy controls (CTR) and women with SUI.

S-E1 level, pmol/L	N	T-PICP	T-PIIINP	T-ICTP	S-E2, pmol/L
<i>CTR, Pre-MP</i>					
≤ 403 (all)	14	0.62*	0.31	0.07	109 (11-316)
≤ 250	11	0.78*	0.51	0.26	97 (11-223)
≤ 220	7	0.89*	0.86*	0.64	97 (11-141)
<i>CTR, Post-MP</i>					
≤ 5756 (all)	15	0.45	0.30	0.03	73 (15-448)
≤ 1900	13	0.76*	0.59*	0.31	60 (15-424)
≤ 500	10	0.72*	0.73*	0.46	38 (15-176)
<i>CTR, no HRT</i>					
≤ 403 (all)	17	0.72**	0.46	0.19	95 (11-316)
≤ 300	15	0.71**	0.56*	0.31	86 (11-170)
<i>SUI, Pre-MP</i>					
≤ 662 (all)	14	0.28	0.49	0.52	195 (33-471)
≤ 475	12	0.18	0.52	0.71*	195 (33-382)
≤ 350	9	0.36	0.30	0.88*	172 (33-372)
<i>SUI, Post-MP</i>					
≤ 6786 (all)	40	0.03	-0.12	-0.13	36 (9-550)
≤ 200	20	-0.09	0.05	0.05	20 (9-93)

HRT = hormone replacement therapy, MP = menopausal. Spearman's rank correlation coefficients. * = $p < 0.05$; ** = $p < 0.01$. (*) = $p < 0.1$

was found in the total group of SUI patients. Similar results were also obtained with the subgroup of SUI patients matched with the controls. In premenopausal SUI patients lowering of the upper E1 limit resulted in significant correlations between E2 and T-ICTP and a tendency ($p < 0.1$) to significant correlations between E2 and T-PIIINP. No significant correlations and no effects of lowering the upper E1 limit were observed in the postmenopausal SUI patients.

When age, parity and BMI were tested against collagen turnover markers in the groups referred to in Table 3 significant negative correlations were found in the controls between T-PIIINP and age from upper E1 limits 400 to 225 pmol/L ($r_s = 0.42-0.61$, all $p < 0.05$), between T-PIIINP and parity from

upper E1 limits 1000 to 600 pmol/L ($r_s = 0.41$, $p < 0.05$ for both) and between T-ICTP and age at the upper E1 limit 250 pmol/L ($r_s = -0.48$, $p < 0.05$). No significant correlations were found in the controls between T-PICP and the one hand and age, parity and BMI on the other and no significant correlations were found in the SUI patients between collagen turnover markers and age, parity and BMI.

Inclusion of supraphysiological E1 levels, like those occurring during oral HRT, did clearly disturb the association between collagen turnover markers and E2 and most of the significant correlations disappear. Our findings are thus in line with the previous reports on E1 as a partial E2 antagonist. The results in the controls without exogenous estrogens also show that the effect of lowering the upper E1 level was not due to disappearance of any synthetic gestagens from HRT preparations.

In contrast to the urologically healthy women, urogenital tissue collagen turnover in women with SUI seems to be rather insensitive to estrogens, even at physiological E1 levels. Interestingly, the effect of estrogens on urogenital tissue collagen turnover in SUI patients was influenced by menopausal status. In premenopausal SUI patients the pattern of correlation coefficients and the effect of lowering the upper serum E1 level showed some resemblance to that in the controls, while there was absolutely no association between E2 and collagen turnover markers in the postmenopausal SUI patients. This suggests some kind of menopause-related, probably irreversible, change in the estrogen sensitivity of urogenital tissue collagen turnover in SUI. Furthermore, our results indicate that E1 at supraphysiological serum levels, such as during oral HRT, may act as a partial E2 antagonist *in vivo*, in accordance with previous *in vitro* studies. Our findings may, at least in part, contribute to an explanation of the less successful results of estrogen replacement therapy on urogenital tissues, epithelium and mucosa in postmenopausal women with SUI and thereby aggravating the symptoms of incontinence (Hextall, 2000; Grady et al., 2001; Hendrix et al., 2005).

Testosteron (paper II)

This study showed significant negative correlations, in SUI patients but not in the controls, between tissue concentrations of all three collagen markers and to total serum T and especially to fT. Multiparity and obesity are well-known risk factors for SUI and when these risk factors were gradually reduced in the SUI patients, the negative correlations between collagen turnover markers and serum T and fT became strong. Effects of androgens on MMPs has been studied in the prostate and also in the ovary in animal experiments and in cell cultures. Most studies report inhibitory effects of androgens (Wilson et al., 1995; Shroen & Brinkerhoff, 1996; Schneikert et al., 1996; Henmi et al., 2001; McCulloch et al., 2004) while some report increased levels of MMPs following androgen treatment (Ouyang et al., 2001; Pang et al., 2004). While tissue concentrations of PICP and PIIINP may reflect either synthesis or breakdown of their respective collagen species, tissue ICTP reflects breakdown of collagen type I. Since the same tissue MMPs and cathepsins are responsible for the degradation of both species of collagen, our finding of negative correlations between all collagen markers and serum T and fT may reflect an inhibitory effect of androgens on collagen degradation in urogenital tissue in SUI patients. As for serum E2, we found no significant difference in serum T and fT between SUI patients and controls. This is in accordance with the lack of difference in urinary androgens reported by Bai and co-workers (Bai et al., 2003). The negative and sometimes strong correlations between serum T and fT and markers of collagen type I and III turnover may suggest that the positive association between urinary androgens and bladder neck descent reported by those workers may reflect an androgen-induced suppression of collagen type I and III turnover, possibly by inhibitory effects on MMPs which may negatively affect the properties of urogenital tissue. Significant correlations between T and fT and markers of collagen turnover were only found in the patients but there were no differences in serum T and fT between SUI patients and controls. This may

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indicate a difference in androgen sensitivity of urogenital tissue in SUI patients and in urologically healthy women. If this is due to differences in androgen receptor content or other factors, including androgen metabolism, is still to be studied.

General conclusions and future prospectives

Urogenital tissue collagen turnover may be stimulated by estrogen in urologically healthy women but not in SUI patients in general but to a certain degree in premenopausal subjects. The latter finding may indicate menopause related changes as one underlying factor behind SUI.

Urogenital tissue collagen turnover is decreased in women with SUI and may reflect an increased androgen sensitivity in the urogenital tissue.

This demands further studies of urogenital tissue androgen metabolism, 5 α reductase activity and androgen receptor content. These studies may further result in randomized trials of the effect of local or systemic treatment of SUI patients with antiandrogens or 5 α reductase inhibitors.

In women with UP the collagen turnover is increased and, at least theoretically, treatment with compounds having inhibitory effects on collagen synthesis as well as degradation may be tried.

Further in the future, when greater knowledge is at hand of hormonal and collagen properties and metabolism, treatment with mesenchymal stem cells for augmentation of the connective tissue could be a possible approach to treatment of SUI as well as UP.

Popular Summary in Swedish

Sammanfattning

Bakgrund

För kvinnor med lång livslängd, aktivt liv, yrkesarbete och sent barnafödande är urininkontinens och livmoderframfall idag ett stort handikapp. Olika undersökningar har visat att 10 procent av alla kvinnor i 40-års åldern har urinläckage och att förekomsten av inkontinens ökar med stigande ålder till att omfatta 50 procent (om den internationella definitionen om minst ett ofrivilligt urinläckage per vecka används). Kostnaderna för samhälle och individer är stora samtidigt som få söker hjälp för sina besvär. Stora framsteg har gjorts när det gäller att utveckla nya metoder för operation av ansträngningsinkontinens och läkemedel för att medicinskt behandla av trängningsinkontinens (se faktaruta). Trängningsinkontinens behandlas inte i denna studie.

Vid framfall kan träning av bäckenbottenmuskulatur och viktnedgång ge lindring men kirurgiskt ingrepp är den behandling som ger bäst resultat; 6 000 operationer utförs årligen i Sverige. Dessvärre är återfallsfrekvensen hög. En förnyad operation kan vara tekniskt svårare och ger sällan ett lika bra resultat som vid första operationen.

Graviditet och förlossning, övervikt, kronisk luftvägssjukdom, tungt arbete och ärftliga faktorer räknas alla till riskfaktorerna för att utveckla ansträngningsinkontinens och framfall. Med ökande ålder tilltar risken för framfall medan

Inkontinens

Ansträngningsinkontinens

Ansträngningsinkontinens (*Stress Urinary Incontinence, SUI*) beror på att ökat tryck i buken vid ansträngning (tungt lyft, hosta, fysisk träning) ger ett tryck på urinblåsan så att slutmuskeln (*uretrasfinktern*) inte förmår hålla tätt. Urinläckage sker utan att trängningar upplevs.

Trängningsinkontinens

Trängningsinkontinens orsakas av ofrivilliga sammandragningar i urinblåsans tömningssmuskel (överaktiv blåsa) som medför plötsliga trängningar att urinera fastän blåsan är långt ifrån fylld.

ansträngningsinkontinens når en höjdpunkt mellan 45–55 år. De egenskaper hos organismen (*patofysiologin*) som ligger bakom dessa tillstånd är inte helt klarlagd. Tidigare studier har emellertid visat på defekt bindväv.

Bindväv

Bindvävens uppbyggnad, hur dess kollagen (se faktaruta) är organiserat och mängden av olika typer kollagen är av stor betydelse för vävnadens styrka och elasticitet. I mineraliserat ben (skelettet) dominerar *kollagen typ I*, vilket också förekommer i bindväv. *Kollagen typ III* förekommer endast i bindväv och är den typ som finns kring urinrör och slida – den urogenitala vävnaden.

Tidigare har man studerat mängden av markörerna PICP (*carboxy-terminal telopeptide* av typ I prokollagen), ICTP (*carboxy-terminal telopeptide* av typ I kollagen) och PIIINP (*amino-terminal propeptide* av typ III prokollagen) som cirkulerar i blodbanan. Dessa markörer har använts för att bedöma kollagenets nedbrytning och nybildning i skelettet (vid dottertumörer, benskörhet och östrogenets påverkan på kollagenomsättning i skelett). Däremot har dessa markörer inte tidigare analyserats direkt ur bindväv för att bedöma nybildning och nedbrytning av det kollagen man önskar studera vid ett specifikt tillstånd.

Kollagen

Kollagen är kroppens dominerande form av bindvävsprotein. Kollagen består av långa fibrer av hoptvinnade kedjor av aminosyror och kan uppnå mycket hög hållfasthet, som i kroppens senor och ledband. Tunnare fibrer av kollagen finns i alla organ och vävnader och har en stödjande och sammanhållande funktion. Källa: NE.

Avhandlingens mål

Syftet har varit att analysera markörer för nybildning och nedbrytning av kollagen i bindväven kring urinrör och slida (*urogenitalt*) samt i serum hos kvinnor med ansträngningsinkontinens eller livmoderframfall och jämföra dessa med friska kontroller. Syftet har även varit att sätta kollagenomsättningen i relation till individens hormonella status (fertil ålder eller klimakterium) samt till östrogen, kroppseget eller tillfört.

Hypotes/Antagande

Frågeställningen har varit att analysera markörer för kollagen i urogenital vävnad och serum från kvinnor med ansträngningsinkontinens respektive framfall och att utröna huruvida en förändrad kollagenomsättning (*metabolism*) skiljer sig åt mellan dessa två tillstånd i jämförelse med friska kontroller.

Artikel I: Markers of collagen synthesis and degradation in urogenital tissue from women with and without stress urinary incontinence (publ. 2005 Neurourology and Urodynamics)

Material och metod

Från 71 kvinnor med ansträngningsinkontinens och 31 friska kvinnor togs vävnadsprover från slidans slemhinna ca 2

centimeter från urinrörsöppningen för att bestämma förekomst och koncentration av markörer för kollagen, och att jämföra dessa med koncentrationen av kollagenmarkörerna i serum

Resultat

Kvinnorna med ansträngningsinkontinens var signifikant äldre, hade högre BMI (*Body Mass Index*) och lägre halt PICP i serum och ICTP i vävnaden jämfört med kontrollgruppen. När hänsyn togs till ålder, BMI, antal förlossningar och hormonell status hade kvinnorna med ansträngningsinkontinens lägre koncentrationer av PICP i serum och lägre nivåer av PIIINP och ICTP i vävnaden jämfört med kontrollgruppen. Lägre halt ICTP i serum sågs hos de klimakteriella kvinnorna med ansträngningsinkontinens som fått någon form av östrogenbehandling, jämfört med de obehandlade. Ett negativt samband till antal förlossningar förelåg för vävnads-PIIINP och PICP och till BMI för vävnads-ICTP.

Slutsatser

Låga nivåer av dessa kollagenmarkörer i bindväven hos kvinnor med ansträngningsinkontinens tyder på nedsatt omsättning av kollagen, vilket kan ha negativ effekt på styrka och elasticitet hos vävnaden.

Artikel II: Endocrine status and markers of collagen synthesis and degradation in serum and tissue from women with and without stress urinary incontinence (publ. 2007 Neurourology and Urodynamics)

Material/metod

Från 58 kvinnor med ansträngningsinkontinens och 30 friska kvinnor togs biopsier från urogenital vävnad för att bestämma förekomst och koncentration av markörer för kollagen, samt serum för analys av *östradiol-17- β* (*E2*), *total testosteron* (*T*) och

sex hormone-binding globulin (SHBG). Fritt testosteron beräknades ur T, SHBG och albumin.

Resultat

Ett positivt samband fanns mellan E2 och PICP hos kontrollgruppen och mellan E2 och ICTP hos kvinnor med ansträngningsinkontinens utan tillfört östrogen. Starka negativa samband fanns mellan serum T och fT å ena sidan och samtliga kollagenmarkörer å andra sidan. Dessa samband stärktes ytterligare statistiskt då antalet förlossningar och/eller BMI-nivå minskades. Inga samband sågs mellan T och fT och markörer för kollagen hos kontrollgruppen. Det förelåg inga skillnader mellan grupperna vad gäller nivåerna av E2, T och fT i serum.

Slutsatser

Östrogen kan ha effekt på urogenital vävnad genom att öka kollagenomsättningen. *Androgener* (manligt könshormon) kan ha negativ effekt på vävnaden genom att hastigheten i kollagenomsättningen minskar, sannolikt genom hämning av *matrix metalloproteas (MMP)*, ett proteinklyvande enzym som här spjälkar kollagen). Känsligheten för manligt könshormon i urogenital vävnad kan antas vara förändrad hos kvinnor med ansträngningsinkontinens jämfört med friska kontroller.

Artikel III: Markers of collagen synthesis and degradation in urogenital tissue and serum from women with and without uterovaginal prolapse (publ. 2008 Molecular Human Reproduction)

Material/metod

48 kvinnor ingick i studien: 24 utan inkontinens men med livmoderframfall och 24 kvinnor utan vare sig inkontinens eller framfall. Prov från vävnad och serum togs för analys av kollagenmarkörer.

Resultat

Högre nivåer av PICP och PIIINP sågs i urogenitalvävnad hos kvinnor med framfall jämfört med kontrollgruppen. Skillnaden kvarstod även sedan hänsyn tagits till ålder, BMI, antal förlossningar och hormonstatus. Inga skillnader sågs mellan grupperna vad gäller markörerna för kollagen i serum.

Slutsatser

Hos kvinnor med framfall ses både en ökad omsättning och nedbrytning av kollagen i bindväven. Denna antas påverka styrka, hållfasthet och elasticitet hos bindväven negativt.

Artikel IV: Different estrogen sensitivity of urogenital tissue from women with and without stress urinary incontinence (submitted)

Material/metod

Hos 54 kvinnor med ansträngningsinkontinens och 29 friska kontrollpersoner analyserades, liksom tidigare, kollagenmarkörerna PICP, ICTP och PIIINP i biopsier från urogenital vävnad och i serum. I denna studie analyserades även östrogenerna E1 och E2 i serum.

Resultat

I hela kontrollgruppen sågs endast ett statistiskt säkerställt samband mellan E2 och T-PICP. När den övre gränsen för E1 i serum sänktes sågs ett samband även mellan E2 och T-PIIINP och slutligen även mellan E2 och T-ICTP. Även dessa samband var statistiskt säkerställda. Detta mönster sågs också i undergrupper av kontrollpersoner före och efter klimakteriet. Hos kvinnorna med ansträngningsinkontinens sågs inget generellt samband mellan E2 och kollagenmarkörer. Sänkta E1-gränser medförde ingen förändring i den totala gruppen av kvinnor med ansträngningsinkontinens liksom hos de kvinnor som uppnått klimakteriet. Inkontinenta kvinnor före klimakteriet visade däremot ett mönster liknande kontrollernas.

Slutsatser

Vid normala E₁-nivåer ökar E₂-omsättningen av kollagen hos kontrollgruppen, men inte vid ansträngningsinkontinens i allmänhet. Men vid en indelning i grupper sågs det en viss effekt hos kvinnor med ansträngningsinkontinens före men däremot inte efter klimakteriet. Urogenital vävnad verkar skilja sig åt vad gäller känslighet för östrogen hos friska kontroller och kvinnor med ansträngningsinkontinens. Hos de senare verkar också skillnaden ha ett samband med klimakteriet. E₁ som cirkulerar i blodbanan och som når mycket höga nivåer vid hormonsubstitution, tycks kunna fungera som en östrogenreceptoragonist likaväl som en partiell E₂-antagonist.

Sammanfattning

Den urogenitala vävnaden hos kvinnor med ansträngningsinkontinens, kvinnor med livmoderframfall och hos friska kontroller skiljer sig åt vad gäller känsligheten för könshormoner och har olika mönster för omsättningen av kollagen. Vid ansträngningsinkontinens noteras en nedsatt omsättning av kollagen och vid framfall en ökad nedbrytning av kollagen, varför det är viktigt att noggrant skilja på dessa tillstånd.

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