multiple childbirths are thought to play a role. Reduction in the elastin content of the connective tissues that support the bladder and urethra would weaken and reduce the elasticity of these supportive structures and contribute to the development of SUI. The maintenance of elastin content is dependent on the balance of protease/anti-protease activity. We recently developed a method of determining elastase activity in plasma and elastase inhibitory capacity of plasma. The aims of this study were to determine (1) whether net elastase activity is lower in the plasma of women with SUI compared to women without SUI and (2) whether the capacity to inhibit elastase is lower in the plasma of women with SUI compared to women without SUI.

Methods: Plasma was prepared from blood taken from women with SUI (n=30) and age matched control women (n=30). Diagnoses of SUI were made by physical and urodynamic evaluation. Plasma (10 μl) was added to an assay mixture containing succinylated elastin substrate to initiate the elastase reaction, in order to measure plasma elastase activity (reported as U/ml ± SEM. In order to measure inhibition of elastase, plasma (10 μl) was added to an assay mixture containing succinylated elastin substrate and the reaction was initiated by the addition of purified porcine pancreatic elastase. Elastase activity in the presence of plasma was compared to that determined in the absence of plasma. Data is reported as percent of elastase activity in the absence of plasma.

Results: The elastolytic activity in the plasma of women without SUI was 4.5±1.5 U/ml, while that of age matched women with SUI was 16.3 ± 3.5 U/ml ($p<0.01$). Thus the elastolytic activity in plasma of women with SUI was threefold the elastolytic activity in plasma of women without SUI. Purified porcine pancreatic elastase was inhibited by unidentified plasma components. The purified elastase activity measured in the presence of 10 μl plasma from normal women was 13.3 ± 1.6% of that in the absence of plasma. However, purified elastase activity measured in the presence of 10 ul plasma from women with Sui was two-fold higher (25.5 ± 2.2%, $p<0.001$).

Conclusions: These data suggest that SUI patients have significantly (threefold) elevated plasma elastolytic activity compared to age-matched women without SUI. At least part of this elevated elastolytic activity can be attributed to a reduced level of certain components in their plasma that control elastase activity, resulting in higher activity of this enzyme. The elevated elastolytic activity in these women may result in increased elastin degradation in cnnective tissues supporting the bladder and urethra and, thus, contribute to the development of SUI.

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COLLAGENASE ACTIVITY IS ELEVATED IN CONDITIONED MEDIA FROM FIBROBLASTS OF WOMEN WITH PELVIC FLOOR WEAKENING.

Aims of Study: Stress urinary incontinence (SUI), the loss of urine during episodes of increased abdominal pressure, is a result of weakened support of the urinary bladder and urethra. The primary structure supporting the bladder and urethra is the collagen-rich endopelvic fascia. A reduction in collagen would be expected to weaken its function in bladder support. A major objective of this study was to determine if weakened support of the bladder and urethra is associated with altered collagen metabolism and to determine if these changes are local or the result of systemic metabolic changes.

Methods: Skin and endopelvic fascia biopsies were obtained from women undergoing vaginal surgery. The experimental group included women with videourodynamic evidence of SUI undergoing bladder neck suspension surgery (n=21). The age matched control group consisted of continent women without evidence of pelvic floor weakening undergoing laparoscopic-assisted vaginal hysterectomy for symptomatic uterine fibroids, endometrial cancer, or benign ovarian pathology (n=11). Biopsies of the lower abdominal skin and endopelvic fascia were taken at the time of surgery, transferred to sterile tissue culture media, and fibroblasts cultured by standard methods. Collagenase activity was determined in the serum-free four-day conditioned media obtained from cultured fibroblasts, by soluble lysis assay. Immunoreactive matrix metalloproteinase 1 (MMP1) levels were determined by ELISA (Biotrak). The capacity for cultured fibroblasts to incorporate [3H]-proline into procollagen was determined.

Results: Mean collagenase activity in the conditioned media from skin was more than 6 fold higher in the SUI group (30.26 U/mg protein) compared to the control group (4.65 U/mg protein). Collagenase activity in the conditioned media from endopelvic fascia fibroblasts was also higher in the SUI group (8.28 U/mg protein) compared to the control group (6.44 U/mg protein), but this difference was not statistically significant. The specificity of the soluble lysis assay for collagenase was confirmed in representative samples by SDS-PAGE fluorography. Immunoreactive MMP1 was more than 10 fold higher in the conditioned media from skin fibroblasts from SUI women (948.9 ng/mg protein) compared to controls (86.72 ng/mg protein). Immunoreactive MMP1 was more than 5 fold higher in the conditioned media from

endopelvic fibroblasts from SUI women (142.40 ng/mg protein) compared to controls (26.62 ng/mg protein). Incorporation of [3H]-proline into procollagen was not significantly different between the two groups, nor was the ration of procollagen a I/III.

Conclusions: These data suggest that fibroblasts from women with weakened pelvic support, such that they have SUI, synthesize and secrete significantly more matrix metalloproteinase 1 resulting in higher collagenase activity in their skin and endopelvic fascia compared to women of similar age without pelvic floor weakening. The finding of increased MMP1 and collagenase activity in the conditioned medium from the fibroblasts of women with SUI, without alterations in collagen synthesis, supports the hypothesis that changes in the collagen composition in women with pelvic floor weakening are due to increased collagen degradation. The fact that the difference was apparent in the skin suggests a systemic change.

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THE PATHOPHYSIOLOGICAL CHANGES OF VAGINAL TISSUE IN WOMEN WITH STRESS URINARY INCONTINENCE: A CONTROLLED TRIAL

Aims of study: Genuine stress incontinence (GSI) is frequently secondary to bladder neck hypermobility. Our understanding of this condition, concerning diagnosis and treatment options, have improved but still there is little known about the pathophysiological process that leads to the tissue weakness. The aetiology of urinary stress incontinence is almost certainly multifactorial. However, collagen, a fibrous protein forming the major structural component of vaginal epithelium, imparts tensile strength to the tissue and has been implicated in the development of urinary stress incontinence. Previous analysis of pre-menopausal nulliparous women with GSI and normal controls demonstrated that nulliparous women with GSI had a significant reduction of total collagen in the vaginal tissue There was an associated reduction in intermolecular collagen cross-linking, suggesting that the underlying defect within this population may be congenital rather than acquired.(1) We have set out to further clarify the pathophysiological changes that are seen in women with bladder neck hypermobility.

Method: Women recruited into this controlled study were pre menopausal. All those with stress incontinence symptoms had the diagnosis of GSI confirmed by conventional cystometric testing. The validated Bristol Female Lower urinary tract symptom questionnaire was used to exclude urinary incontinence in the control group. The International Continence Society's female pelvic organ prolapse grading system was used to assess genitourinary prolapse and women were withdrawn if the score was greater than 1. A tissue sample was taken peri-urethrally from the anterior vaginal wall using Eppendorfer punch biopsy forceps. The tissue was stored at -80°C before undergoing biochemical analysis. Total collagen content content was determined by hydroxyproline analysis and sulphated proteoglycan assay using dimethylmethylene blue. The collagen intermediate cross-links dehydro-hydroxylysinonorleucine (HLNL) and dihydro-hydroxylysinonorleucine (DHLNL) and mature cross links hydroxylysyl-pyridinoline (Pyr) and histidino-hydroxylysinonorleucine (HHL) plus the advanced glycation end-product pentosidine were quantified either by a modified amino acid analysis procedure or by a high performance liquid chromatography. The metalloproteinase activity was measured using substrate gel electrophoresis and protein content assayed by microkjeldahl analysis. The data underwent non-parametric testing (Mann-Whitney) using SPSS for windows.

Results: 61 women were recruited in total (28 in the GSI group and 33 in the control group). The mean age in the GSI group was 43 (range 26-53) and in the control group 40 (range 27-51). The mean parity in each group was 2.8 and 2.2 respectively. The glycation end products are expressed as mole of collagen per mole pentosidine where an increase in levels indicates a decrease in pentosidine present.