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.

	Control (n = 33) Median (range)	Incontinence (n = 28) Median (range)	Significance
Total collagen (%dry wt)	50.6 (23.0 – 79.2)	38.7 (13.0 – 57.9)	0.002
Protein Content (%dry wt)	89.2 (58.3 – 99.6)	69.5 (4.4 – 93.5)	0.017
Proteoglycan (μg/mg dry wt)	7.9 (4.1 – 13.2)	22.3 (8.0 – 42.1)	0.002
Metalloproteinase (/μg coll) MMP 2 expression	3.27 (0.20 – 8.01)	8.13 (4.9 – 39.6)	< 0 001
Immature cross-links (/mole coll) HLNL	0.11 (0.00 – 0.21)	0.21 (0.01 – 0.55)	< 0.001
DHLNL	0.05 (0.00 – 0.20)	0.01 (0.01 – 0.38)	0.001
Mature cross links (/mole coll) Pyridinoline	0.14 (0.04 – 0.45)	0.25 (0.01 – 0.50)	0.017
HHL	0.16 (0.02 0.34)	0.21 (0.01 – 0.38)	0.081
Glycation end products (mole/mole) Pentosidine	333 (90 – 1221)	432 (122 – 1153)	0.515

Conclusions: We have confirmed the previous findings that genuine stress incontinence is associated with a reduction in total vaginal collagen due to an alteration in vaginal collagen metabolism. Furthermore there is an increase in de novo synthesis of collagen indicated by the extent of intermediate cross links. The rise in MMP2 (a gelatinase) also demonstrates an increase in turnover. The ratio of intermediate/mature cross links is the same in both groups but the incontinence group has a similar increase in both cross links. This suggests normal maturation with increased collagen synthesis which is accompanied with a fall in advanced glycation products indicating collagen turnover. The reduction in tissue protein, including collagen may have been exaggerated by the dilutional effect of proteoglycans and other extracellular matrix substances. This reduction in vaginal collagen suggests it has lost some of its supporting capacity.

1. *Br J Obstet Gynaecol* 1997; **104**(9):994-997.

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THE BIOCHEMICAL PROFILE OF VAGINAL TISSUE IN WOMEN WITH GENITOURINARY PROLAPSE: A CONTROLLED TRIAL

Aims of study: Genitourinary prolapse can result in physical, psychological and social discomfort. Childbirth is involved in the development and progression of genitourinary prolapse however, its actiology is almost certainly multifactorial. Collagen, a fibrous protein, forms the major structural component of vaginal epithelium imparting tensile strength to the tissue and has been implicated in the development of genitourinary prolapse. A pilot study demonstrated that premenopausal women with genitourinary prolapse had a reduction in total vaginal epithelial collagen as well as an increase in immature cross-linking when compared with age-matched controls. Furthermore, both matrix metalloproteinase and acid cathepsin collagen proteinase activity increased suggesting that in prolapse the overall reduction in total collagen is due to increased metalloproteinase activity despite some increased synthesis of new collagen as a repair mechanism.[1] We set out to clarify the mechanism of the underlying connective tissue abnormality in women with genitourinary prolapse.

Method: All of the women recruited into this controlled study were Pre menopausal. The presence or absence of genitourinary prolapse was determined using the International Continence Society female pelvic organ prolapse grading system. The prolapse group had a score greater than 1. The Bristol Female Lower urinary tract symptom questionnaire was used to exclude women with urinary incontinence. 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 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. Differential scanning calorimetry (DSC) was used to determine the denaturation temperature of the collagen providing information on collagen quality. The data underwent non-parametric testing (Mann-Whitney) using SPSS for windows.

Results: 56 women were recruited in total (23 in the prolapse group and 33 in the control group). The mean age in the prolapse group was 40 (range 28-50) and in the control group 40 (range 27-51). The mean parity in each group was 2.9 and 2.2 respectively. The glycation end products are expressed as mole of collagen per mole pentosidine where a reduction in levels indicates an increase in pentosidine present.

	Control (n = 33) Median (range)	Prolapse (n = 23) Median (range)	Significance
Total collagen (%dry wt)	50.6 (23.0 – 79.2)	27.7 (16.7 – 37.3)	< 0.001
Protein Content (%dry wt)	89.2 (58.3 – 99.6)	85.3 (41.1 – 99.2)	0.48
DSC (Pk Ht °C)	67.7 (65.9 – 69.8)	66.5 (65.1 – 68.5)	0.06
Proteoglycan (µg/g wet wt)	5944 (3103 – 9594)	3711 (2020 – 14865)	0.04
Metalloproteinase (/µg coll) MMP 2 expression	3.27 (0.20 – 8.01)	15.42 (3.35 – 42.14)	< 0 001
Immature cross-links (/mole coll)			
HLNL	0.11 (0.00 – 0.21)	0.18 (0.04 – 0.72)	< 0.001
DHLNL	0.05 (0.00 – 0.20)	0.06 (0.01 – 0.40)	0.7
Mature cross links (/mole coll)			
Pyridinoline	0.14 (0.04 – 0.45)	0.42 (0.04 – 1.26)	< 0.001
HHL	0.16 (0.02 0.34)	0.49 (0.08 – 2.07)	< 0.001
Glycation end products (mole/mole) Pentosidine	333 (90 – 1221)	113 (51 – 268)	< 0.001

Conclusions: Women with genitourinary prolapse appear to have an alteration in collagen metabolism causing a decrease in total collagen therefore reducing its ability to provide structural support. The increase in intermediate cross linking indicates de novo synthesis is occurring but not at a level to restore the overall collagen content. The extracellular components (proteoglycans and protein) decrease making it unlikely that dilution of the tissue leads to the reduction in collagen. Gelatinase activity (MMP2) indicates turnover of the collagen is occurring but it is the new collagen rather than the old being degraded which is indicated by the increase in advanced glycation end products. The peak temperature at denaturation is reduced in the prolapse group although not significantly and as there is no reduction in cross links this would suggest that the prolapse collagen is damaged. Enthalpy values measuring the energy required to denature collagen should corroborate this and is part of our ongoing work.

1. *The Lancet* 1996; 347:1658-1661.

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QUANTITATIVE ELECTROMYOGRAPHIC ANALYSIS OF LEVATOR ANI AND EXTERNAL ANAL SPHINCTER MUSCLES OF NULLIPAROUS WOMEN

Aims of study: To introduce the use of digital quantitative electromyography (QEMG) of the levator ani and external anal sphincter, and establish reference values for these muscles. QEMG is both reproducible and independent of the operator, and is a technique routinely used in the analysis of striated muscles outside the pelvis. We present a study using QEMG to evaluate pelvic muscles in nulliparous subjects.

Methods: Fifteen nulliparous women without symptoms of pelvic floor dysfunction underwent EMG examination of the levator ani (LA) and the external anal sphincter (EAS) using a concentric needle electrode with a recording area 0.07mm² and diameter of 0.45mm (Medelec NDMC 37). The levators were sampled via a transvaginal approach at two sites on each side: on the levator near the insertion of the levator arcus into the ischial spine, and 1 cm medial to the first site. Muscle bellies palpated at these sites were presumed to be either iliococcygeus or puborectalis. Two sites on the left and right of the posterior external anal sphincter were sampled just inside the mucocutaneous junction. The resulting signal was filtered (range 5Hz-10KHz) and amplified and recordings made at three levels of voluntary activation at each site: at rest, at partial activation, and at maximal activation. Epochs of up to 20sec of each site and activation level were recorded on digital audio tape (Sony DTC-A8). Analysis of taped signals was performed on a Medelec Synergy electromyograph capable of analyzing motor unit action potentials (MUAPs) and the interference pattern (IP). Measurements were made of MUAP amplitude, area, amplitude/area ratio, duration, number of phases and turns, and of the activity, envelope amplitude (env amp), and number of small segments of the interference pattern for both muscles. Normal ranges were generated and compared to those established for other striated muscles.