be masked due to the extent of pelvic floor relaxation. However, little knowledge exists why some patients with prolapse develop GSI and some do not.

Aims of study: The status of paraurethral connective tissue in postmenopausal female patients was investigated for relevant changes of paraurethral connective tissue between continent and incontinent women with genital prolapse.

Methods: Before pelvic reconstructive surgery patients underwent a complete urogynecologic assessment including history, urinalysis, evaluation of residual bladder volume, medium fill cystometry, urethral pressure profile measurements (UPP) at rest and during stress, and a clinical stress test in the supine and standing position. A pronounced prolapse was repositioned during UPP measurements and during a clinical stress test to assess or exclude GSI. All patients underwent pelvic floor reconstructive surgery (anterior colporrhaphy: n=19, sacrospinous fixation: n=8). Patients with proven GSI received additional tension-free vaginal tape procedure.

During pelvic floor reconstructive surgery biopsies from both paraurethral regions were obtained. Biopsies were investigated for localization and distribution of both collagen (types I, III, IV, V, VI) and glycoproteins (fibronectin, laminin, vitronectin) using immunofluorescent microscopic techniques. Frozen biopsies were incubated with primary polyclonal antibodies (1:25 dilution in PBS) at room temperature for 60 minutes. Three washings with a 0.2% PBS-BSA solution and exposure to Trimethylrhodamin-isothiocyanat (TRITC)-conjugated second antibodies (swine anti-rabbit immunglobulins [Dakopatts, Denmark] diluted 1:20 dilution in PBS) were performed to visualize the protein. After another washing with PBS-BSA solution, photographs of the samples were taken (Kodak Ektachrome 400) for documentation.

Results: GSI was present in 10/19 women (Group A, mean age: 56,2 years), whereas 9/19 patients (Group B, mean age: 59,6 years, $p > 0.05$) were continent.

Irrespective of the presence or absence of GSI all types of collagen (I,III, IV, V, VI) were found in the biopsies of the whole study group. The tissues of Group A patients showed a marked weaker immunohistochemical reaction of type I, III and VI collagen compared with the specimen of group B patients.

No difference of Type IV and V collagen was observed between the biopsies of group A and B patients. Type V collagen was located in the subepithelial connective tissue zone of the stroma, touching the basement membrane and forming a fibrillar meshwork. Type IV collagen was selectively found in the zone of basal membrane and vessel walls.

Among the structural glycoproteins fibronectin and laminin were found in the specimen of all patients. However staining of fibronectin was less pronounced than that of collagen. Nevertheless, fibronectin was distinctly found in the extracellular matrix. The stroma revealed a fine fibrillar matrix reaching to the basement membrane. Laminin showed a similar distribution in the basement membrane as type IV collagen.

Vitronectin was be observed in the paraurethral connective tissue of all group B patients, whereas this glycoprotein was lacking in the biopsies of group A women. In vitronectin positive tissues the stained structures were granular-like or fibrous and sometimes amorphous. A striking association of vitronectin with elastic fibres was seen.

Conclusions: There is a complex architecture of the extracellular matrix in the female paraurethral region with marked differences between postmenopausal, continent women and patients with GSI, irrespective of the presence of pelvic floor relaxation. Our findings suggest a selective and altered metabolism of connective tissue in the paraurethral region responsible for the onset of GSI in patients with pelvic floor relaxation.

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Title (type in CAPITAL LETTERS, leave one blank line before the text):

ALTERATIONS IN THE STRUCTURAL AND MOLECULAR CHARACTERISTICS OF THE PELVIC FLOOR TISSUES IN DIABETES MELLITUS (DM).

Introduction and objective- The absence of insulin and insulin like growth factors (IGF) has been reported

to play an important role in impaired contractility seen in DM. Insulin and IGF is required by several cell-types including connective tissue fibroblasts and also the smooth and striated myocytes. In addition to stimulating growth, insulin and it's congeners also promote the production of secondary growth factors as fibroblast growth factor (FGF) and connective tissue growth factor (CTGF) peptides. DM disrupts various growth factors signaling pathways mediated by PKC isoforms. The objective of our study was to interrogate the structural and molecular changes occurring in pelvic floor tissue seen in DM.

Methods- Samples of the levator ani striated muscle and fibroconnective tissue of the control (WT) and transgenic rat model of DM were cryosectioned and digitally imaged with an epifluoresence microscope (Leica). PKC isoforms Cy-3 (α, β, ε, δ, η, ξ) were examined by indirect immunofluoresence against C-terminal antigens. Sections were simultaneously doubly or triply stained to depict surface glycoproteins (Oregon green labeled wheat germ agglutinin), or actin (Texas red phalloidin) and nuclear DNA (bisbenzamide).

Results- The connective tissue sheath covering striated pelvic muscle (epimysium), individual cells, and fiber bundles (endomysium and perimysium, respectively) is severely diminished in DM. Connective tissue covered fascicles that enter the muscle, carrying nerves and vessels are also decreased in number and size in DM. This is apparent from i. the decreased staining of sialylated and glucuronylated proteins detected by the lectin and wheat germ agglutinin, ii. the thinned basophilic areas in conventional H&E stain, iii. By the attenuated autofluorescence of elastin, and iv. Decreased cell number indicated by nuclear stain. In muscle from diabetic animals the endomysial connective tissue between cells also appears particularly susceptible to rupture and premature tear. In addition, changes in expression and distribution of PKC isoforms, or mitochondrial Hsp75 suggest phenotypic remodeling of striated muscle, vascular and connective tissues in DM. The epimysium in diseased muscle is reduced to 15-45 microns compared to 65-150 microns in controls. Similarly the endomysium between muscle cells is reduced from 4.8 ± 0.3 to 2.7 ± 0.4 microns in diseased muscle.

Conclusions- These findings suggest that DM induce fundamental changes in the intracellular structure and signaling pathways of the pelvic floor tissues. These molecular changes may explain the pathological morphology and contractile dysfunction seen in lower urinary tract and pelvic floor tissues in DM.

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Title (type in CAPITAL LETTERS, leave one blank line before the text):
THE DENSITY OF PRESUMABLY SENSORY SP- AND CGRP-CONTAINING NERVE FIBERS CORRELATES WITH THE FUNCTIONAL STATUS OF THE LOWER URINARY TRACT IN PATIENTS WITH MULTIPLE SCLEROSIS. A PRELIMINARY STUDY.

Aims of Study:

Multiple sclerosis (MS) is a chronic inflamation-demielinization process of the nervous system . Lower urinary tract dysfunctions occurs in about 96% of MS patients, in 12% they occurs at the very beginning phase of the MS or even they precede the onset of MS itself. The most common functional finding in these patients is detrussor hyper-reflexia combined in some cases with detrussor-sphincter dyssynergia. It is suggested, that hyper-reflexia in patients with MS may be due to activation of C-fibers afferents. In our pilot study we decided to evaluate the functional status of the lower urinary tract in the