

ARE AGE-RELATED CHANGES IN THE PELVIC CONNECTIVE TISSUE RELATED TO FUNCTION?

Hypothesis / aims of study

Several studies in adults have suggested that the pelvic floor muscles are composed partly of smooth muscle (1,2). It has been reported that a distinct smooth muscle layer on the medial surface of the pelvic floor exists in adults. However, in neonates this layer is absent (1). The fact that smooth muscle tissue is only present in adults and not in neonates and embryos has resulted in speculation about its origin and function.

In this study we examined the distribution of smooth muscle tissue in the pelvic floor and connective tissue of the pelvis. Additionally we investigated whether the presence of smooth muscle is related to age.

Study design, materials and methods

To characterize the smooth muscle content of pelvic tissue we studied (immuno) histochemically stained sections of human foetal, infant and adult pelvises. Histological sections of the pelvic organs and muscles were prepared from two complete female foetal pelvises (14 & 19 weeks of gestation), one male infant pelvis (7 years), one adult male and one adult female pelvis (age > 60 years). The sections were cut in the transverse plane and stained immunohistochemically for the presence of striated muscle tissue, using a monoclonal antibody directed against myosin heavy chain (Upstate, clone A4.1025), smooth muscle tissue, using a monoclonal antibody directed against smooth muscle actin (Sigma, A-2547) and nerve tissue, using a polyclonal antibody against Neurofilament 68 kD (Chemicon, AB1983). Also, representative sections were stained with haematoxylin-azophloxine (overview staining) and Gomori's trichrome (collagen fibres).

Additionally, biopsies of the pelvic floor muscle and fascia of eight female and seven male adult cadaveric pelvises were taken at two positions (ventral and dorsal) in each pelvis. The ventral sample of the pelvic floor muscle and fascia was taken laterally to the urethra; the dorsal sample was taken laterally to the rectum. These samples were also sectioned and stained immunohistochemically for smooth and striated muscle, nerve tissue and histochemically with haematoxylin-azophloxine as also Gomori's trichrome.

Results

The foetal sections show no smooth muscle actin expression in the connective tissue of the pelvis. Smooth muscle of the blood vessels, internal urethral sphincter and the gut is however stained positively (Fig. 1). The adult male and female pelvis in contrast revealed a high expression of smooth muscle actin in the connective tissue surrounding the organs and striated muscles. Striated muscle tissue was as expected negative for smooth muscle actin, demonstrating the specificity of the antibody. The connective tissue of the 7-year-old male infant shows some expression of smooth muscle actin in the connective tissue but to a much lesser extent than in adults.

Samples of two female and three male biopsy specimens of the pelvic floor and surrounding fascia clearly demonstrated smooth muscle actin expression. These smooth muscle cells were always situated in the connective tissue around or in the striated muscle of the pelvic floor (Fig. 2). The striated levator ani muscle tissue was again negative for smooth muscle actin. Nerves innervating the smooth muscle bundles were not observed.

Interpretation of results

In contrast to the active function of striated muscle of the pelvic floor, the connective tissue in the human pelvis has probably a passive function of pelvic organ support and functions as a compensatory mechanism to the continuous stress of intra-abdominal pressure. This intra-abdominal pressure probably induces stretch to the connective tissue.

Recent research has shown that fibroblasts can differentiate into so-called 'myofibroblasts'. These myofibroblasts express smooth muscle actin and have a smooth-muscle-like phenotype. Interestingly, in an experimental set-up myofibroblasts tend to increase actin expression if they are exposed to mechanical stress. The smooth muscle actin expression upregulates the cells contractile activity (3).

Since the smooth muscle in the pelvis is not found in foetal stage but is present later in connective tissue and the connective tissue is exposed to mechanical stress due to intra-abdominal pressure we postulate that these smooth muscle cells are in fact myofibroblasts differentiated from fibroblasts.

Concluding message

From our findings it can be seen that in humans from the foetal stage to old age progressive differentiation of connective tissue takes place whereby increasing amounts of smooth muscle in the pelvic tissue is demonstrable.

References

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2. Br J Obst Gyn (1989) 96:15-23.
3. Nat Rev Mol Cell Biol (2002) 3(5):349-63.

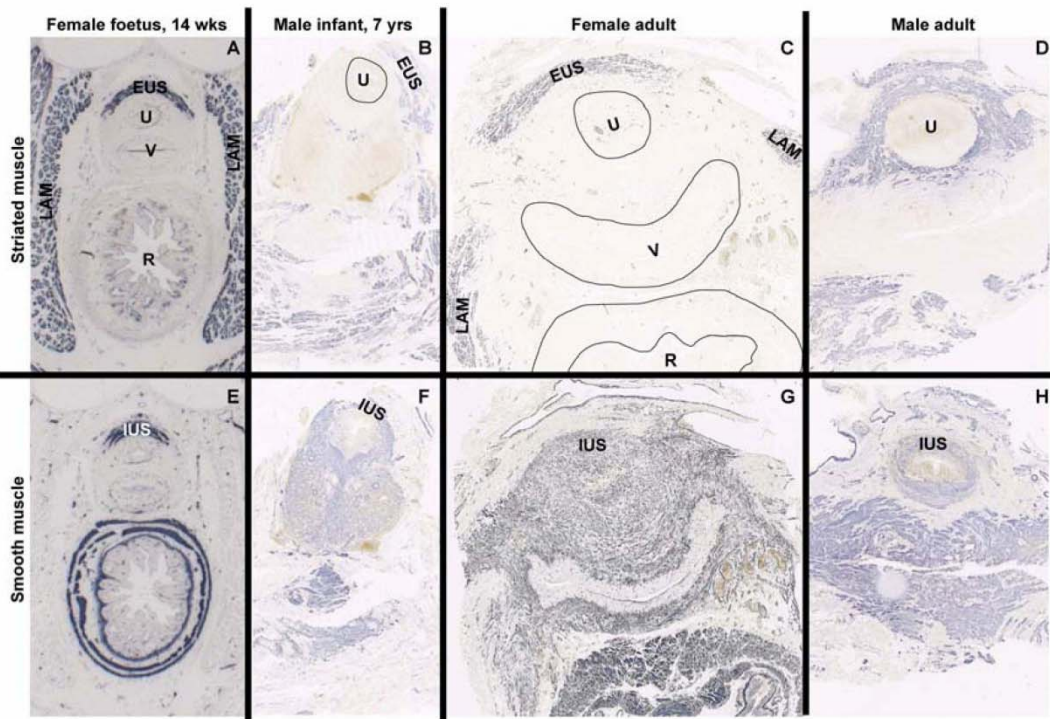


Figure 1. Immunohistochemically stained sections of striated muscle (A-D) and smooth muscle (E-H) of a female foetus, a male infant, a female adult and a male adult. Smooth muscle actin expression is absent in the foetus except in the rectum and internal urethral sphincter (E). Increased expression is visible with increasing age (F and G/H). Note that images are taken at different magnifications. EUS, external urethral sphincter; IUS, internal urethral sphincter; LAM, levator ani muscle; R, rectum, U, urethra, V, vagina.

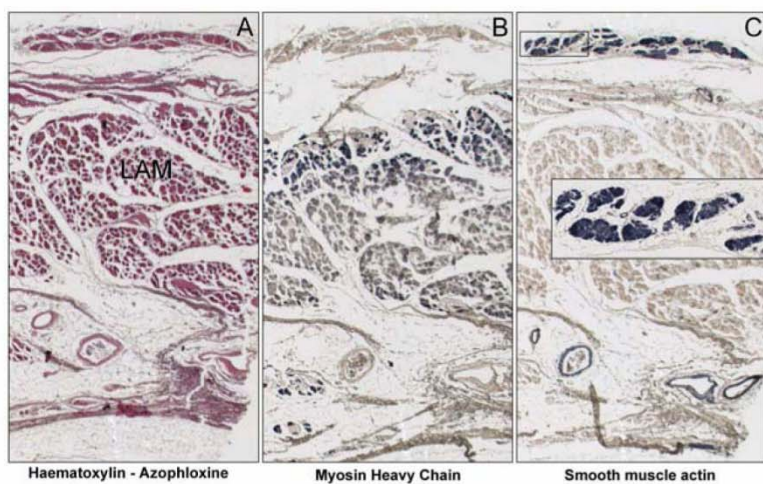


Figure 2. Serial sections of biopsy specimens (adult male) of the pelvic floor and surrounding fascia clearly demonstrating smooth muscle actin expression in the connective tissue on the superior surface of the levator ani muscle (LAM). **A.** Overview staining (Haematoxylin – Azophloxine). **B.** Immunohistochemically stained section for striated muscle (myosin heavy chain) staining the LAM. **C.** Immunohistochemically stained section for smooth muscle (smooth muscle actin). Note the smooth muscle actin expressing cells in the connective tissue layer. Box: magnification of the smooth muscle actin expressing connective tissue layer.

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HUMAN SUBJECTS: This study did not need ethical approval because only cadaveric specimens were used. and did not follow the Declaration of Helsinki - with approval by the ethics committee - in the sense that this was not applicable since only only cadaveric specimens were used. Informed consent was not obtained from the patients.