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# SMOOTH MUSCLE ANCHORS THE PELVIC ORGANS TO THE PELVIC FLOOR

#### Aims of study

The pelvic floor plays a central role in continence and pelvic organ support. The striated muscles of the pelvic floor provide active support and the fascial connective tissue is believed to provide the passive support necessary to counteract the effects of constant and intermittent elevations of intra-abdominal pressure. The presence of smooth-muscle cells in the pelvic connective tissue has been demonstrated in several studies. Smooth muscle was found in the adult pubourethral, pubovesical and puboprostatic ligaments, the uterosacral ligaments and the tendinous arch of the pelvic fascia. A smooth muscle termed "rectourethralis" in the male and "rectovaginalis" in the female that is continuous with the longitudinal muscle layer of the rectum has also been reported. The longitudinal smooth-muscle layer of the rectum has a distal attachment in the perimysia of the muscle fibres of the LAM and external anal sphincter at the level of the levator hiatus. This layer anchors the rectum to the pelvic floor. This smooth muscle anchor of the posterior compartment (rectum) to the LAM has been previously described both in anatomical and embryological studies and has also been illustrated in anatomical atlases. Our main goal was to establish whether in addition to the posterior compartment, the anterior (urethra) and middle (vagina) compartments are also anchored to the pelvic floor by smooth muscle tissue.

### Study design, materials and methods

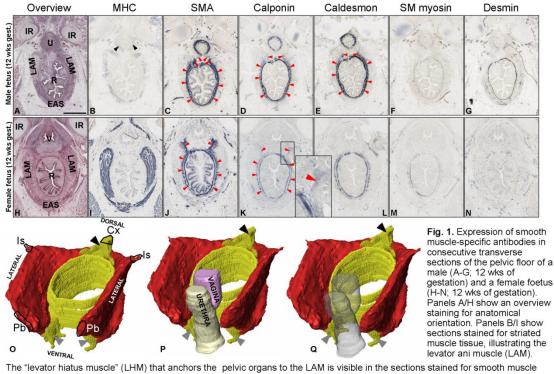
Large transverse sections were prepared of the *en bloc* excised pelvic organs and surrounding tissue from individuals of different ages (three foetuses (two female one male, 12 and 18 wks gest.), one infant (male, 7 years) and three adults (1 male, 77 yrs and 2 females, 54 and 86 yrs). Immunohistochemical stainings were performed with antibodies directed against  $\alpha$ -smooth muscle actin, caldesmon, calponin and desmin, which are all proteins that are typically present in smooth muscle tissue, and against striated-muscle myosin-heavy chain, a protein typically present in striated-muscle tissue. Haematoxylin-azophloxine was used as an overview staining. 3D reconstructions were prepared (1, 2).

#### **Results**

In the foetuses, cells that stain with the smooth-muscle markers SMA, calponin, and caldesmon were demonstrable in the connective tissue that connects the medial surface of the puborectalis portion of the LAM, with all the pelvic organs (urethra, vagina and the rectum; Fig. 1). Smooth muscle, therefore, anchors the pelvic organs to the striated muscle of the pelvic-floor at the site where the organs transit through the levator hiatus. This smooth "levator hiatus muscle" (LHM) is continuous with the longitudinal smooth-muscle layer surrounding the rectum. The LHM also extends into the median raphe which connects the LAM to the coccyx (Fig. 1C/J/O/P/Q). Smooth-muscle myosin heavy chain was expressed very weakly (Fig. 1F/M) and we could not demonstrate desmin in the LHM (Fig. 1G/N). The smooth-muscle markers were first identifiable in the 12-week old foetal specimens, with the female specimen being slightly less advanced than the male specimen. The specimens of the 7-year-old infant and adults also reveal a strong expression of smooth-muscle markers in the connective tissue that fills the space between the puborectalis muscle and the pelvic organs (data not shown in abstract). Allowing for changes in size, the staining pattern laid down at the early foetal stage, remains unchanged during subsequent postnatal development.

#### Interpretation of results

Our study demonstrates that already in the foetus, at the level where the still relatively long foetal urogenital sinus and rectum transit the LAM, the connective tissue between the pelvic organs and the LAM contains smooth muscle tissue. This important finding demonstrates that this tissue is also an integral component of the pelvic organ-support system. This component is formed in the early foetus and remains present throughout life, expanding in a coordinated fashion with the increasing size of the pelvis and pelvic organs. We demonstrate that smooth muscle is not restricted to the posterior compartment (rectum), but also anchors the anterior (urethra) and middle compartment (vagina) to the pelvic floor at the level of the levator hiatus. This new concept of support of the anterior and middle compartments should encourage us to reconsider our concept of the function of the lower urogenital tract. The clinical importance of our findings is that smooth muscle rather than (dense) connective tissue anchors the pelvic organs to the pelvic floor at the site of the hiatus. An obvious difference between smooth muscle and connective tissue is the potentially more controlled stretch of the junction, but its behaviour under tensile stress, e.g. during delivery, is completely unknown. Additional studies of the smooth muscle cells of the pelvic floor in young and ageing adults are therefore urgently required to understand the functional consequences of our findings.



The "levator hiatus muscle" (LHM) that anchors the pelvic organs to the LAM is visible in the sections stained for smooth muscle actin (SMA; panels C/J, red arrowheads). Note the expression of calponin (D/K; red arrowheads) and caldesmon (E/L; red arrowheads) in the LHM. The box between panel K and L shows a magnification of the calponin expression in the female. The expression of caldesmon is relatively weak in the female fetus. Smooth muscle myosin heavy chain is expressed very weakly (F/M). Desmin (G/N) was not detectable in the LHM. Panels O-Q show a three-dimensional reconstruction of the striated LAM and the smooth levator hiatus muscle in a female foetus (12 wks of gestation). The structures are seen from a cranial oblique view, without urethra and vagina (O) and with urethra and vagina shown either solid (P) or transparent (Q). The LAM is shown in red, the longitudinal smooth muscle of the rectum in bright yellow and the levator hiatus muscle (LHM, grey arrowheads) in a darker yellow tint. The LHM surrounds the foetal vestibule and rectum, and is intimately connected to the medial surface of the LAM. Similarly, smooth-muscle cells are also found in the median raphe of the LAM that connects the LAM dorsally to the coccyx (black arrowheads). Note that the LHM is continuous with the longitudinal smooth muscle layer of the rectum (transition from dark to bright yellow). For orientation, the three bony attachments of the LAM (Pb, Is, Cx) are illustrated. Cx, Coccyx, EAS, external anal sphincter muscle; IR, inferior ramus of pubic bone; IS, Ischial spine; LAM, levator ani muscle; MHC, striated muscle myosin heavy chain; PB, pubic bone; PS, pubic symphysis; R, rectum; SMA; α-smooth muscle actin; U, urethra; V, vagina; Ve, vaginal vestibule. Bar = 1 mm.

## Concluding message

The tissue connecting the pelvic organs with the medial border of the puborectalis muscle is made up largely of smooth-muscle cells. This "levator hiatus muscle" forms, together with the striated muscles of the pelvic floor, an integral and essential component of the pelvic organ support system.

#### <u>References</u>

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- Wallner C, van Wissen J, Maas CP, Dabhoiwala NF, DeRuiter MC, Lamers WH (2008). The contribution of the Levator Ani Nerve and the Pudendal Nerve to the innervation of the Levator Ani Muscles; a study in human fetuses. Eur Urol 54(5):1136-42

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Is this a clinical trial?	No
What were the subjects in the study?	HUMAN
Was this study approved by an ethics committee?	No
This study did not require eithics committee approval because	only cadaver material was used.
Was the Declaration of Helsinki followed?	No
This study did not follow the Declaration of Helsinki in the sense	only cadaver material was used.
that	
Was informed consent obtained from the patients?	No