Architectural Changes in Extracellular Matrix of The Endopelvic Fascia After Oral Intake of Vitamin D Hyperdose in Rats

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ABSTRACT

º **Vitamin D deficiency is largely spoken of as being associated with several different diseases**. Most of the associations are **inferred by the levels of 25(OH)D** in the plasma, which has no direct correlation to the activity of its active metabolites – i.e. 1,25(OH)D – since it follows the patterns of a **steroid hormone**, with negative and positive feedbacks and other molecular interactions, instead of behaving as a vitamin.

º **Pelvic organ prolapse (POP) has also been linked to lower serum levels of D vitamin**, although the exact mechanism for this increase in risk is not totally understood.

º **One of the potential mechanisms for this increased risk is the influence vitamin D has on the architecture of the extracellular matrix**, main component of the structures of the pelvic support apparatus and responsible for its mechanical properties.

º **The aim of this study was to examine the arrangement of collagen fibers, the most prevalent fibrous protein present in the extracellular matrix at the endopelvic fascia,** to capture any different morphological pattern between subjects with a normal diet and those under a vitamin D supplementation.

METHODS

º This is an **experimental study investigated in female healthy adult Wistar rats** (8 weeks old, approximately 200-220g).

º The **treatment group** received cholecalciferol diluted in arachid oil by gavage (in the dose 37.5mcg/Kg/day, equivalent to **1,500Ui/Kg/day**), and the control group received only the oil, for 18 consecutive days.

º After euthanasia, a surgical procedure was undertaken extracting **tissues fo the endopelvic fascia that are representative of the pelvic organs ligaments at DeLancey's Level 1 of support** (Fig. 1).

º Samples were immersed in Tissue-Tek and cut in **50 micrometer thick slices** and placed on the sheets for microscopic evaluation with **SHG (second harmonic generation) technique, which requires no tissue preparation**.

º For the purpose of interpretation, t**he rats were also separated into groups according to the estrous phase: high estrogenic influence (proestrus and estrus) and low estrogenic influence (metaestrus and diestrus)** identified by histology under conventional light microscopy with H&E (hematoxylin and eosin).

º Tissues were analyzed using TCS SP8 CARS Confocal Microscope (Leica) and F and Epi-SHG were used to detect SHG from collagen fibers.

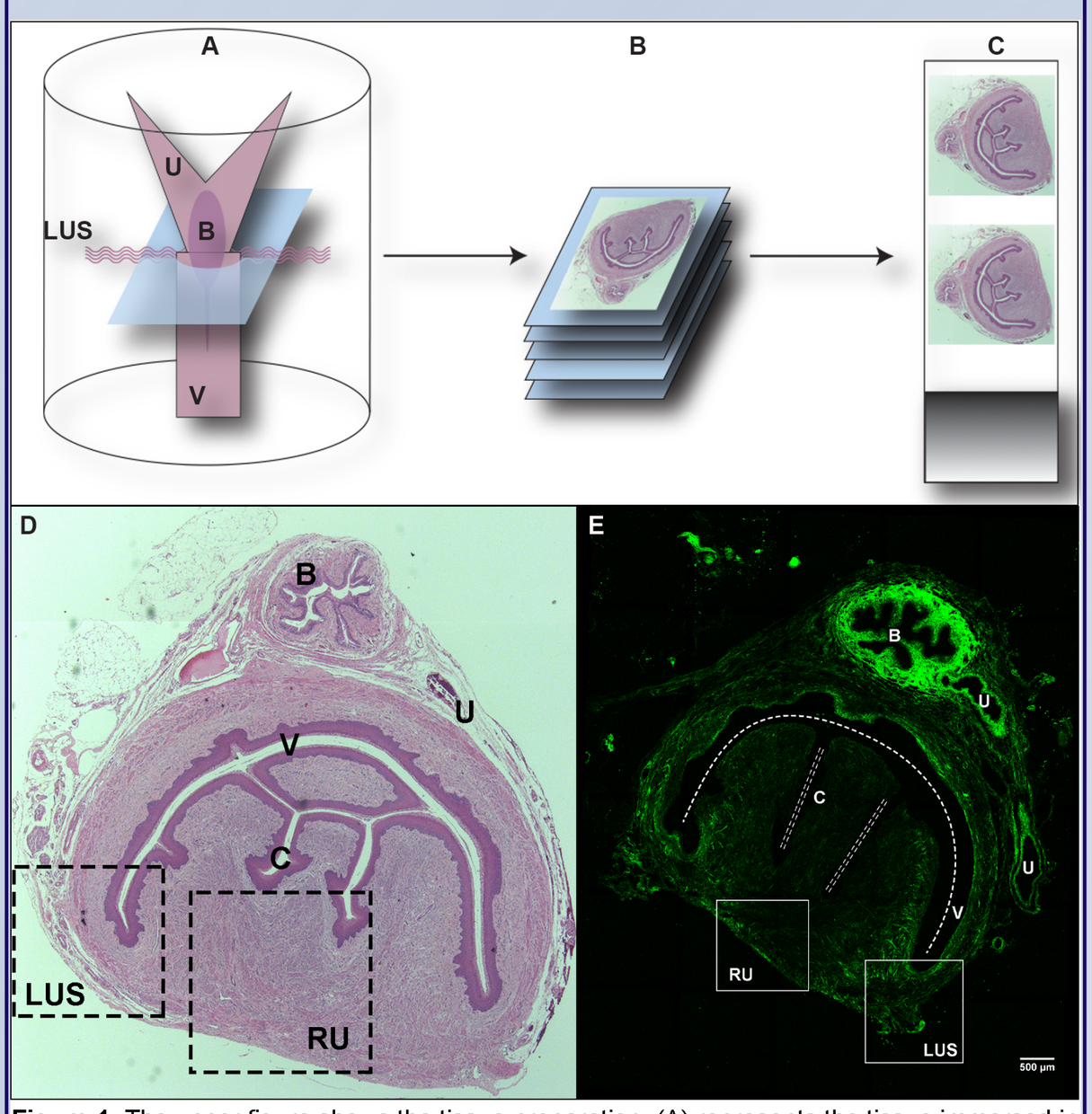

Figure 1. The upper figure shows the tissue preparation: (A) represents the tissue immersed in Tissue-Tek forming a bloc and the blue rectangle shows the region of interest. (B) The bloc is taken to the cryostat where it is cut into slices 50 micrometers thick and (C) laid on the sheet. D and E are H&E and SHG (second harmonic generation) images, respectively, with the identification of the structures present in every sample: uterossacral ligament (LUS), retro uterine region (RU), vaginal lumen (V), cervical lumen (C), ureter (U) and bladder (B).

RESULTS

º We found that **collagen fibers have a consistently undulated pattern and more random disposition in the treated group whereas it assumes a more taut and oriented appearance in the control group when under high estrogenic influence** according to the estrous cycle.

º **The collagen quantification showed statistical significance with a higher ammount of the substance in the treated group** compared to the control group also **under a high estrogenic environment** (Fig. 2).

º These findings suggest that **there is a difference in the morphological presentation of the extracellular matrix of subjects under high dose intake of vitamin D compared to subjects under normal intake of the vitamin, pointing to a probable change in the mechanical properties of the tissue** and that **there might be an interaction of the metabolic pathways of the two steroid hormones cholecalciferol and estrogen** at least regarding the amount of collagen deposited in the tissue.

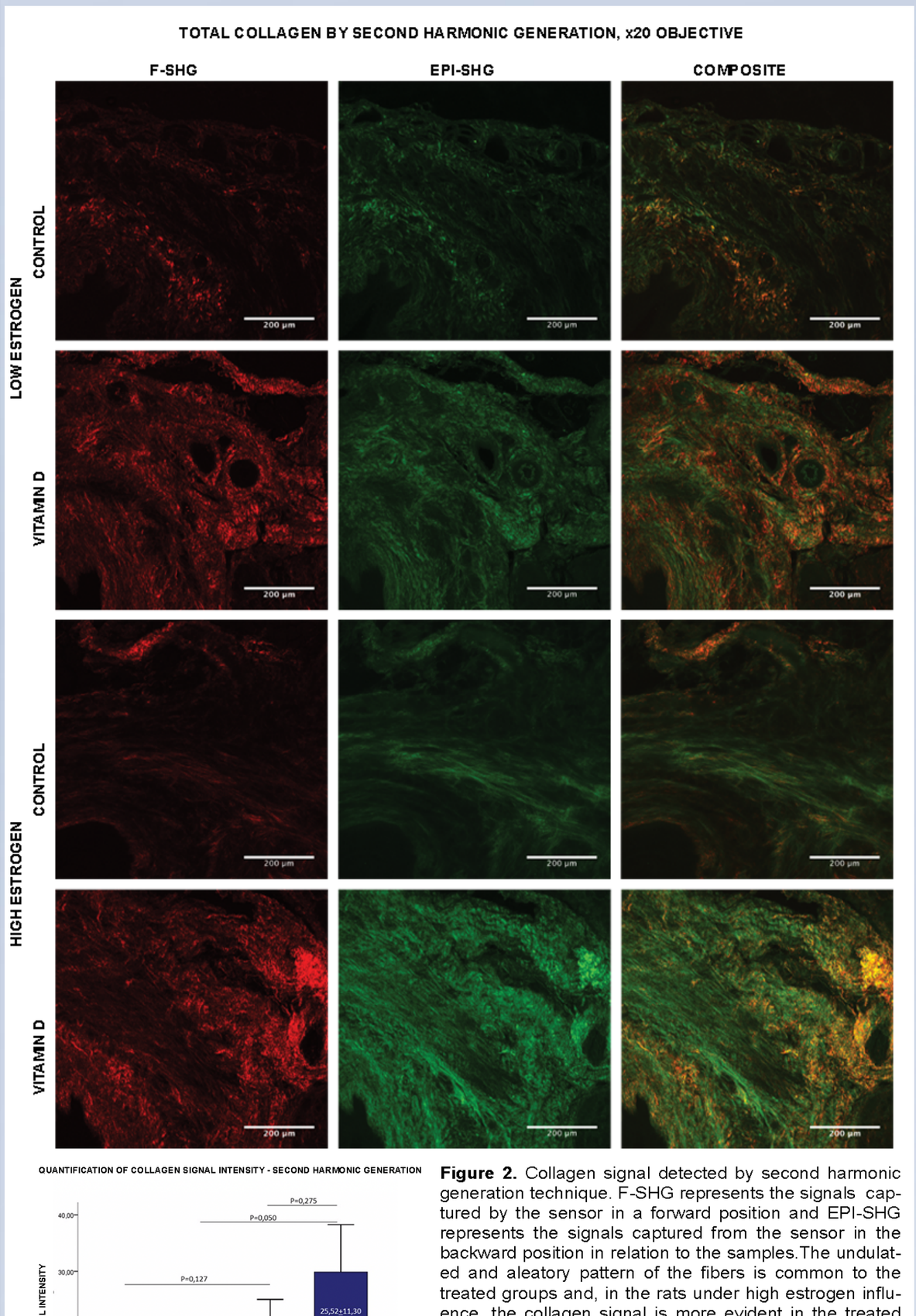

Figure 2. Collagen signal detected by second harmonic generation technique. F-SHG represents the signals captured by the sensor in a forward position and EPI-SHG represents the signals captured from the sensor in the backward position in relation to the samples.The undulated and aleatory pattern of the fibers is common to the treated groups and, in the rats under high estrogen influence, the collagen signal is more evident in the treated group. The graphic underneath shows the comparison of the collagen signal intensity aquired by second harmonic generation at the lamina propria of the upper vagina of female rats under low and high influence of estrogen (according to the estrous cycle) of the control and treated (vitamin D) groups. The values refer to the mean and standard deviation of the ammount of collagen signal in each group.

CONCLUSIONS

º **Oral high dose intake of vitamin D changes the framework and composition of the extracellular matrix at the level of the endopelvic fascia**, which can impact the properties and resilience of the tissue.

º Our novel findings provide new exciting insights into the mechanical influence of the vitamin D in the pelvic tissues. This may explain the higher prevalence of pelvic floor disfunctions if similar results are shown in human, given that the structure of the tissue dictates its function.

º In summary, **new research are needed in the biomechanical field to approach a clinical significance but we have shown a tangible relation between vitamin D exposure and collagen fiber architecture**.

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