**Objectives:**
The tendon rupture rate and leads to disability.

**Methods:**
Orthopaedic Surgery, Magdeburg, Germany

**Background:** Pathological (or heterotopic) calcification is the deposition of calcium-containing crystals in soft tissues that normally do not calcify. The deposition of these crystals in tenons such as the rotator cuff and the Achilles tendon is known as calcific tendinopathy (CT). CT is a painful condition, which increases tendon rupture rate and leads to disability.

**Objectives:** To understand what inhibits calcification, in order to provide new strategies to treat a condition for which existing therapies are ineffective.

**Methods:** We investigated the role of the neurotransmitter hydrogen sulfide (H\(_2\)S), and in particular of the H\(_2\)S-producing enzyme cystathionine \(\gamma\)-lyase (CSE) in CT. In vitro, we induced calcification in tenocytes from WT and CSE KO mice or we treated WT tenocytes with different H\(_2\)S donors. In vivo, calcification was assessed in a surgery-induced murine model of CT (tenotomy of the Achilles tendon) and in a spontaneous model of CT (aging). Samples obtained from patients with rotator cuff or Achilles tendon CT were also analyzed. To investigate the underlying mechanisms of the CSE+H\(_2\)S effect, we focused on the bone morphogenic proteins (BMPs) pathway. We additionally explored if altered extracellular matrix (ECM) organization, due to lysyl oxidase (LOX) activity and aberrant collagen-crosslinking, could be involved in CT. In this context, we studied if H\(_2\)S could affect LOX expression and activity.

**Results:** In vitro, tenocyte calcification was inhibited by exogenous H\(_2\)S-donors, while it was exacerbated in CSE KO tenocytes. The protective role of CSE-H\(_2\)S was confirmed in vivo. In aged mice, microtomography analysis revealed exacerbated Achilles tendon calcification in CSE KO mice compared to WT. In the surgery-induced model of CT, an inverse correlation between calcification and CSE expression in operated Achilles tendon was seen over time. Similarly, inverted correlation between calcification and CSE expression was found in human CT samples.

Reduced calcification in tenocytes exposed to H\(_2\)S was accompanied by decreased expression of genes coding for BMP2, BMP4 and decreased activation of the BMP signaling pathway (pSMAD1/5/8). On the contrary, BMPs expression and BMPs-pathway activation were exacerbated in CSE KO tenocytes compared to WT tenocytes.

We next investigated whether ECM disorganization could play a role in CT. Tenocytes cultured in calcification media and treated with the pan-inhibitor of lysyl oxidases (LOX, LOXL1-4) -(aminopropionitrile (BAPN) showed decreased calcification. This pointed to a potential beneficial role of LOX inhibition, therefore decreased collagen-crosslinks, in CT. By analysis of LOXs gene expression in WT and CSE KO tenocytes cultured in calcifying condition, we found much higher expression (4-fold) of LOX2 and LOXL4 in CSE KO tenocytes. Moreover, H\(_2\)S-donors inhibited LOX activity. Altogether, these results suggest that decreased H\(_2\)S could lead to aberrant LOX expression and activity, excessive collagen-crosslinking, and, ultimately, calcification. Further experiments are ongoing to prove these hypotheses.

**Conclusion:** We suggest targeting H\(_2\)S production by CSE, or supplying an H\(_2\)S donor, is of therapeutic relevance to pathological calcification in the context of CT and can modify the disease course.

The anti-mineralizing effect of H\(_2\)S in tendons could be due to both inhibition of the BMPs pathway and suppression of abnormal LOXs activity.

**Disclosure of Interests:** None declared.

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**References:**


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