Can a common diabetes drug prevent our tendons from ageing?

- Ageing affects all our body, including tendons.
- Ageing tendons can make the joints stiff and painful, making life difficult for the elderly.
- By preventing the ageing of our tendons, we could improve the life of millions of people.
- Professor James H-C Wang at the University of Pittsburgh, USA, and colleagues have discovered a new role for a known diabetes medication called metformin in keeping our tendons young.

Ageing affects the structure and function of the musculoskeletal system in our body, including the muscles, bones, and tendons. Tendons are tough and strong bands of connective tissue that connect muscles to bones and are important for the stability and functionality of our joints. But ageing tendons can make our joints stiff and often very painful as we age. This tendon disorder due to ageing is called tendinopathy, which is characterised by inflammatory and degenerative changes in the tendon. Achilles tendon, the strongest tendon in our body that connects the lower end of our calf muscles to our heel, is prone to such injury, making everyday activities such as walking difficult and painful for elderly individuals.

What happens when tendons age?

Ageing cells in tendons produce and release molecules that can cause inflammation and degeneration of the structures. Some of these molecules include pro-inflammatory cytokines, which are proteins that mediate tissue inflammation. Professor James H-C Wang and colleagues at the University of Pittsburgh, USA, have shown that a protein called high mobility group box 1 (HMGB1) can function as an inflammation mediator and as a key molecule that is responsible for the development of tendinopathy due to mechanical overloading/overuse.

> **Metformin decreased the levels of inflammation and the number of deteriorating cells in ageing tendon, while it caused a significant increase in tendon stem cells.**

Ageing increases the levels of HMGB1 in the tissues, and depending on the structural state, HMGB1 can have many functions. One form of HMGB1 that helps tissue regeneration can get modified into an inflammatory mediator form. This inflammatory form of HMGB1 may be mainly responsible for the development of ageing-related tendinopathy.

A new hope

Medications currently used to treat Achilles tendinopathy are steroids and non-steroidal anti-inflammatory drugs (NSAIDs). These can help with the symptoms by reducing the levels of inflammation temporarily but don't fully alleviate the problem or stop the degeneration process. They can only be used for a short period of time because of their potentially severe side effects, such as intestinal bleeding, ulcers, stroke, and kidney damage due to long-term use. Since there is an obvious need for new drugs against tendinopathy, in the quest for new treatment strategies an old drug – metformin – captured the attention of Wang and colleagues.

Metformin is commonly used for reducing the levels of blood sugar in diabetic people. Lately, metformin has also been shown to have an effect against tissue inflammation and ageing and is currently being tested against a few ageing-related diseases such as stroke, cancer, heart disease, among others. Since there is evidence that metformin has anti-ageing and anti-inflammatory properties, the researchers decided to test the medication against ageing-related tendinopathy in mice and measure the levels of damage-related molecules in them, including HMGB1.

Through the magnifying glass

The research team used a total of 130 mice for the experiment. They initially took samples of the Achilles tendons of both young (2.5–4.5 months old) and old mice (14–19 months old). The samples were studied using several methods including examination under the microscope after staining the tendinous tissues (figure 1).

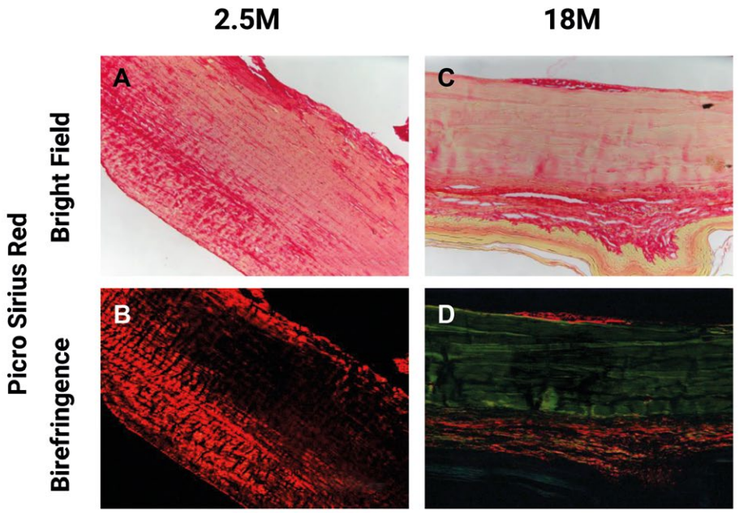


Figure 1. Ageing tendons show degenerative changes. Young tendon is formed by strong collagen fibres and stained with red under bright light microscopy (A). In contrast, ageing tendon is formed by loose collagen fibres and stained with yellow under bright light microscopy (C). The thick collagen fibres in young tendon are formed by collagen type I (red in B), while the loose collagen fibres in ageing tendon are formed by collagen type III (green in D).

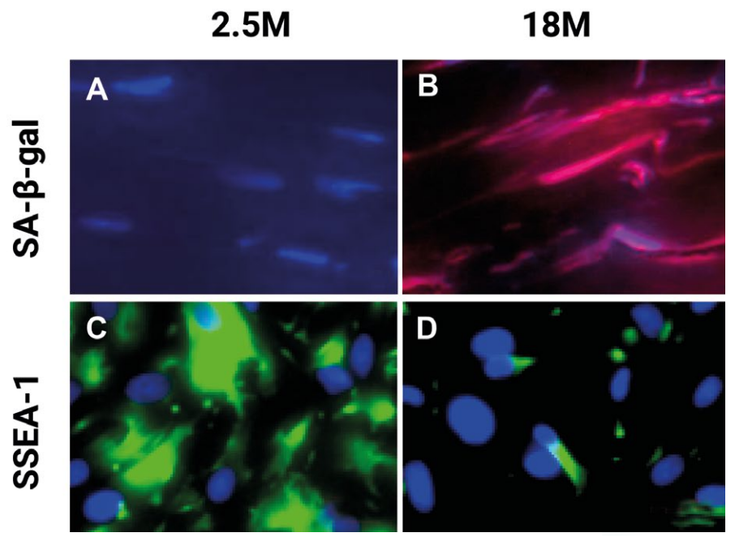


Figure 2. Ageing cell marker, SA-β-gal, was not detected in young tendon (A), but abundant staining is seen in ageing tendon (red in B). A large number of cells from young tendon were stained positive for SSEA-1, a stem cell marker (green in C), but only a few cells in ageing tendon expressed SSEA-1 (green in D).

They next used quantitative real time polymerase chain reaction (pRT-PCR), a method that measures how much RNA (a molecule that serves as a template for the composition of a specific protein) is produced by a specific gene. Both types of tendons, young and old, were then visualised using specialised stains to identify certain inflammation molecules (immunohistochemistry) and tendon cells were isolated from both types of samples and grown in containers. Some of the containers were then treated with different concentrations of metformin. These treated cells were also stained and examined under the microscope. Comparisons were made both between treated and non-treated cells as well as between samples from young and ageing tendons.

A second group of mice including old and young animals were divided into two groups. The first group were given metformin in the form of an injection inside their body cavity (lower abdomen) and the second were given a saline (water and salt) injection instead (the control group). Samples of Achilles tendons were finally taken from both groups of mice, stained, and then examined under the microscope and compared.

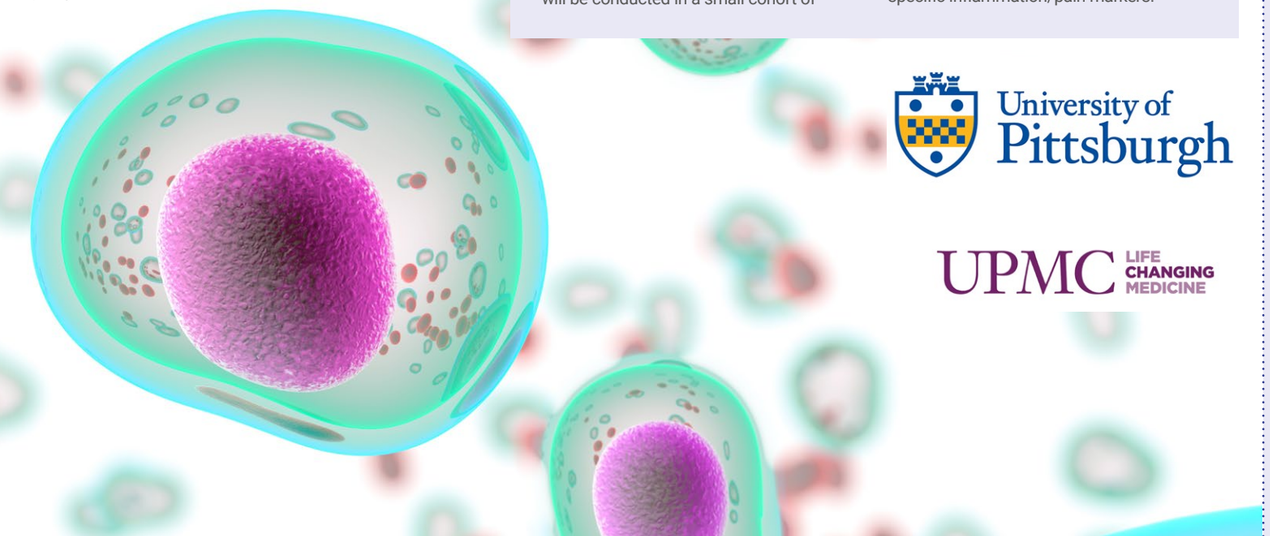
HMGB1 could be used as a biomarker of tendon ageing and as a target for developing specialised treatments in the future.

A potential new therapy

The experiments confirmed that tendons from old mice showed typical tendinopathy features including inflammation and degeneration when examined under the microscope. In addition, the amount of age-associated and inflammatory molecules, including HMGB1, was higher in the tendons from old mice compared to those from the younger animals. The researchers also noticed that compared to young tendons, the ageing tendons had a higher number of ageing cells but lower number of stem cells, the 'mother cells' from which all other specialised cells come from (figure 2).

The cells and tendons that had treatment with metformin showed less age-related deterioration and lower levels of tissue damage related HMGB1 molecule, suggesting that metformin works on keeping HMGB1 levels low. Metformin treatment also decreased the levels of inflammation and the number of damaged cells, while it caused a significant increase in stem cells. This finding is very important since stem cells replace old and damaged cells in tendons with new cells, consequently keeping them strong and healthy.

These encouraging results suggest that metformin could potentially be used in humans for preventing the ageing of tendons. Such treatments will be increasingly important as the world's population is living longer. The results also showed that HMGB1 could serve as a biomarker of tendon ageing and as target for developing specialised age-induced tendinopathy treatments in the future.



Personal response

What inspired your research on improving the health of tendons?

Tendon injury such as tendinopathy is a highly prevalent disease in athletes, military, and the general population that causes significant pain, disability, healthcare cost, and lost productivity. Injured tendons heal poorly, making them susceptible to further injury and rupture. We are building on our extensive tendinopathy-related research for the past two decades. The major drawbacks of current treatment options for tendinopathy have prompted us to develop novel strategies that are efficacious and safe.

Understanding the mechanisms of tendinopathy development and progression is key to developing new therapeutic strategies. Tendon inflammation in early stage of tendinopathy is often accompanied by chronic pain, disability, tendon degeneration, and rupture. Mechanisms that regulate tendon inflammation are the major focus of our study. The identification of the potent inflammatory molecule, HMGB1, as a major causative factor for the development of tendinopathy has inspired us to target HMGB1 as the first step in the development of therapeutic strategies for the prevention and treatment of tendinopathy. Considering that there isn't a repurposed drug on the market for the prevention and treatment of tendinopathy, we are excited to test the new repurposed drug – metformin, which is already in clinical use for diabetes treatment.

Do you have any plans to test metformin on humans? What would a study like this look like?

We have already made plans to test metformin in humans. The initial studies will be conducted in a small cohort of Achilles tendinopathy patients selected from the high volume of patients vising our UPMC clinics. Metformin will be administered orally or as a topical application of the metformin lotion we formulated in our lab. Currently, we are testing the efficacy and toxicity of the metformin lotion formulation in animals.

How would you further assess the functionality of elderly people's tendons with and without metformin in living animals or humans?

Chronic non-resolving pain, inflammation, and swelling, and impaired performance are the major characteristics of tendinopathy. The assessment of pain in animals involves observing surrogate measures of pain and signs of animal well-being and making a judgement. Standardised methods and equipment are available for this assessment. Other standardised methods to evaluate tendon pain in animals will be used. Finally, functional evaluation of the treated tendons will be performed using ultrasound imaging and mechanical testing.

Assessment of functionality in humans will be mainly performed by standardised pain questionnaires such as VAS (Visual Analogue Scale) and VISA-A (Victorian Institute of Sports Assessment-Achilles). Qualitative questionnaires will assess Achilles tendon pain and function by assessing severity of pain during activity and at rest. Functional recovery tests will be scored using the VAS combined with a battery of evaluations specifically designed for Achilles tendinopathy. Ultrasonography will be performed to examine tendon structural changes. Additionally, microdialysis will be used to measure specific inflammation/pain markers.

Details

e: wanghc@pitt.edu
w: sites.pitt.edu/~mechbio/

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Collaborators

Dr Jianying Zhang, Dr Roshawn Brown, Dr MaCalus Hogan, Dr Kentaro Onishi

Bio

James H-C Wang PhD is a professor at the Department of Orthopaedic Surgery, University of Pittsburgh. Wang researches tendinopathy mechanisms and novel intervention strategies for the prevention and treatment of tendinopathy and other musculoskeletal injuries focusing on cell and tissue mechanobiology and functional tissue engineering.

Jianying Zhang PhD is a research associate professor at the Department of Orthopaedic Surgery, University of Pittsburgh. Her main research interest is the mechanisms for the onset of tendinopathy and the role of inflammatory mediators. She combines her experience as a pharmaceutical scientist towards developing novel drugs and drug delivery methods.

Roshawn Brown MD is a resident at the Department of Physical Medicine and Rehabilitation, University of Pittsburgh, whose career interests are in the field of musculoskeletal rehabilitative medicine.

MaCalus V Hogan MD, MBA is Professor and Chairman of Department of Orthopaedic Surgery, University of Pittsburgh. As the Chief of Foot and Ankle Surgery at the University of Pittsburgh Medical Center (UPMC), his interests are in musculoskeletal regenerative medicine with a focus on tendon, ligament, and cartilage bioengineering.

Kentaro Onishi DO is assistant professor at the Departments of Physical Medicine and Rehabilitation and Orthopaedic Surgery, University of Pittsburgh. He is a clinician scientist focusing on tendon research and clinical treatment, and he is a leading musculoskeletal ultrasound expert.

Further reading

Zhang, J, et al (2022) Metformin improves tendon degeneration by blocking translocation of HMGB1 and suppressing tendon inflammation and senescence in ageing mice. *Journal of Orthopaedic Research*, 1–15.

Zhao, G, et al (2019) HMGB1 mediates the development of tendinopathy due to mechanical overloading. *PLOS ONE*, 1–20.

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