Ageing causes structural and cellular alterations in the mouse muscle-tendon junction

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Background

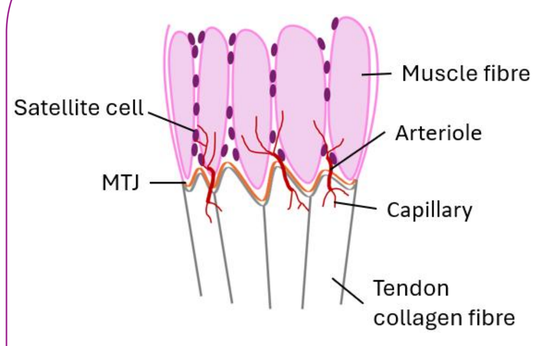


***Figure 1**. Schematic illustration of MTJ.*

- The muscle-tendon junction (MTJ) is a specialised interface that transmits contractile force from muscle to tendon (Fig. 1).
- The MTJ is more prone to injury, and its vulnerability increases with age.
- Current treatments are not so effective, resulting in scar tissue formation and high re-injury rates.
- However, the mechanisms underlying MTJ ageing and associated functional decline are poorly understood.

The aim of this study: to characterise age-related structural and cellular changes in the MTJ using high-resolution imaging techniques.

Methods

Sample acquisition

- Achilles tendon and gastrocnemius muscle junctions (Achilles MTJs) were subsequently harvested from male C57BL/6 mice.
- Two age groups: young (3 months old) and old (23 months old).
- Samples were subsequently processed for µCT imaging, 3D immunolabeling, or 2D analysis (n = 4 per age group, Figure 2).

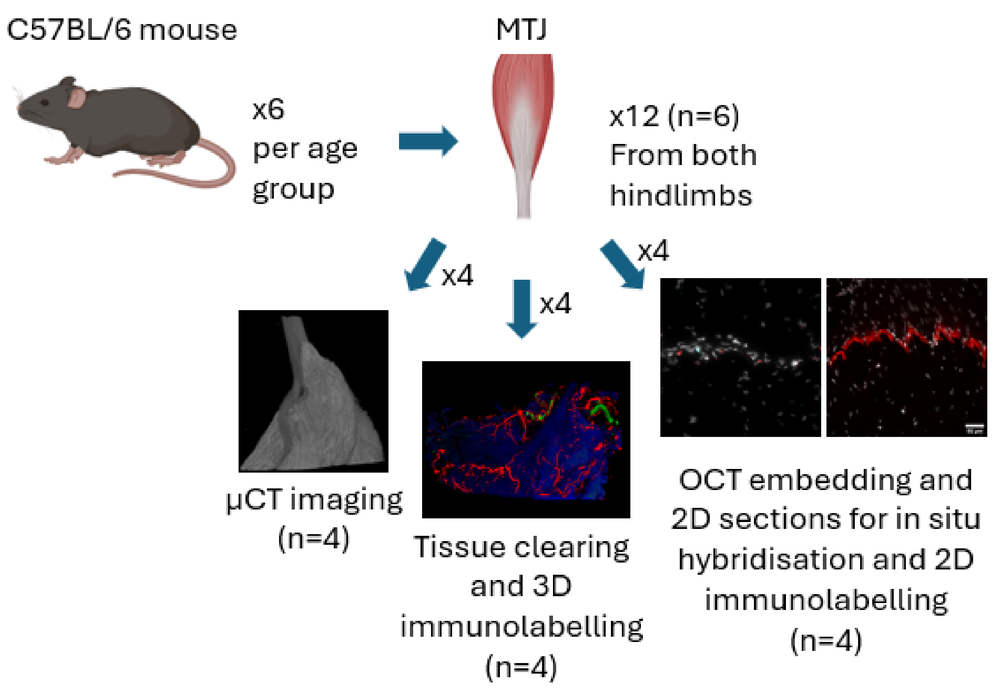


***Figure 2**. Overview of sample allocation for each method.*

Sample processing

- µCT imaging: mouse MTJs were stained with 1% phosphotungstic acid to enhance the contrast prior to imaging. Reconstructed images were analysed using Avizo and CTAn.
- 3D immunolabelling: samples were permeabilised, immunolabelled (using vascular endothelial cell marker, VWF and skeletal muscle marker, LAMA2) and cleared (using Histo-1 and 2) prior to imaging.
- In situ hybridisation: samples were OCT embedded and labelled using and RNAscope Multiplex Fluorescent Reagent Kit (Bio-Techne).

Discussion & Conclusion

- This study provides the first comprehensive investigation of structural and cellular alterations with age in the mouse MTJ.
- High-resolution µCT and confocal imaging revealed that ageing leads to a reduction in muscle fibre diameter, pennation angle, and vascular volume at the muscle-tendon unit, alongside an expansion of the MTJ interface area.
- Cellular analysis demonstrated that vascular and MTJ-specific cells are particularly susceptible to senescence.
- These findings highlight that ageing drives structural remodelling and cell-type-specific senescence within the mouse MTJ, which may contribute to impaired tissue functionality and repair capacity.

Funding

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Results

- µCT image analysis shows significant decreases in muscle fibre diameter and pennation angle at the MTJ with age (Fig. 3b&d).
- The MTJ surface area, normalised to total surface area, showed a trend towards an age-related increase (Fig. 3c).

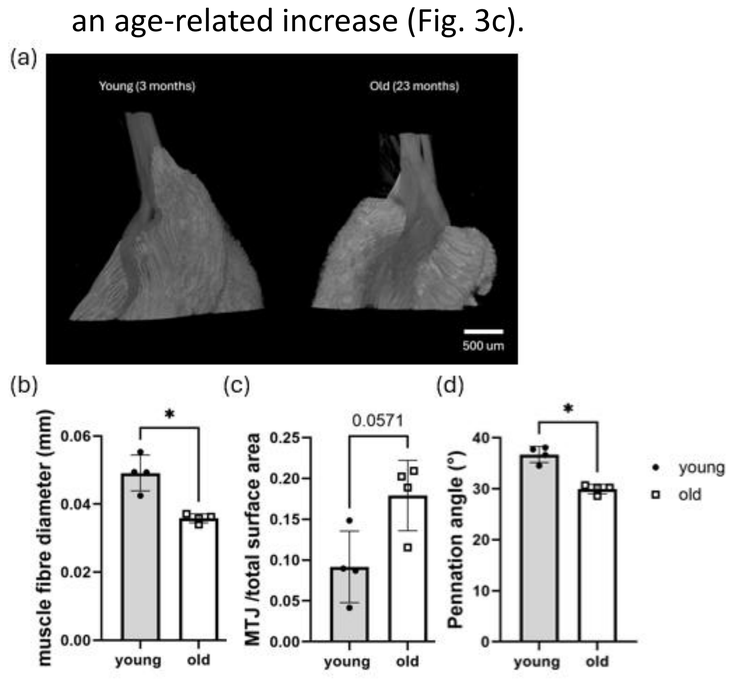


***Figure 3.** (a) Representative 3D reconstructed µCT images of young and old MTJ in longitudinal view. Scale bar is 500 µm. Quantitative analysis of µCT images showing (b) muscle fibre diameter, (c) MTJ surface area normalised to total surface area, and (d) pennation angle at the MTJ. Data are presented as mean ± SD from analysis of 4 young and 4 old mice. Mann-Whitney test was used to calculate the significance between young and old MTJs. *p<0.05*

- Immunolabeled 3D image analysis shows a significant decrease in muscle-tendon unit vascularity with age (Fig. 4b).
- LAMA2-labelled volume showed no significant change with age (Fig. 4c).

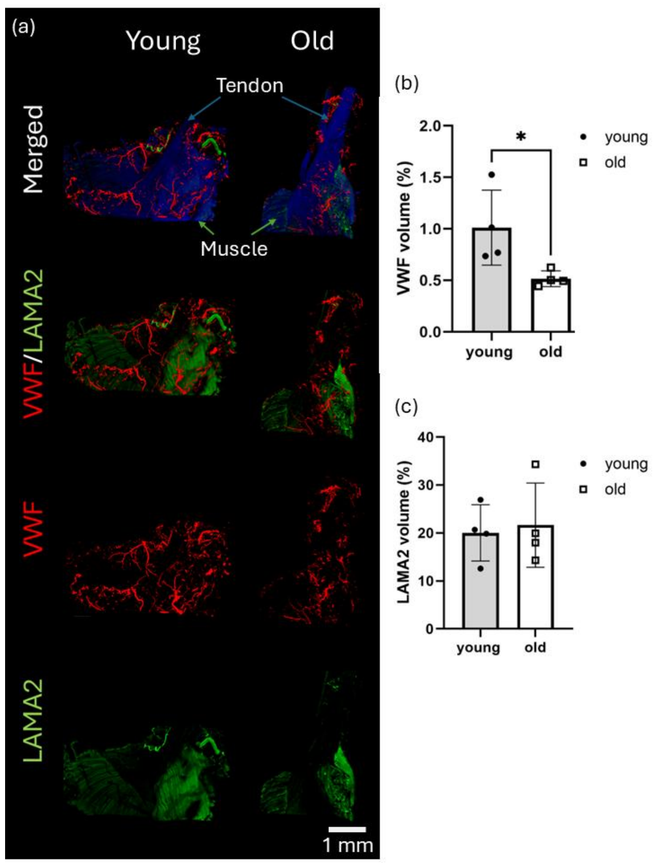


***Figure 4.** (a) Representative reconstructed 3D immunolabelled images of young and old mouse MTJ with surrounding muscle and tendon (n = 4 per age group) with endothelial cell marker, VWF (red), muscle marker, LAMA2 antibodies (green), and DAPI (blue). Scale bar is 1 mm. Immunolabelled volume of (b) VWF (endothelial cell marker) and (c) LAMA2 (skeletal muscle marker) in young and old MTJ normalised using DAPI labelled volume. Data are presented as mean ± SD (n = 4). A Mann-Whitney test was used to calculate the significance between young and old MTJs. *p<0.05.*

- In old MTJs, senescence markers p16 and p21 were predominantly co-localised with the MTJ marker Col22 and endothelial cell marker VWF (Fig. 5)

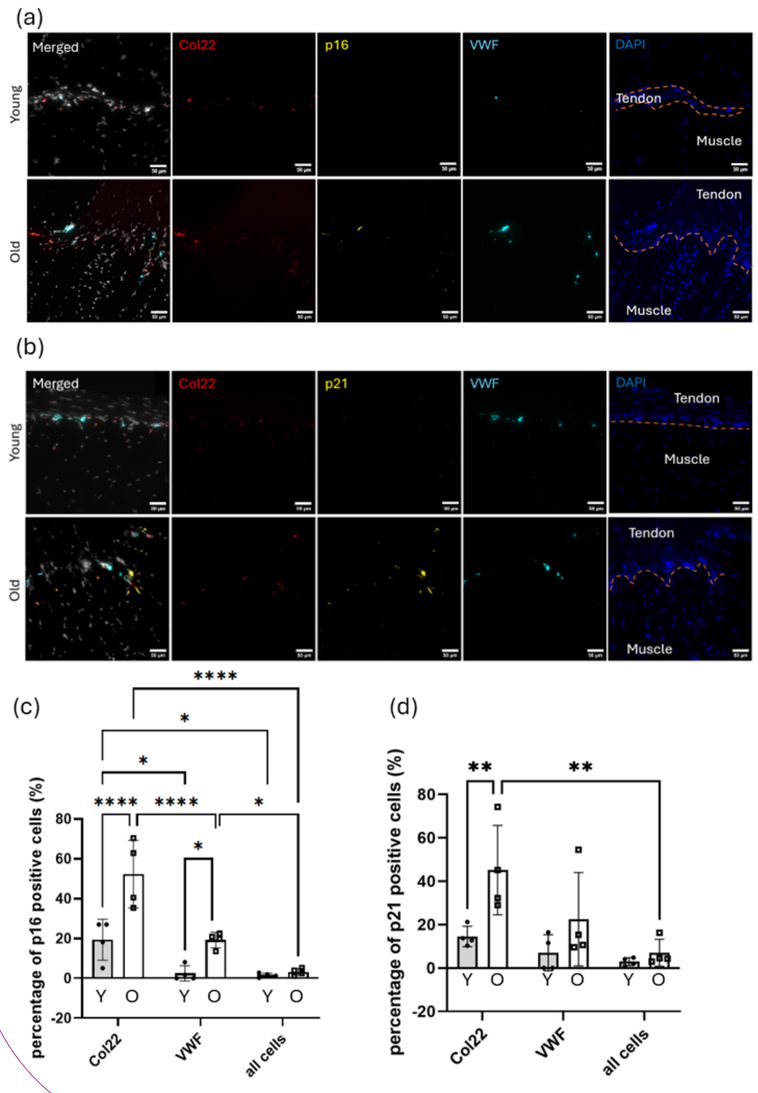


***Figure 5.** Representative in situ hybridisation images of young and old mouse MTJ (n = 4 per age group) showing senescence markers (a) p16 and (b) p21.Red: Col22 (MTJ marker); yellow: (a) p16 and (b) p21; cyan: VWF (endothelial cell marker); grey: DAPI. Blue DAPI image on the right shows the interface between the muscle and tendon indicated by orange dashed lines. Scale bar is 50 µm. Percentage of (c) p16 and (d) p21 positive cells were measured in Col22-positive MTJ-specific cells, VWF-positive endothelial cells, and all cells in the field of view. Y: young; O: old. Two-way ANOVA followed by Tukey's multiple comparisons test was used to calculate the significance between the age groups and cell populations. Data are presented as mean ± SD: *p<0.05 **p<0.01 ****p<0.0001*