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Contribution of oxidative stress to the degeneration of rotator cuff entheses

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Background: Rotator cuff degeneration is one of the multiple factors that lead to rotator cuff tears; however, the precise mechanism of such degeneration still remains unclear. In this study, we investigated the supraspinatus tendon enthesis to clarify the link between rotator cuff degeneration and oxidative stress in antioxidant enzyme superoxide dismutase 1 (*Sod1*)–deficient mice ($Sod1^{-/-}$).

Methods: The supraspinatus tendon and humeral head were isolated and fixed to prepare histologic sections from wild-type and $Sod1^{-/-}$ male mice at 20 weeks of age. Hematoxylin-eosin staining was performed to assess the histomorphologic structure. To investigate the collagen fibers, we examined spatially aligned collagen fibers using a polarizing microscope and assessed the amount of collagen using immunohistochemical staining. To analyze the tissue elasticity, we measured the tissue acoustic properties using scanning acoustic microscopy.

Results: The $Sod1^{-/-}$ mice showed histologic changes, such as a misaligned 4-layered structure and fragmented tidemark, in the enthesis. *Sod1* loss also decreased the amount of brightly diffracted light and type I collagen, indicating collagen downregulation. The scanning acoustic microscopy analysis showed that the speed and attenuation of sound were increased in the nonmineralized fibrocartilage of the $Sod1^{-/-}$ mice, suggesting decreased mechanical properties in the supraspinatus enthesis.

Conclusion: *Sod1* deficiency–induced degeneration is associated with impaired elasticity in the supraspinatus tendon enthesis, recapitulating human rotator cuff degeneration. These results suggest that intracellular oxidative stress contributes to the degeneration of rotator cuff entheses.

Level of evidence: Basic Science, Histology, Animal Model.

Keywords: Rotator cuff degeneration; enthesis; oxidative stress; *Sod1*; collagen fibers; scanning acoustic microscopy; mechanical property

Rotator cuff tear is a common orthopaedic disease, resulting in shoulder pain and dysfunction. However, the causative factors of the tear are still debated. Traditionally, extrinsic factors, such as acromion morphology, the presence of spurs, and shoulder overuse, contribute to the

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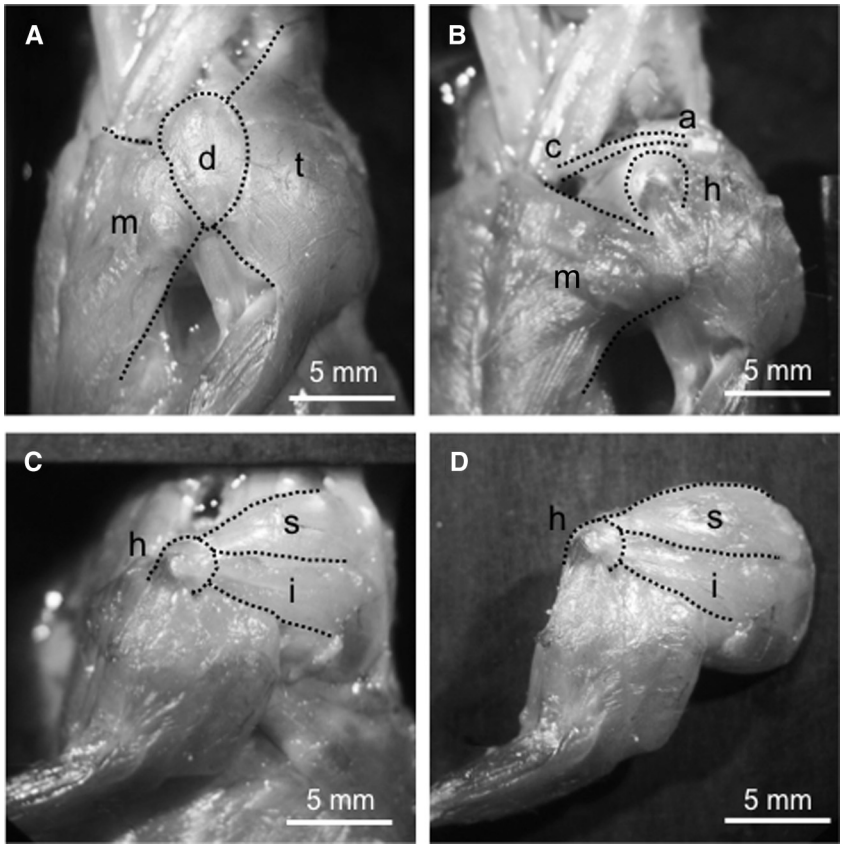

Figure 1 Preparation of supraspinatus enthesis. (**A**) After the skin and subcutaneous tissue of the upper body were removed, the pectoralis major (*m*), deltoid (*d*), and trapezius (*t*) muscles were observed. (**B**) After the deltoid and trapezius muscles were removed by use of microscopy, the clavicle (*c*), acromion (*a*), and humeral head (*h*) were identified. (**C**) After the clavicle and acromion were removed, the supraspinatus (*s*) and infraspinatus (*i*) muscles and tendons were observed. (**D**) The supraspinatus and infraspinatus muscles and tendons and the humeral head were removed in 1 lump.

tears.[6,11] On the other hand, epidemiologic studies have also proposed that intrinsic factors, including aging, inflammation, oxidative stress, and hypovascularity, are attributable to rotator cuff tears.[16,20] Rotator cuff degeneration is one of the characteristics of the tear and aging in rotator cuff entheses. Pathologic changes in rotator cuff degeneration have been reported to include thinning and disorientation of collagen fibers, as well as the loss of cellularity, vascularity, and fibrocartilage mass at the site of cuff insertion and so on.[4,11] However, the precise mechanism of age-related degeneration remains unclear.

We previously focused on the relationship between tissue degeneration and oxidative stress, which is known to accumulate in several organs with increasing age. Oxidative stress involves an imbalance between oxidation caused by reactive oxygen species and reduction elicited by antioxidant systems, leading to the initiation and progression of age-related diseases, such as diabetes, hypertension, atherosclerosis, osteoporosis, and neurodegenerative diseases.[8] In previous reports, we also showed that an antioxidant enzyme, superoxide dismutase 1 (*Sod1*), regulates the intracellular reduction-oxidation balance and its deficiency induces deleterious effects on skeletal tissue, such as low-turnover osteoporosis[12] and the exacerbation of unloading-induced bone loss.[7]

On the basis of this knowledge, we investigated the supraspinatus tendon enthesis in *Sod1*-deficient mice to elucidate the deleterious effects of oxidative stress on rotator cuff entheses.

Methods

Animals

Sod1-deficient mice ($Sod1^{-/-}$) were purchased from the Jackson Laboratory (Bar Harbor, ME). The $Sod1^{-/-}$ mice were backcrossed with C57BL/6NCrSlc mice (Nilson SLC, Shizuoka, Japan) 5 to 6 times. The mice were maintained and housed with 5 mice per cage with a 12-hour light-dark cycle and allowed free access to food and drinking water. The spontaneous activity of the male mice was measured in the cage using an activity-monitoring system (ACTIMO; Bio Research Center, Tokyo, Japan). The mice were studied according to protocols approved by the Animal Care Committee of the authors' institutions.