Enhancement of transforming growth factor beta 1 and type I collagen expressions in tenocytes through low-magnitude vertical vibration stimulation

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INTRODUCTION
Whole-body vibration training is widely used in rehabilitation and sports activities to improve muscle strength, balance and flexibility. Our previous study also found that vertical vibration (VV) treatment at frequencies of 8 to 10 Hz can stimulate the expression of ECM proteins and MRFs in myoblasts and, in turn, increase myotube formation (1). However, the effect of vertical vibration training and their molecular mechanisms on the tenocytes remain undefined. This study was undertaken to address the hypothesis that VV may act as an anabolic stimulus of tenocytes to enhance the expression of extracellular matrix (ECM) proteins and transforming growth factor beta 1 (TGF-β1), which may have the potential to improve tendon properties for accelerating tendon healing.

METHODS
Tenocytes were isolated from achilles tendon of Taiwan porcine with type I collagenase digestion for 24 hours at 37°C. Tenocytes were maintained in Dulbecco’s Modified Eagle Medium: Nutrient Mixture F-12 medium (DMEM-F12; Gibco BRL, Grand Island, NY, USA) containing 10% fetal bovine serum (FBS; Gibco BRL, Carlsbad, CA, USA) in a humidified atmosphere of 5% CO2 at 37°C. Cell viability was evaluated using MTT assay. Cell cycle profile was detected using flow cytometry. Using real-time PCR, ELISA and immunofluorescence studies, we examined the effect of VV treatment with frequencies of 5, 8 or 10 Hz on the expression of ECM proteins, type I collagen, as well as TGF-β1 in tenocytes.

RESULTS:
Our results showed that there was no significant difference in the cell viability between the VV-treated groups after 3 days (Fig. 1A). As shown in Fig. 1B, there was no significant difference in the cell cycle profile of VV-treated cells (5, 8 and 10 Hz) compared to control cells (0 Hz). TGF-β1 is an important determinant of the production of extracellular matrix and matrix deposition in healing tendons (2). We first investigated whether VV stimulation regulated the expression of TGF-β1 in tenocytes. Real-time PCR analysis showed that TGF-β1 gene expression increased in tenocytes in a dose-dependent manner with VV stimulation (Fig. 2A). We confirmed the expression of TGF-β1 in VV-stimulated tenocytes using ELISA analysis, and the results showed that the expression of TGF-β1 proteins was significantly increased in VV-treated groups (5, 8 and 10 Hz) in a dose-dependent manner (Fig. 2B). Type I collagen is the most abundant ECM in tendon structure. We next investigated whether VV stimulation regulated type I collagen expression in tenocytes using real-time PCR for up to 3 days. In the 5 Hz VV-treated group, gene expression of type I collagen was significantly increased at days 3 (Fig. 3). In the 8 Hz and 10 Hz VV-treated groups, gene expression of type I collagen was significantly increased in a dose-dependent manner at days 2 and day3. We confirmed the effect of VV treatment on the expression of collagen in tenocytes at days 3 with Sirius Red staining for total collagen determination and immunofluorescence analysis for type I collagen detection (Fig. 4). After VV treatment, Sirius Red staining (Fig. 4A) and quantitative results (Fig. 4B) revealed a significant increase in the total collagen expression in each VV-treated group (5, 8 and 10 Hz). Consistent with the real-time PCR results, type I collagen staining (Fig. 4C) and quantitative results (Fig. 4D) showed that the expression of type I collagen was significantly increased in VV-treated groups (5, 8 and 10 Hz) with the highest type I collagen expression in the 10 Hz VV-stimulated group.

DISCUSSION
In this study, we showed that low-magnitude VV stimulation is safe and effective at stimulating tenocyte-related gene such as type I collagen expression in tenocytes. TGF-β1 is known to be upregulated by exercise in intact tendons (3) and healing tendons (2), which also a potent upregulator of collagen expression (4). Our results support these and showed that VV-treated tenocytes increased the expression of TGF-β1 and type I collagen. According to this study, we suggest that VV can act as an anabolic stimulus of tenocytes to trigger the expression of type I collagen and TGF-β1 proteins, which may have the potential to improve tendon properties for accelerating tendon healing.

REFERENCE
1. C. Z. Wang et al., J Appl Physiol 109, 840 (Sep).

Fig. 1. Effect of VV stimulation on the viability and cell cycle profile of tenocytes. (A) MTT assay (B) Cell cycle analysis

Fig. 2. VV stimulation augments the expression of TGF-β1. (A) TGF-β1 mRNA. (B) TGF-β1 protein concentration.

Fig. 3. Effect of VV stimulation on type I collagen mRNA expression.

Fig. 4. Sirius Red staining (A) and quantitative results (B) of total collagen. Type I collagen staining (C) and quantitative results (D)