Effect of reduced Mechanical Stress on Development of the Femoral Head

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INTRODUCTION

Mechanical stress is essential for joint and skeletal development. It has been reported that the reduced mechanical stress on bones and joints in microgravity environments leads to osteoporosis. Previous studies have been focused on mice with completed bone formation. In recent years, decreased opportunities for exercise have become an issue, especially among young people. Physical exercise is necessary for healthy joint formation. As represented by developmental dysplasia of the hip (DDH), joint dysplasia in children and young people is a risk factor for future joint diseases such as osteoarthritis. The process of skeletal development during development involves bone formation by endochondral ossification. In endochondral ossification, bone formation occurs by hypertrophy of chondrocytes. Mechanical stress also plays an important role in this process. In fact, not only genetic factors but also mechanical stresses applied to joints, such as postnatal lifestyle, are associated with the development of DDH. However, previous studies have focused on adults, and the effects of decreased mechanical stress on the developing hip joint are still unknown. Therefore, the purpose of this study was to clarify the effect of mechanical stress reduction on the development of the femoral head, which is a component of the hip joint.

METHODS

This study was approved by the Ethics Committee of Saitama Prefectural University and strictly adhered to the on-campus animal experiment guidelines (approval number: 2022-1). This study used 4-week-old female mice (C57BL/6, n = 15). We reproduced the reduced mechanical stress by hindlimb suspension. We divided mice into three groups: the Short-Term Hind limb Unloading group (STHU; n=5) for 4 weeks of hindlimb suspension, the Long-Term Hind limb Unloading group (LTHU; n=5) for 8 weeks of hindlimb suspension, and the Sham group (n=5). The time point for tissue collection was set at 16 weeks old. The STHU group had 4 weeks of hindlimb suspension followed by 8 weeks of cage activity period, and the LTHU group had 8 weeks of hindlimb suspension followed by 4 weeks of cage activity period (Fig,1A). At 16 weeks old, we collected the right and left hip joints. The right hip joint performed bone morphological analysis by Micro-CT, and the left hip joint performed histological analysis. To analyze the effect of decreased mechanical stress on skeletal development, the hip joint was scanned with a micro-CT (Sky Scan 1272, Bruker, Massachusetts, US). Settings were as follows: pixel size 7.5 µm, voltage 60 kV, current 165 µA, frame averaging 3, filter AI 0.25 mm. We analyzed bone volume/tissue volume fraction (BV/TV) for the femoral head using 3D data analysis software (CT Analyzer; Sky Scan). For histological analysis, the collected left hip joint was embedded in paraffin, and sections were prepared. Subsequently, we performed safranin-O staining and fast green staining. Immunofluorescent staining with collagen type 2 (Col 2) and collagen type X (Col X) was performed using the avidin-biotinylated enzyme complex method. Then, we calculated the staining intensity of Col 2 and the positive cell ratio for Col X in the femoral head. In addition, we performed TRAP staining to evaluate osteoclast activity. The osteoclast surface (Oc.S/BS, %) of the femoral head was then calculated as an analysis of osteoclast activity. All data were statistically analyzed using RStudio version 1.2.5019 and compared among the three groups using Tukey's post hoc test.

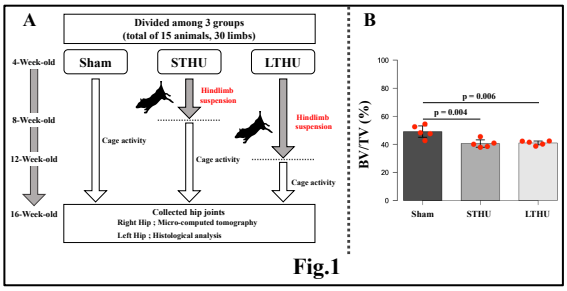

Fig.1

RESULTS SECTION

Micro-CT analysis, the BV/TV in the STHU and LTHU groups were significantly lower than that in the Sham group (Sham vs STHU, p = 0.004; Sham vs LTHU, p = 0.006) (Fig,1B). The staining intensity of Col 2 in the STHU and LTHU groups was significantly higher than that in the Sham group (Sham vs STHU, p = 0.012; Sham vs. LTHU, p = 0.007) (Fig,2B). Col X positive cell ratio in the STHU and LTHU groups was significantly lower than that in the Sham group (Sham vs STHU, p = 0.009; Sham vs LTHU, p = 0.014) (Fig,2C). There was no significant difference in Oc.S/BS at the femoral head (Fig,2D).

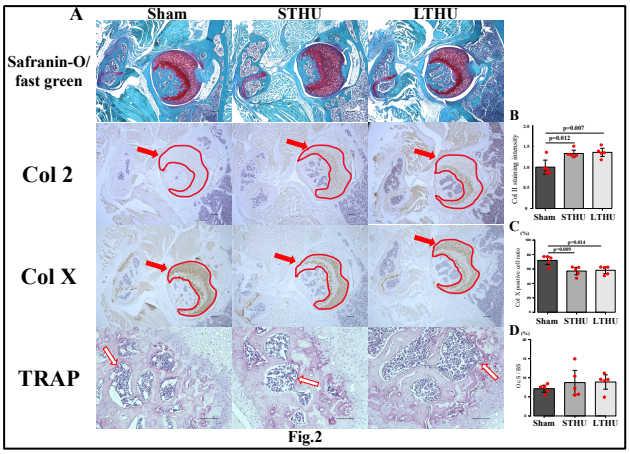

Fig.2

DISCUSSION

This study examined the effects of decreased mechanical stress during development on skeletal development. In developmental stages, bone modeling occurs, in which epiphyseal cartilage is replaced by bone. Previous studies in adults have shown that hindlimb suspension decreases BV/TV. This study evaluated the osteogenic process of the femoral head of the hip, which is most subjected to mechanical stress due to weight bearing. As a result, we found that the BV/TV of the femoral head decreased. To further reveal this mechanism, we evaluated the hypertrophy of chondrocytes by immunohistochemical staining of Col 2 and Col X, and osteoclast activity by TRAP staining. The results showed that the staining intensity of Col2 was higher and the positive cell rate of Col X, a marker of hypertrophic chondrocytes, was lower in the STHU and LTHU groups that underwent hindlimb suspension. These results suggest that decreased mechanical stress suppresses endochondral ossification due to hypertrophy of chondrocytes. In addition, there was no change in osteoclast activity. Previous reports have suggested that osteoclast activation is responsible for the decrease in BV/TV in adults. These results suggest that decreased mechanical stress during development may prolong the duration of bone maturation, and could be influenced by external factors such as lifestyle. Furthermore, there were no significant differences between the STHU and LTHU groups in all analyses. This indicates that the reduction of mechanical stress during development may have short-term but also long-term effects.

SIGNIFICANCE/CLINICAL RELEVANCE

This study found that a decrease in mechanical stress during development inhibits chondrocyte hypertrophy and delays endochondral ossification. Furthermore, the results suggest that the effects of delayed endochondral ossification during development may continue for a long time.