Transcutaneous CO_2 application suppresses disuse osteoporosis and muscle atrophy in a rat hindlimb suspension model

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INTRODUCTION:
Transcutaneous CO_2 application promotes fracture healing and osteogenesis via angiogenesis [1]. In the present study, we utilized a rat hindlimb suspension model to investigate the preventive effect of this treatment on disuse osteoporosis and muscle atrophy.

METHODS:
Twenty-one 11-week-old male Sprague-Dawley rats were randomly divided into the hindlimb suspension (HS), hindlimb suspension with transcutaneous CO_2 application ($HSCO_2$), and control groups. In the HS group, the rats were suspended using their tails to maintain a 30-degree head-down tilt, so that the hind limbs did not touch the ground [2]. In the $HSCO_2$ group, hindlimbs were similarly suspended but with CO_2 application i.e., a hydrogel facilitating CO_2 absorption was applied to both hindlimbs and sealed in a polyethylene bag filled with 100% CO_2 gas for 20 min, five times a week. After three weeks, the femurs, tibias, and gastrocnemius muscles were harvested. Radiographic, histological, and biomechanical assessments of the harvested samples were performed.

RESULTS:
No significant difference in body weight was observed among all groups on day 0, but body weight significantly decreased in the HS and $HSCO_2$ groups on day 21 in comparison to the control group (Fig.1). Microcomputed tomography demonstrated that both the trabecular (Fig.2a,b) and cortical (Fig.2a,c) bones of the femurs in the HS group showed significant bone atrophy, while improvement was noted in the $HSCO_2$ group. The muscle weights of the gastrocnemius in the HS and $HSCO_2$ groups were significantly decreased compared to those in the control group but were not significantly different between these two groups (Fig.3b). The myofiber cross-sectional area in hematoxylin and eosin (H&E) staining decreased in the HS and $HSCO_2$ groups compared to the control group and increased in the $HSCO_2$ group compared to the HS group (Fig.3a,c). Histological analysis of the proximal tibia by H&E staining showed relatively more adipocytes in the HS and $HSCO_2$ groups than in the control group, and fewer adipocytes in the $HSCO_2$ group than in the HS group (Fig.3d,e). TRAP staining showed decreased osteoclast surfaces and numbers in the $HSCO_2$ group compared to the other groups (Fig.3f,g). ALP staining in the $HSCO_2$ group showed significantly increased osteoblast surfaces and numbers compared with those in the HS group (Fig.3h,i). Immunohistochemistry for VEGF and PGC-1α revealed that larger areas were positively stained in the $HSCO_2$ group than in the HS group (Fig.3j-m). The biomechanical maximum load in the three-point bending test of the femoral midshafts showed no significant difference; however, those in the compression test of the distal femur and femoral neck were significantly larger in the $HSCO_2$ group than in the HS group.

DISCUSSION:
PGC-1α is known as a critical switch of cell fate decisions of skeletal stem cells, and promotes osteoblastic differentiation while suppressing adipogenic differentiation [3]. In the present study, transcutaneous CO_2 application suppressed disuse osteoporosis by decreasing marrow adipose tissue and increasing the number of osteoblasts. In conjunction with immunohistochemistry, the data suggests that PGC-1α acts as a key player for osteogenesis via angiogenesis.

SIGNIFICANCE:
Transcutaneous CO_2 application was effective in preventing disuse osteoporosis and muscle atrophy in a rat hind limb suspension model.

REFERENCES: [1] Koga T, et al. Journal of Bone and Joint Surgery. 2014. [2] Morey-Holton ER, et al. Bone. 1998. [3] Yu B, et al. Cell Stem Cell. 2018.

Fig.1

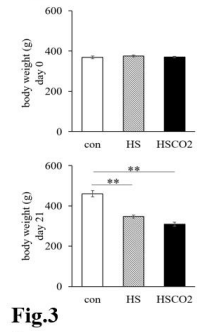

Fig.2

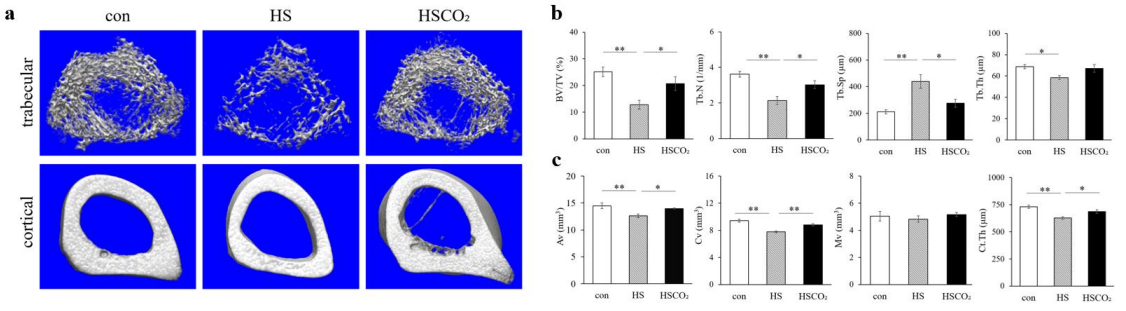

Fig.3