The Effect of Mechanical Unloading on the Osteogenic Differentiation Potential of the Bone Marrow Cells

INTRODUCTION
Mechanical loading plays an important role in bone homeostasis. Although osteoblasts seem to be the principal mediator of osteopenia in hindlimb unloading through tail suspension model (Morey-Holton & Globus, 1998), the proliferation and/or differentiation potential of the osteoprogenitors in response to unloading is still unclear. The present study was designed to investigate the effects of hindlimb unloading for 9 days on (i) the rat tibial trabecular bone architecture, (ii) the colony formation and differentiation potential of bone marrow cells from the femurs and (iii) the growth and differentiation potential of the bone marrow-derived mesenchymal stem cells (BM-MSCs).

MATERIALS & METHODS
Animal experimental design
Four-months old female Wistar rats were randomized to 2 groups of 5 animals to be either tail-suspended for 9 days (HU) or to act as controls (CTL) (Holy et al., 1996). All experiments were conducted according to the institutional guidelines for animal welfare.

Bone architecture evaluation
Bone volume and structure changes in the proximal tibia in response to 9 days of unloading were quantified by micro-X-ray computed tomography (Skyscan 1172, Aartselaar, Belgium). µCT images were made within the mid sagittal planes in the metaphyseal region of the bones. The trabecular volume of interest (VOI) in the axial direction was delineated by a reference point 1 mm distally from the growth plate over a length of 3.38 mm (= 200 slices). For each transverse slice, the VOI was established manually in the area of trabecular bone.

Growth and differentiation potential BM-MSCs
The bone marrow cells were then cultured, MSCs at passage 1 replated and cultured for 4 passages for growth (population doubling time) and differentiation potential: ALP staining and optical density (OD) measurement of Alizarin Red and OD measurement of Oil Red O staining for osteogenic and adipogenic differentiation respectively.

Statistical analysis
Data were analyzed with a Student’s t-test and a two-way analysis of variance (ANOVA) to assess the statistical significance of comparisons between both groups.

RESULTS
Effect of HU on colony formation and differentiation potential of bone marrow cells
Mechanical unloading significantly affected the number of fibroblastic progenitors and the number of osteo- and adipoprogenitors (Table 2).

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Table 2. Number of colonies/10 cm² (mean ± SEM) after 14 days of culture of bone marrow cells of CTL and HU rats in standard medium, and after 21 days of culture in osteogenic or adipogenic medium. Values with the same mark are significantly different from each other (Student’s t-test, p<0.05).

The results of the expressions of Cbfa1 and OC mRNAs in induced cells are shown in Fig. 1. The expression of the two osteogenic gene markers tested increased with increasing time of osteogenic induction for both CTL and HU condition. The expression of OC mRNA in cells induced from bone marrow cells was significantly lower for HU compared to CTL rats.

DISCUSSION
In this study, a multi-scale approach including µCT, cellular and molecular analysis was adopted. The µCT technique validated our model of osteopenia. Absence of mechanical loading by gravity for 9 days resulted in alterations in the skeletal microarchitecture. There was a tendency towards a lower percent bone volume for HU compared with CTL, which is in line with reports in the literature (David et al., 2003).

Exploring the underlying mechanism of these bone changes was aimed for in the in vitro osteoprogenitor model. A striking decrease in the number of fibroblastic progenitors in response to 9 days of unloading was observed, while their adipogenic and osteogenic commitment potential was preserved. The expression of OC mRNA was significantly decreased in osteoprogenitor cells isolated from unloaded bone marrow. The decreased growth and differentiation potential of the cultured BM-MSCs support our findings that skeletal unloading affects the osteogenic differentiation potential of the bone marrow cells.

REFERENCES