INTRODUCTION: Axial micromotion of bone fragments enhances callus formation during fracture repair or limb lengthening [1]. The purpose of this study is to show that compression force provided by axial shortening is converted into hypoxic stimuli caused by collapse of vascular lumens, which directly enhances membranous bone formation via the induction of Hypoxia inducible factor (HIF)-1α-mediated Vascular endothelial growth factor (VEGF) production.

METHODS: In 18 Japanese white rabbits, after 10 mm lengthening of the left tibia with an external fixator (Orthofix M-100, Verona, Italy), the callus was shortened by 2 mm. Anteroposterior radiographs of the five tibiae in the control and shortened group were taken just after harvesting for radiographic observation. The bone mineral density (BMD) of the callus was measured using dual-energy X-ray absorptiometry (model QDR-1000, ultra-high-resolution mode, Hologic, Bedford, MA). Four harvested tibiae of each control and shortened group were prepared for histology. HE, safranin O, Mallory-azan and TRAP staining were performed. Furthermore, immunohistochemical staining was performed for VEGF, its receptors (VEGFR1 and 2), CD31, proliferating cell nuclear antigen (PCNA), and HIF-1α. The percentage area of vascular lumens was calculated as a ratio of pixel numbers enclosed by CD31-positive cells to the total pixel numbers in the field, using image analysis software. VEGF protein concentrations and alkaline phosphatase (ALP) activities in the tibial callus (n = 4 in each group) were measured using commercially available kits, respectively.

RESULTS: Radiographs and quantitative evaluation of corrected bone mineral density showed a significant increase in mineralization in the shortened callus (57.3% versus 36.2%, p = 0.001, Figure 1). Histologically, greater osteoblast proliferation and more vigorous trabecular bone formation were noted in the shortened calluses than in the controls. In the front of membranous bone formation in the shortened callus, there was a significant decrease in mean percentage area of vascular lumens (1.8% versus 4.5%, p = 0.009), which seemed attributable to compressive force by axial shortening. Production of VEGF protein was significantly increased (422.5 ± 83.2 versus 142.7 ± 113.0 pg/mg protein, p = 0.007). Mean ALP activity was also 2.5 times higher in the shortened group (224.6 ± 32.1) than that in the controls (80.0 ± 6.12 K-AU/mg protein) (n = 4, p = 0.001). There were also increased numbers of TRAP-positive osteoclasts and PCNA-positive cells in the front of membranous bone formation in the shortened group. A marked increase of VEGF, its receptors and HIF-1α expression in osteoblasts was also observed in this area of the shortened group.

DISCUSSION: There is a clear relationship between increased VEGF production and enhancement of bone formation [2,3,4]. However, mean proportion of vascular lumens in the front of membranous bone formation in the shortened group was significantly smaller than in that of the controls. The fact that both decrease in vascularity and strong immuno-reactivity for HIF-1α were observed may imply that vascular lumens collapsed under the compression force by axial shortening, resulting in the high expression of HIF-1α via local hypoxia. In addition to the roles of VEGF in new bone formation through angiogenesis, VEGF has been shown to have the direct effects on new bone formation in vitro and in vivo, including enhancement of differentiation, migration and ALP activity of osteoblasts [5,6]. In the shortened group, these direct actions of VEGF on bone formation may play an important role after starting the axial shortening. Enhancement of membranous bone formation by static compression or axial dynamization may be at least partly attributable to HIF-1α-mediated VEGF induction following the local hypoxia caused by collapse of vascular lumens.

REFERENCES