ABSTRACT INTRODUCTION:
In distraction osteogenesis, the new bone formation seems to be contrast to Wolff’s law that bone forms under compression. In our previous studies (1,2,3), we have also shown the active biological responses in newly formed osseous tissue during distraction. We, therefore, hypothesize that continuous distraction may create micro-strain across the distraction site and hence whole system is more sensitive to compression stimulation (weight bearing). Neo-osteogenesis and mineralization will then be initiated. This study is to investigate the effect of weight bearing on the regeneration activities in the newly formed osseous tissue in large animal model – goat. The results were compared with those animals without weight bearing during the distraction.

METHODS:
In this study, The protocols were approved by the Animal Ethics Committee, CUHK. 24 Skeletal immature goats (26.7 ± 2.6 Kg body weight) were undergone standardized distraction procedures. Postoperatively, 12 animals were assigned to the weight bearing (WB) group and 12 to the non weight bearing (NWB) group. The animals rested for 7 days before the distraction with rate at 1 mm/day and 6 days/week. The distraction was carried out for four weeks and kept for five more weeks for consolidation. Adequate analgesic was given i.m. every 6 hours for 6 days to ensure animal can weight bear on the operated limb. For the weight bearing group (n = 12), the animals were allowed immediate free walking after surgical treatment. For the non-weight bearing group (n = 12), the animals were held in a special cage (Fig. 1), which allowed the left tibia suspending in the air.

Venous blood sample was taken weekly for the measurement of the bone specific alkaline phosphatase (ALP). Weekly radiograph was also taken to monitor distraction. The radiographs were digitized by PACE System (diagnostX 2048, Germany) and analyzed by Image Analysis System (Universal Imaging Corporation, USA) to measure callus width and mineralized tissue. The animals in each group were euthanized at the end of the 2nd, 5th and 9th week to obtain the tissue for histological and immunohistochemical (TGFb1) studies. Immunohistochemical results were evaluated by image analysis. Student’s t-test were used to compare the means of different groups with p<0.05 regarding as significant difference.

RESULTS:
All the animals completed the distraction procedures. For radiographic analysis, the callus width of WB group was significantly larger than that of NWB group at week 2 and 5 (p = 0.028 and 0.041 respectively). Significantly more mineralized tissue in WB group was also found for all time points, with mineralized area in WB group being 326.3 ± 57.5 mm² while that in NWB group 101.7 ± 66.4 mm². For ALP measurement, the activity in WB group was much increased throughout the process while that in NWB group showed initial drop and gradual increase during distraction phase. The difference in ALP activity was statistically significant in distraction phase.

For histological study, the newly formed woven bone in WB group was thick and interconnected while that in NWB group was thin and aligned with direction of distraction force (Fig. 2). The percentage of woven bone formed 2mm away from the bone formation front in WB group was 63.0 ± 10.3% and that in NWB group was 43.5 ± 9.8% (p<0.05).

For immunohistochemistry, the expression of the TGFb1 in the newly formed osseous tissue was obviously higher in WB group than that in NWB group at week 2 and 5 (p = 0.008 and <0.001 respectively).

DISCUSSION:
In the present study, we have shown that the compressive force generated by weight bearing walking enhances bone formation both quantitatively and qualitatively during distraction osteogenesis. This strongly supports our hypothesis. The increase of ALP activity and TGFb1 expression were caused by the additive effects of continuous distraction and the weight bearing stimulation. With the aid of ALP and TGFb1, the amount of bone formation with weight bearing was also much larger, as assessed by callus width and degree of mineralization. Histologically, less interconnected woven bone in non-weight bearing group also implies that compression stress has positive effect on bone formation and the architecture of the new bone in distraction osteogenesis. Less difference in consolidation phase was observed because the effect of weight bearing is less marked without distraction force. The newly formed bone is less sensitive to the changes of strain during mineralization.

REFERENCES:

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