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Mechanical Load Modulates the Stimulatory Effect of BMP2 in a Rat Nonunion Model

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Introduction: Local application of bone morphogenetic proteins (BMPs) at the fracture site is known to stimulate bone regeneration. However, recent studies illustrate that the BMP-initiated mineralization may be enhanced by additional mechanical stimulation. Therefore, bone healing was monitored *in vivo* in order to investigate the effect of mechanical loading on the initiation and maturation of mineralization after cytokine treatment. We hypothesized that the mechanical stimulation would further enhance the efficacy of BMP2 treatment.
***Method*:** Female Sprague-Dawley rats underwent a 5-mm defect, stabilized with an external fixator. Type I collagen scaffolds containing 50 μg of BMP2 diluted in a solvent or solvent only were placed into the defects. The BMP2-treated specimens and control specimens were then each divided into two groups: one that underwent mechanical loading and a nonloaded group. *In vivo* loading began immediately after surgery and continued once per week for the entire 6-week experimental period. For all groups, the newly formed callus tissue was quantitatively evaluated first by *in vivo* microcomputed tomography at 2, 4, and 6 weeks and further by histologic or histomorphometric analysis at 6 weeks postoperation.
Results: Mechanical stimulation with BMP2 treatment significantly enhanced mineralized tissue volume and mineral content at 2 weeks. Histological analysis demonstrated a significantly greater area of fibrous connective tissue including bone marrow in the stimulated group, suggesting reconstitution of the endosteal canal and more advanced bone remodeling present in the mechanical loaded group. Both groups receiving BMP2 underwent massive bone formation, achieving bony bridging after only 2 weeks, while both control groups, receiving solvent only, revealed a persisting nonunion, filled with fibrous connective tissue, prolapsed muscle tissue, and a sealed medullary canal at week 6.
Conclusion: Mechanical loading further enhanced the efficacy of BMP2 application evidenced by increased mineralized tissue volume and mineralization at the stage of bony callus bridging. These data suggest that already a minimal amount of mechanical stimulation through load bearing or exercise may be a promising adjunct stimulus to enhance the efficacy of cytokine treatment in segmental defects. Further studies are required to elucidate the mechanistic interplay between mechanical and biological stimuli.

Introduction

Segmental bone defects resulting from high-energy trauma, bone tumors, and revision surgery[1,2] represent a challenge for regeneration and current surgical and grafting techniques. Complications such as delayed healing, nonunions, or resulting limb length differences can lead to a significant reduction in the patient's quality of life.[3,4] Due to limitations associated with current treatment strategies, alternative approaches have been investigated, including the development of bone graft substitutes or osteobiologics,[5] which incorporate osteoconductive matrices and osteoinductive proteins. Bone morphogenetic proteins (BMPs), members of the transforming growth factor-beta superfamily that are well known to be osteo- and chondroinductive,[6] are commercially available for clinical use, but their application is restricted to limited applications.[7] Local application of BMPs has been extensively studied in animal models of bone healing to examine their regenerative capacity. However, promising experimental results have only been partly recapitulated in human patients.[8] Furthermore, supraphysiological dosages[9,10] of BMP2 and BMP7 are frequently required to reach effective bone formation and still with inconsistent results.[11]

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It is clear that endogenous BMP2 plays a significant role during bone healing, as several studies have demonstrated the expression of BMPs and their inhibitors during normal and compromised fracture healing in animal models.[12–16] As well, it is widely accepted that mechanical loading through controlled *in vivo* axial compressive external loading[17–19] or dynamization through reduced fixation stiffness to allow more interfragmentary movement during locomotion[20,21] can influence the healing process. Studies on small bone defects in rats that heal uneventfully have shown that early dynamization, reducing external fixation stiffness at 1 week postosteotomy, impairs healing, whereas late dynamization 3 or 4 weeks postosteotomy enhances healing.[20,22] Additionally, *in vitro*[23–25] and *in vivo*[26–30] studies have shown that endogenous BMP2 expression is influenced by mechanical loading.

Only few studies have investigated the role of mechanical loading in combination with exogenous BMP2 application on critical-sized segmental defect healing,[31–34] but with varying results. Boerckel *et al.*[31] recently demonstrated that early dynamization through reduction in fixation plate stiffness significantly inhibited vascular invasion into the defect and reduced bone formation in comparison to a constant stiff fixation plate with a low BMP2 dose. In contrast, Glatt *et al.*[34] suggested that early loading of the mechanical environment by varying external fixation stiffness after local application of BMP2 enhanced structural parameters of rat femoral critical-sized bone defects.

The use of mechanical loading in terms of weight bearing or exercise may be a promising therapy to augment BMP2 treatment of segmental bone defects. However, the combined effect of both mechanical and biological stimuli requires further study, and dosages of biologics such as BMP could eventually be minimized. Therefore, the aim of the present study was to investigate if mechanical loading would improve the efficacy of an osteoinductive growth factor locally applied in a rat femoral large segmental bone defect model over a 6-week time course. We hypothesized that weekly controlled *in vivo* axial compressive mechanical loading would further enhance the efficacy of BMP2 treatment.

Materials and Methods

Operative procedure and experimental design

Thirty-two female 12-week-old Sprague-Dawley rats (weight 250–300 g; Charles River) underwent diaphyseal femoral osteotomies of the left limb, resulting in a 5-mm critical defect. The operative procedure has been previously reported[35]; in brief, rats were administered ketamine hydrochloride (60 mg/kg, Ketamin 50 mg, Actavis®; München-Riem) and medetomidine (0.3 mg/kg; Domitor®; Pfizer), and also the antibiotic clindamycin-2-dihydrogenphosphate (45 mg/kg; Ratiopharm). An incision was made across the lateral aspect of the thigh, through the fascia, exposing the femur by separating the gluteus superficialis and biceps femoris muscles. The external fixator was attached, allowing a 7.5-mm offset. A 5-mm defect was created in the diaphysis of the femur using an oscillating saw by performing a double-transverse osteotomy. Rats were locally treated at the osteotomy site with either 50 μL of recombinant human BMP2 (rhBMP2) (1 mg/mL; Prof. Sebald) diluted in an aqueous low-concentrated hydrochloric acid (10 mM) or 50 μL aqueous low-concentrated hydrochloric acid (10 mM) only, with a collagen sponge (Lyostypt®; B. Braun) serving as a carrier. Animals tolerated the experimental procedure well; however, some animals developed pin infections and were excluded from the study, leaving the following groups: (1) collagen sponge with solvent (Control, $n=4$), (2) collagen sponge with solvent + mechanical loading (Control-load, $n=4$), (3) collagen sponge with rhBMP2 (BMP2, $n=7$), and (4) collagen sponge with rhBMP2 + mechanical loading (BMP2-load, $n=8$). Following surgery, the rats were housed two per cage and allowed to resume normal activity and given unrestricted access to food and water. All animals were sacrificed 6 weeks after surgery. All animal experiments were carried out according to the policies and procedures established by the NIH Guide for Care and Use of Laboratory Animals. The study was approved by the local legal representative (LAGeSo Berlin, G0071/07 and G0210/08).

Fixator design and characterization

Two designs of external unilateral fixators were used to stabilize the defect, depending on whether the rats would undergo *in vivo* mechanical stimulation or not. In non-mechanically loaded groups, the defect fragments were stabilized with a fixator that contained two carbon fiber-reinforced polysulfon plates bound by two countersunk screws and additionally secured by two locking nuts (Fig. 1C1).[35] A second fixator design allowed axial loading of the healing zone in groups intended for mechanical stimulation (Fig. 1C2).[36] The fixator design allowed disassembly such that the fracture pins remained within the frame, but the defect bridging bar could be detached to allow mechanical loading. In all fixators four titanium threaded (0.65-mm core diameter/1.2-mm outer diameter) pinsfixed to the crossbar by thread tightening through compression of the fixator ensured fragment fixation. The offset distance, the free length of the pins between the rat's lateral femoral surface and the inner side of the fixator bar, was always 7.5 mm. To ensure comparability of fixator stiffness values for the two designs, the mechanical competence of all fixators was determined nondestructively *in vitro*. Both fixator designs were mounted to harvested cadaveric femurs of female 12-week-old Sprague Dawley rats ($n=6$/fixator design) to undergo axial compression and torsional testing using published protocols.[36] The fixator design of the mechanically stimulated group resulted in an axial stiffness of 62.02 ± 13.52 N/mm (mean ± standard deviation) and a torsional stiffness of 15.35 ± 2.69 N/mm, and the fixator design of the unstimulated group produced an axial stiffness of 59.05 ± 14.45 N/mm and a torsional stiffness of 14.57 ± 2.03 N/mm.

In vivo *mechanical loading*

After surgery and at weekly intervals thereafter, animals from the Control-load and BMP2-load groups were subjected to an *in vivo* biomechanical loading. All animals received intraperitoneal injection of anesthesia, regardless if they were subscribed to the loaded or nonloaded groups. For the mechanical loading, the external fixator was constrained to a custom-made setup, containing a precision linear actuator (M-230; Physik Instrumente) controlled by a Labview script (LabVIEW 8.5; National Instruments).[36] While the proximal

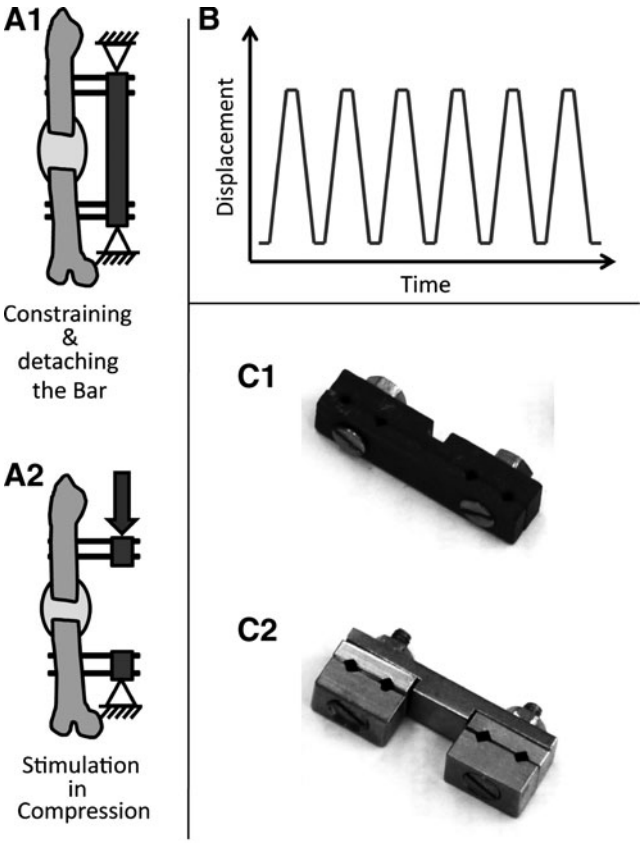

FIG. 1. Schematic representation of **(A1)** constraining the external fixator within the custom-made setup and after the **(A2)** detachment of the fixator bar before the loading was started. **(B)** The trapezoidal waveform used for mechanical loading. **(C1)** Photo of the fixator used on the non-mechanically loaded groups and **(C2)** on the groups that underwent mechanical loading.

fixator site was clamped, the axial opposed fixator site was attached to the linear actuator. The load-bearing fixator bar was then detached, thereby permitting only an axial deformation. The loading protocol consisted of six compression cycles, where each compression included 500-μm displacement at a constant rate of 10 μm/s, followed by a dwell (resting phase) of 40 s, where the compression was kept constant. Then the actuator was removed back to the initial position, where it rested again for another 40 s.

Micro-computed tomography

At 2, 4, and 6 weeks postoperation, bone healing was assessed by *in vivo* microcomputed tomography (microCT) (vivaCT 40; Scanco Medical; 55 kVp, 145 μA, 150 ms integration time) at an isotropic resolution of 35 μm. Analysis was performed using a semi-automated segmentation of cross-sectional tomograms to derive the volume of interest (VOI), defined by the periosteal callus as the outer boundary and the endosteal callus as the inner boundary, excluding the cortical bone. The total callus VOI included the 5-mm defect region and 0.5 mm in the proximal and distal directions from the borders of the original osteotomy. A global threshold of 50% of the mineral density of the intact limb, equivalent to 351 mg HA/ccm, was used to distinguish mineralized tissue (bone and calcified cartilage [BV]) from poorly mineralized and unmineralized tissue. Outcome measures included mineralized callus volume, which includes BV (mm^3), total callus volume (TV, mm^3), tissue mineral density (TMD, mg HA/ccm), and tissue mineral content (TMC, mg HA), defined as BV multiplied by TMD, with TMD measured using only voxels whose intensity exceeded the threshold. *In vivo* microCT-derived results for the BMP2-load and Control-load groups were previously published to show establishment of the *in vivo* mechanical loading system,[36] whereas in the current study the data are used to demonstrate the influence of loading on tissue formation compared to novel nonloaded BMP2-treated and nontreated groups after a healing time course of 6 weeks.

Histology and histomorphometry

After 6 weeks postsurgery, rats were anaesthetized and sacrificed by an intracardial potassium-chloride injection. Femora were harvested and fixed directly in formaldehyde for 48 h and subsequently decalcified in ethylenediaminetetraacetic acid for approximately 4 weeks at 37°C. Fixed and decalcified tissues were dehydrated in graded ethanol up to 100%, transferred to xylene, and embedded in paraffin. Four-micrometer-thick longitudinal sections were prepared on a customary microtome (Leica RM 2125) and placed on glass slides. Quantitative histomorphometry was performed to analyze tissue differentiation for a single fixed region of interest (ROI), using semiautomated software (KS400 3.0 software; Carl Zeiss MicroImaging GmbH). The composition of the callus tissue was quantified after staining with Movat Pentachrome by measuring the area occupied within the osteotomy gap by bone (yellow), cartilage (blue to green), and fibrous connective tissue formation (pink to purple), with the fibrous connective tissue also including bone marrow elements. Tissue areas (mm^2) were measured and tissue fractions (%) calculated based on the ROI. Qualitative analysis of remodeling of the newly formed bone was also performed by staining sections with Picrosirius red and analyzed with polarized light microscopy. Additionally, the callus width was calculated by ImageJ (Rasband, W.S., ImageJ, U. S. National Institutes of Health; http://imagej.nih.gov/ij/, 1997–2011).

Statistical analysis

Differences in the amount of bone formation and mineralization, cartilage formation, and fibrous tissue formation between the mechanically loaded and nonloaded groups were determined using either an independent-group *t*-test or a Mann–Whitney *U*-test, depending on normality, determined by a Shapiro–Wilk test. Analyses were performed using standard statistical software (SAS® 9.1; SAS Institute, Inc.). A p-value of < 0.05 was considered as significant.

Results

Mechanical loading modulated the efficacy of BMP2 treatment

Bony bridging was achieved by 2 weeks in calluses of all animals treated with BMP2 (Fig. 2). In contrast, no animals from the solvent-only-treated Control group achieved bony bridging over the 6 weeks experimental time period. At 2

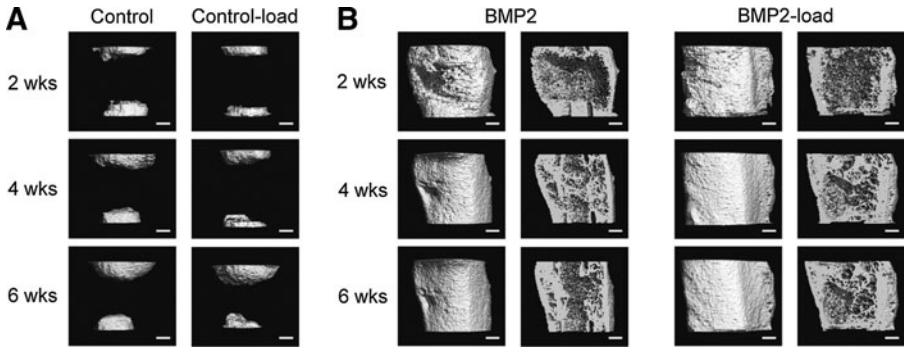

FIG. 2. MicroCT images with **(A)** 3D renderings of calluses from the Control and Control-load groups at weeks 2, 4, and 6 postoperation; **(B)** 3D renderings and transversal cut images of calluses from the BMP2 and BMP2-load groups at weeks 2, 4, and 6 postoperation. Scale bars = 1 mm. MicroCT, micro computed tomography; 3D, three dimensional; BMP, bone morphogenetic protein.

weeks, the BV ($p = 0.003$), TMC ($p = 0.002$), and TV ($p = 0.002$) of the BMP2-load group was significantly greater compared to the BMP2 group (Fig. 3). The TV was also significantly greater in the BMP2-load than in the BMP2 group at 4 ($p = 0.003$), and 6 ($p < 0.001$) weeks postosteotomy. Similar to the microCT data, histomorphometric analysis demonstrated that the BMP2-load group had a significantly greater total callus area at 6 weeks postoperation than the BMP2 group ($p = 0.013$) (Table 1). An increase in callus width also occurred in the mechanically loaded BMP2 group (BMP2 6 ± 0.9 mm; BMP2-load 6.9 ± 0.9 mm). Both the bone and fibrous connective tissue in the callus contributed to the increased total callus area and width, although only the fibrous connective tissue (mainly bone marrow) was significantly greater in the BMP2-load group compared to the BMP2 group ($p = 0.002$) (Fig. 4A, B). At 6 weeks postosteotomy, both the BMP2-load and BMP2 groups had a similar limited amount of cartilage, primarily present in the periosteal callus bone tissue. The osteotomized ends of the cortical bone were mainly surrounded by newly formed woven bone. Picrosirius red staining under polarized light microscopy demonstrated that periosteal calluses from the BMP2 and the BMP2-load groups consisted of a mixture of woven and lamellar bone (Fig. 4A1–B2). However, qualitative assessment suggested that the BMP2-load group appeared to have more advanced remodeling with greater amounts of lamellar bone compared to woven bone present in the periosteal bridged callus tissue.

Mechanical loading alone did not enhance bone defect healing

At 2 weeks postoperation, the mechanically loaded control group (Control-load) had significantly less BV ($p = 0.032$), TMC ($p = 0.029$), and TV ($p = 0.019$) than did the nonloaded Control group (Fig. 3). After 4 and 6 weeks of healing, mechanical loading in the control animals had no influence on bone healing parameters. Histomorphometric analysis showed similar total callus area as well as similar amounts of bone, cartilage, fibrous connective tissue, and muscle tissue within the callus of both mechanically loaded and nonloaded control specimens (Table 1). Only moderate bone formation (Control $3.2 \pm 2.3\,mm^2$, Control-load $3.1 \pm 1.4\,mm^2$) was measured over the 6-week period, and no bony bridging was achieved in both control groups. Furthermore, the control groups showed a similar defect repair result with a fibrous connective tissue and prolapsed muscle tissue filled gap and rounded cortical bone ends, without newly formed bone

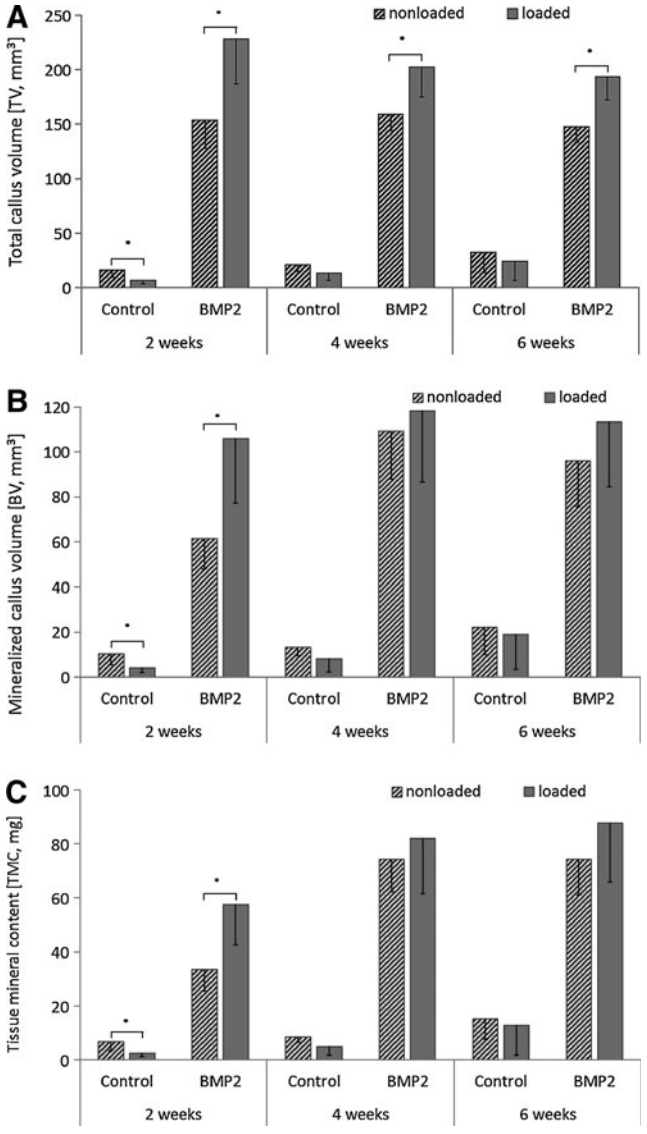

FIG. 3. *In vivo* microCT data **(A)** TV, **(B)** BV, and **(C)** TMC for each nonloaded and mechanically loaded Control and BMP2 group at 2, 4, and 6 weeks postoperation (mean ± standard deviation, $^*p < 0.05$). TV, total callus volume; BV, bone and calcified cartilage; TMC, tissue mineral content.

Table 1. Absolute Data of the Histomorphometric Analysis and Callus Width Measurements for All Four Groups (Mean ± Standard Deviation)

Parameter/group	*Control* n = *4*	*Control-load* n = *4*	*BMP2* n = *7*	*BMP2-load* n = *8*
Total area (mm^2)	18.9 ± 9.3	17.6 ± 1.0	24.3 ± 4.5[a]	32.1 ± 5.7[a]
Bone area (mm^2)	3.2 ± 2.3	3.1 ± 1.4	8.6 ± 2.2	9.2 ± 4.1
Cartilage area (mm^2)	0.1 ± 0.1	0.0 ± 0.0	0.1 ± 0.1	0.1 ± 0.1
Fibrous connetive tissue area (mm^2)	13.2 ± 8.3	12.8 ± 1.6	15.7 ± 3.8[a]	22.8 ± 3.1[a]
Muscle area (mm^2)	2.4 ± 2.3	1.7 ± 1.3	0.0 ± 0.0	0.0 ± 0.0
Callus width (mm)	5.0 ± 0.8	4.8 ± 0.7	6.0 ± 0.9	6.9 ± 0.9

[a]BMP2 different from BMP2-load.
$p < 0.05$; *t* test.
BMP, bone morphogenetic protein.

tissue. Using the Picrosirius red polarization method, a permanent bony sealing of the medullary canal by lamellar bone was obvious, covered by a layer of collagen fibers.

Discussion

To understand the combined effect of mechanical loading and exogenous BMP2 stimulation, we treated nonhealing critical-sized defects in rats with BMP2 or only a solvent in collagen scaffolds and compared healing outcomes at three different time points with and without weekly controlled *in vivo* axial compressive mechanical loading.

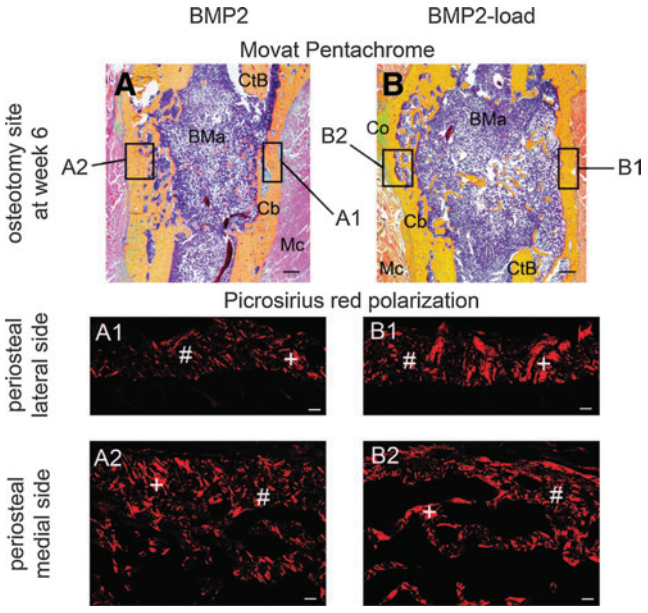

FIG. 4. Periosteal bony bridging of the osteotomy gap 6 weeks after surgery **(A, B)**. Note the bony bridging independent from the cortical bone, and the remodeling of the periosteal bony callus 6 weeks after surgery **(A1–B2)** on the lateral side **(A1, B1)** and the medial side **(A2, B2)** with predominant lamellar bone in the BMP2-load group (B1). Woven bone (#) is recognizable on the unorganized mesh pattern, whereas lamellar bone (+) shows organized thick bands. Cortical bone (CtB), callus bone (Cb), bone marrow (BMa), fibrous connective tissue (Co), and muscle tissue (Mc). Movat Pentachrome staining, scale bars = 500 μm. Picrosirius red polarization method, scale bars = 100 μm. Color images available online at www.liebertpub.com/tea

Local treatment of 50 μg BMP2 enhanced bone formation at 2 weeks postosteotomy with bony bridging achived at this time point in all specimens. In contrast, no animals from the solvent-only-treated control group achieved bony bridging after 6 weeks postosteotomy. We assume that there was a homogenous distribution of the BMP2 within the collagen sponge and thus throughout the osteotomy site based on our histological analysis that showed bony bridging of the BMP2-treated defects. Local application of BMP2 at the fracture site has been demonstrated in a number of preclinical[37–39] and clinical[40] studies to stimulate bone healing. Similar to mechanical loading, the timing of BMP2 treatment seems to have an effect on bone healing. Murnaghan *et al.*[41] reported that locally applied BMP2 at days 0 and 4 has an enhanced effect compared to application at day 8 in mice. In our study, the application of exogenous BMP2 at day 0 revealed bony bridging of a critical-sized defect in the BMP2-treated specimens and thus an effective time point for administration of this growth factor.

Without BMP2, mechanical loading did not enhance bone defect healing, and led to lower callus volume, mineralized callus volume, and callus mineralization after 2 weeks, although there was no influence of loading after 4 and 6 weeks. Although many studies have examined the influence of mechanical loading during normal healing in rats,[17–22] no studies have thus far examined the influence of mechanical loading alone on critical-sized defect healing. Claes *et al.*[20] demonstrated that early loading through dynamization (reduced fixation stiffness) after osteotomy surgery (1 mm defect in rat) delays uneventful bone healing. They suggested that any improved bone healing found after early dynamization in previous studies[42,43] was due to closure of the osteotomy gap rather than increasing the interfragmentary movement, which is known to influence healing.[44] Our results support these findings, in that early loading decreased bone formation in our nonunion model when BMP2 treatment was absent.

In the present study, once per week mechanical loading enhanced healing in bone defects treated locally with 50 μg rhBMP2 at 2 weeks postosteotomy. The total callus formation, bone formation and bone mineralization was greater after 2 weeks of healing, at which time bony bridging had occurred in all loaded and nonloaded group specimens. As bony bridging was already achieved by 2 weeks after local application of BMP2, the loading may have enhanced the bone formation by stimulating vascular remodeling, as seen

in uneventful osteotomy healing.[45] Mechanical loading is known to enhance migration and proliferation of mesenchymal stem cells (MSCs)[46] and their regulation of angiogenesis,[47] and BMP2 stimulates osteogenic differentiation of MSCs.[48–50] Thus, the combination may have led to mechanical loading-induced MSC migration and proliferation and exogenous BMP2-induced MSC differentiation. Additionally, a stimulatory effect of mechanical loading on osteoblast-like cells[51] and an osteoblastic differentiation induced by BMP2 under loading in a three-dimensional bioreactor system has been documented[52] *in vitro*. Kopf *et al.*[52] have shown that BMP2 and mechanical loading cooperatively regulate the early signaling in the BMP pathway, indicating the mechanical environment as a trigger for bone metabolism.[53] Wang *et al.*[54] could show that after axial strain BMP2 was increased in osteoblasts and culture medium 4–12 h after mechanical stimulation and decreased at 24 h. Currently, there remains a paucity of *in vivo* data examining the molecular mechanism between BMP2 stimulation and mechanical load, which needs to be addressed in the future, but the findings could explain our enhanced bone formation by combined treatment of both mechanical loading and exogenous BMP2. Our data are consistent with a previous study in a rat critical-sized defect model that showed BMP2 in combination with early (day 0) dynamization, performed through reduction in fixation plate stiffness[31] led to enhanced defect healing.

In our study at later time points, 4 and 6 weeks measured by microCT and at 6 weeks measured by histomorphometry, BV in the loaded BMP2 group was maintained at levels similar to those observed at 2 weeks. At 4 and 6 weeks, BV in the BMP2 group increased to similar BV levels measured in the BMP2-load group. However, TV slightly declined over time but continued to be significantly greater in the BMP2-load compared to the BMP2 group. Histomorphometric analysis at 6 weeks also demonstrated that although there was a nonsignificant increase in bone tissue with loading the major contributor to the increased total callus area was by fibrous connective tissue. These data suggest that once bony bridging was achieved the loading regime no longer enhanced bone formation and may have actually slowed or hindered the reduction in TV callus size required to regain the architecture of the original bone.

In contrast to our study, Boerckel *et al.*[31] showed that late (week 4) compared to early (day 0) loading in combination with exogenous BMP2, led to an increased bone formation. However, a higher BMP2 dosage was applied in the late dynamization compared to the early dynamization groups in their study, which could explain the enhanced defect repair they observed independent of loading. Additonally, it is difficult to compare the influence of loading between these studies because we used once a week controlled loading while their loading was by dynamization through reduction in fixation plate stiffness. Although not directly transferable, our treatment strategy of BMP2 in combination with mechanical stimulation could be in principle be adapted in humans during the healing process as the inter-fragmentary strain allowed in our loading model is slightly lower than that allowed by an external fixator that has already been used clinically.[55,56] Our data show that a very small amount of interfragmentary movement sufficiently stimulated bone formation in combination with BMP2 in a rat segmental bone defect model. However, the optimal timing and magnitude of loading in combination with BMP2 application requires further investigation.

In conclusion, we demonstrated that the combined effect of early controlled *in vivo* axial compressive mechanical loading administered once weekly combined with locally applied exogenous 50 μg BMP2 significantly enhanced bone defect healing in rats at 2 weeks. However, continued loading after bony bridging was achieved, which led to an increased amount of fibrous connective tissue, mainly bone marrow, resulting in a larger total callus at week 6. Exogenous BMP2 application alone led to bony bridging of all calluses after 2 weeks of healing and a smaller callus width. Mechanical loading alone without BMP2 application did not enhance the formation of bone; in fact, mechanical loading alone led to lower callus volume, mineralized callus volume, and callus mineralization after 2 weeks, although there was no influence of loading after 4 and 6 weeks. Biophysical therapies, whereby growth factor treatment is augmented with load bearing or exercise, could be an effective strategy to achieve bony bridging and successful clinical outcomes. Lower doses of growth factors could potentially be used in combination with mechanical loading administered before bony bridging and should be investigated in the future, as this strategy may attenuate complications associated with ectopic bone formation as well as reduce costs.

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Disclosure Statement

The authors state that no competing financial interests exist.

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