INTRODUCTION
Immobilization is frequently used treatment for bone injuries despite resulting osteopenia. In bone, immobilization results in temporary uncoupling of the bone turnover, where rapid initial increase in bone resorption is later accompanied by decrease in bone formation. Despite the deleterious effects, many treatment protocols of bone fractures still require a substantial period of immobilization even after operative stabilization with internal implants.

The number of osteoporotic fractures has increased markedly during the past few decades. Despite the high prevalence of osteoporotic fractures, the regenerative capacity of osteoporotic/osteopenic bone remains somewhat controversial and poorly understood.

The purpose of the present study was to find out whether osteopenic mouse bone has the same regenerative potential as normal, healthy bone.

MATERIALS AND METHODS
A total of 188 female C57Black/DBA mice, age 3 months, weighing 23–27 g, were used in this study. The study protocol was approved by the institutional committee for animal welfare.

A standardized cylindrical (diameter 0.9 mm) metaphyseal bone defect of the distal femur was created unilaterally both in immobilization-induced osteopenic mice (n=70) and in non-immobilized age-matched control animals (n=70). After creation of the bone defect, the animals in both groups were further divided into two groups: postoperative three-week cast-immobilization (Im-Im and Mo-Im) groups, and unrestricted weight-bearing (Im-Mo and Mo-Mo) groups.

A total of 48 control animals were subjected to 3 weeks of immobilization alone (Im-c, n=24) or were used as unoperated normal controls (Mo-c, n=24).

The mice were followed for 3 weeks after creation of the defect. For the molecular biologic analysis six animals from each group were sacrificed 1, 2 and 3 weeks postoperatively. RNA was extracted from metaphyseal defect area. The tissue specimens were frozen in liquid nitrogen, pulverized with a morcel and the RNA was separated in guanidine isothiocyanate after ultracentrifugation in CsCl gradient. For Northern analyses, 10 µg aliquots of total RNA were denatured with formaldehyde, electrophoresed on 1 % agarose gels, transfered onto nylon membranes, and hybridized with 32P -labeled cDNA probes for osteocalcin (Ocn), cathepsin K (CtsK), and for mouse 28S rRNA. The bound probes were quantified using a phosphoimager.

For histologic analysis 3 animals per group was used. Bones were decalcified and processed for routine paraffine sectioning, stained with Van Gieson and analyzed with histomorphometric quantification of the new bone and cartilage.

Biomechanical testing was performed using a standardized cantilever-bending technique. Distal condyles of the femurs were loaded in anteroposterior direction at a constant rate of 1.0 mm/min until failure in a universal testing device. The force-displacement curves recorded were used to measure ultimate bending force to failure (N) and bending stiffness (N/mm).

Statistical analyses were based on ANOVA. A p-value <0.05 was considered statistically significant.

RESULTS
Biomechanical testing demonstrated that immobilization for three weeks results in substantial decrease in the ultimate bending force and bending stiffness (Table 1). The ultimate bending force was significantly reduced in the defects that were subjected to preoperative immobilization. Unrestricted postoperative loading did not significantly improve the biomechanical properties of the defects in osteopenic bone.

Northern analyses demonstrated that the general expression profiles for osteocalcin, chondroblast and osteosclerotic markers were strikingly similar in all the groups (Fig 1). Unrestricted mobilization significantly increased the mRNA levels for cartilage specific genes in one- and two-week time-points. This was also reflected in histomorphometric analyses

as increased amounts of cartilage-spicaes within the defect rims. Loading also increased the osteoblastic marker gene mRNA levels at the three-week time-point. For the osteosclerotic marker, the highest Cathepsin K mRNA levels were seen in Im-Im group at two weeks of the defect healing.

Table 1. Cantilever bending test of the distal femurs.

<table>
<thead>
<tr>
<th>group</th>
<th>Failure load (N)</th>
<th>Stiffness (N/mm)</th>
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<tbody>
<tr>
<td>A_Mo-C</td>
<td>11.4 ± 1.1 **</td>
<td>33.6 ± 4.9 **</td>
</tr>
<tr>
<td>B_Im-C</td>
<td>8.8 ± 1.4</td>
<td>22.1 ± 5.0</td>
</tr>
<tr>
<td>C_Im-Mo</td>
<td>9.0 ± 0.8</td>
<td>23.9 ± 5.8</td>
</tr>
<tr>
<td>D_Mo-Mo</td>
<td>10.4 ± 1.2 ▲</td>
<td>38.0 ± 3.2 §§ ▲</td>
</tr>
<tr>
<td>E_Mo-Im</td>
<td>9.9 ± 1.6</td>
<td>33.7 ± 14.1</td>
</tr>
<tr>
<td>F_Im-Im</td>
<td>8.9 ± 1.4</td>
<td>26.8 ± 5.4 □</td>
</tr>
</tbody>
</table>

**p<0.01 A vs B; §§p<0.01 C vs D; ▲p<0.05 E vs F ▲p<0.05 D vs F.

DISCUSSION
Biomechanical testing revealed that normal defects heal within three weeks near to the strength of normal intact bone when the mice were allowed to mobilize freely. Immobilization during the postoperative period reduces the ultimate bending strength in normal bone. When the animals were subjected to preoperative immobilization, the defects were significantly weaker after three weeks of healing. However, the ultimate bending force and stiffness was near to the level of intact osteopenic bone.

The results indicate that the primary healing response in terms of gene expression is similar in osteopenic bone as it is in the normal bone. RNA analyses also demonstrated that loading significantly modulates the magnitude of gene expression in chondroblastic and osteoblastic lineages. This data shows that loading during the defect healing also increases the strength of the defects in normal bone. However, postoperative loading did not significantly increase the bone strength in previously osteopenic bone.

This study demonstrates that biomechanical loading is an important modulator of the bone healing process and still a vast amount of molecular biologic studies are warranted to really understand the complexity of the cellular signals related to bone regeneration.

ACKNOWLEDGMENTS
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