INTRODUCTION:
During physiologic homeostasis and in case of infection, the immune system and the skeleton are deeply interdependent on each other. Both systems closely interact, due to shared anatomical compartments, cell precursors and molecular mediators. This project was aimed at further elucidating how activation of the innate immune system by lipopolysaccharide (LPS) influences the process of bone regeneration following fracture. The hypothesis of this study was that fracture healing would be impaired due to LPS induced activation of the innate immune system.

METHODS:
A standard closed femoral fracture was created in 8-10 week old wildtype (WT) mice and mice receiving LPS. Repeated intraperitoneal injections of LPS (2 mg/kg body weight) starting 24 h before fracture and repeated thereafter every 3 post OP days induced activation of the innate immune system. Animals were followed for biomechanical testing and µCT analysis up to 14 and 21 days, respectively (N=8/time point). Statistical comparisons between groups were performed using the Mann-Whitney U-test.

RESULTS:
Biomechanical testing demonstrated a significantly lower torsional stiffness at day 14 (p=0.01) in the LPS injected mice in comparison to the WT group (Fig. 1).

DISCUSSION:
The results of this study have shown impaired fracture healing in mice following induction of immune responses by LPS. The biomechanical analyses provide strong evidence for this conclusion supporting the results of Reikeras et al. [1]. The µCT analyses provide new information on differences of morphology and morphometric measurements of the callus tissue. Mineralisation of the regenerative tissue is delayed. This is a major and severe failure compared to the normal healing cascade. Activation of selective immune cell populations critically influences fracture healing [2]. On the one hand, macrophages are mandatory for successful fracture healing, as their depletion delays resorption of the soft callus. On the other hand, LPS induced over activation of the innate immune system interferes with mineralisation of the callus tissue thereby delaying healing. Cells of the innate immune system including macrophages and neutrophils are key players in the response to LPS. LPS signals are transduced via toll like receptor-4 (TLR-4) and potently induce the release of pro-inflammatory cytokines, including TNF-α. This might cause an altered balance between osteoblast and osteoclast. This could explain the reduced mineralisation. It is yet unclear which direct effect LPS has on osteoclast number and activity in our in vivo study. Further analyses are necessary to identify the underlying molecular and cellular mechanisms. We conclude that the innate immune system is mandatory for successful fracture healing. However, the degree of its activation is crucial. This molecular interplay might offer new therapeutic targets to enhance fracture healing.

REFERENCES:
2. Toben, D et al. Fracture healing is accelerated in the absence of the adaptive immune system, J Bone Miner Res, in press

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Fig. 1. Relative torsional stiffness of fractures. Note significantly lower torsional stiffness at day 14 (a) in LPS (green) compared to WT (blue).

Fig. 2. 3D reconstructions of representative µCT sections during the course of fracture healing in WT (A) and LPS (B) mice. Scale bars = 1 mm.

Fig. 3. Bone volume of fractures. Note significantly lower bone volume at day 21 (a) in LPS (green) compared to WT (blue).

Immunological aspects of fracture healing: LPS and the innate immune system