Early life stress induces an osteocatabolic response in female mice, but has no long-term effects on fracture healing

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INTRODUCTION: Early life stress (ELS) affects approximately 40% of the world's population (Kessler et al., 2010), and is an acknowledged risk factor for the development of several somatic and affective disorders, including obesity, posttraumatic stress disorder (PTSD) and depression. The latter seem to occur in a sex-dependent manner, with women being more vulnerable (Rincel et al., 2019; Vafaei et al., 2016). Moreover, disorders like PTSD and depression are associated with osteoporosis and increased bone fracture risk (Foertsch et al., 2017; Gebara et al., 2014) and ELS with reduced height in adulthood, suggesting shorter long bones and an effect on bone metabolism (Batty et al., 2009). Intriguingly, factors that are known to influence bone homeostasis in physiological and pathophysiological conditions, for instance an inflammatory phenotype, are also associated with ELS (Baumeister et al., 2016; Danese et al., 2007). However, as direct effects of ELS on bone and the inflammatory response after bone fracture are unclear so far, this study aims to investigate the effects of ELS on bone tissue and fracture healing in a murine model for ELS. We investigated whether these effects are sex-specific, and if similar findings can also be observed in humans.

METHODS: The maternal separation (MS) procedure was used to induce ELS in C57BL/6N mice (Schimmele and Langgartner et al., 2025). Pups were separated from their mother for three hours per day for the first 14 days of their life. Control pups (noMS) were only handled but not separated. For evaluation of bone homeostasis, mice were euthanized 6-7 weeks after noMS/MS, and µCT measurements of the femur, and histological staining and qPCR analysis of paraffin-embedded femur sections were performed. To study fracture healing, MS was combined with a standardized right femur osteotomy at an age of 12 weeks, and mice were euthanized 3 hours, 1 day, 10 days and 21 days post fracture. Depending on the time point, cytokine assays, flow cytometry, histological staining, biomechanical testing and µCT measurements were used to characterize the inflammatory response, functional outcome and fracture callus tissue formation. Statistical analysis was conducted using a non-paired t-test or a two-way ANOVA. All animal experiments were approved by the local animal welfare committee (Regierungspräsidium Tübingen, Germany) and were performed in compliance with international regulations for laboratory animal welfare and handling. For the unfractured mice, group size was n = 10-12/ group, for the fracture analysis n = 4-11/group. In order to further assess the effect of ELS in humans, 84 post-menopausal women were screened for osteoporosis history, radiological and biochemical bone parameters, and were asked to fill out the standardized childhood trauma questionnaire (CTQ) to assess for prevalence and severity of ELS. Spearman's correlation analysis was performed between the measured bone parameters and the ELS scores. The study was conducted in compliance with the Declaration of Helsinki and the International Conference on Harmonization Principles of Good Clinical Practice. The research protocol was approved by Local Ethics Committees and all participants gave informed consent allowing their anonymized information to be used for a data analysis.

RESULTS: We first analyzed the general effects of ELS on bone in male and female mice. µCT parameters did not differ between noMS and MS mice, both in male and female mice. In female MS mice, osteoclast activity (measured by OcS/BS; no MS 14.83 ± 2.82 vs. MS 19.56 ± 2.47 %, p value = 0.0007), and the relative gene expression of Cathepsin K, IL-6 and RANKL were significantly increased (Fig.1). Because we only saw an effect of ELS in female mice, we further analyzed possible consequences of ELS on bone health in women. A correlation analysis between bone parameters and ELS scores in post-menopausal women revealed emotional abuse being negatively correlated with the spine T score (r = -0.28; P = 0.010), the spine BMD (r = -0.31; P = 0.009) and the diagnosis of osteopenia (r = -0.28; P = 0.009), while being positively correlated with the diagnosis of osteoporosis (r = 0.30; P = 0.005), among other significant correlations. This suggests that ELS is a risk factor for the development of osteoporosis in postmenopausal women. Next, we assessed the effects of ELS on fracture healing in male and female mice. Male mice subjected to MS showed a reduced inflammatory response three hours after fracture, characterized by decreased expression of IL-10 (hematoma: no MS 3978 ± 1787 vs. MS 1930 ± 1546 µg/ml; plasma: no MS 11.35 ± 28.16 vs. MS 0.73 ± 2.19 µg/ml), RANTES (hematoma: no MS 7396 ± 2237 vs. MS 3656 ± 1781 µg/ml; plasma: no MS 244 ± 112 vs. MS 116 ± 120 µg/ml) and TNFα (hematoma: no MS 1675 ± 721 vs. MS 665 ± 509 µg/ml; plasma: no MS 3.39 ± 4.80 vs. MS 0.51 ± 1.52 µg/ml) in the fracture hematoma and plasma. However, one day after fracture there were no significant differences in immune cell composition in fracture hematoma, bone marrow, or spleen. Furthermore, the composition of the fracture callus and bending stiffness assessed at days 10 and 21 after fracture did not differ between MS and noMS males. No significant differences were seen in female mice for all four time points and measured parameters.

DISCUSSION: Our results imply that ELS enhances osteoclast activity and associated markers in female mice, which is in line with an increased risk for osteoporosis in post-menopausal women who have experienced ELS. Further studies are needed to elucidate whether changes in structural parameters will occur in older female MS mice as well. Interestingly, in terms of fracture healing, ELS only affects the early inflammatory phase in male mice, while these alterations did not result in long-term effects on callus tissue formation and functional outcome. In conclusion, ELS affects bone and inflammatory response towards fracture in a sex-specific manner. This might be due to sex hormones like estrogen and testosterone, which are known to influence bone health and inflammatory status during life.

SIGNIFICANCE: Our preclinical data indicates that ELS leads to an osteocatabolic response in females. Clinical data underscores the importance of ELS to be considered as a risk factor for the development of osteoporosis in postmenopausal women.

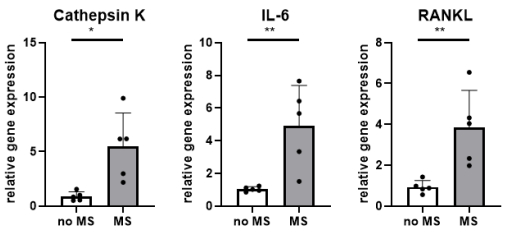

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Fig. 1: Relative gene expression analysis of bone parameters in female mice subjected to maternal separation (MS) or sham-handling (no MS). n=5. *0.05>p≥0.01; **0.01>p>0.001.