Sexual Dimorphism in Bone Trace Minerals following Fracture

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INTRODUCTION: Trace minerals such as magnesium (Mg) and zinc (Zn) concentrate in bone and may have key roles in bone healing through osteogenic signaling, immune modulation, and resorption inhibition. The therapeutic potential of Zn and Mg appear to be concentration- and time-dependent, but how endogenous levels fluctuate after fracture remains unclear. Sex differences in bone physiology may further complicate mineral dynamics, as sex specific factors influence osteoblast and osteoclast activity. This study aimed to investigate temporal changes in bone Mg and Zn levels at early time points following fracture and to assess if sex-specific differences in mineral homeostasis occur in a rat femur fracture model.

METHODS: Thirty-one skeletally mature diabetic-resistant BB Wistar rats (12 males, 19 females) were studied conforming to an Institutional Animal Care and Use Committee-approved protocol. Closed mid-diaphyseal femur fractures stabilized with an intramedullary rod were created in 19 rats, while 12 rats served as pristine controls. Fractured and contralateral femurs were harvested at 3- and 7-days post fracture (dpf). Ten millimeters of diaphyseal bone from the contralateral femurs and from the femurs of the pristine rats were collected while 5 mm portions of the fractured bone proximal and distal to the fracture site were also collected. The bone samples from each femur were digested in nitric acid, diluted, and analyzed for Mg and Zn content via ICP-MS. Standard curves were generated per batch and values extracted accordingly. Ratios were calculated between the pristine and the 3 dpf and 7 dpf samples to normalize variation between standard curves from each ICP-MS batch. The ratios from the 3 and 7 dpf specimens were compared to the baseline pristine values using paired t-tests. Significance was set at $p<0.05$.

RESULTS SECTION: Results are expressed as Ratio ± SD. In males, Mg and Zn levels at the fracture site were significantly decreased at 3 dpf (Mg: –28.3%; Zn: –24.5%) with partial recovery by day 7. In females, Mg and Zn levels showed a statistically insignificant decrease at 3 dpf but dropped significantly by day 7 (Mg: –32.2%; Zn: –18.4%). Sex comparisons revealed distinct temporal patterns with males exhibiting early decreases in fracture site bone Mg and Zn levels, while females demonstrated a delayed but more pronounced decreases in bone fracture site Mg and Zn levels.

DISCUSSION: Fracture-induced disruption of trace mineral homeostasis exhibits clear sexual dimorphism. The paired t-test findings support that time shapes fracture-site mineral dynamics, with males showing early depletion and partial recovery, while females exhibit delayed but more pronounced decreases. The trace minerals exiting the bone after fracture may be influencing the local environment to enhance or regulate callus formation. For instance, Zn can promote chondrogenesis and Mg can modulate macrophage activity.[1,2] The delayed loss of Mg and Zn from the fracture-site bone in female rats may be influenced by estrogen-mediated buffering of mineral balance. These differences highlight the importance of sex as a biological variable in fracture healing and suggest that mineral-based therapies may need to be tailored by dose and timing for each sex. Limitations include the absence of systemic mineral and hormone measurements and use of a well-nourished animal model that may not reflect human dietary deficiencies in trace minerals.

SIGNIFICANCE/CLINICAL RELEVANCE: This study identifies sex-specific temporal differences in Mg and Zn dynamics during early fracture healing, suggesting that therapeutic strategies involving trace mineral supplementation or mineral-releasing implants may require sex-, dose-, and time-dependent adjustments. Recognizing these patterns may inform personalized approaches to fracture care and improve outcomes in bone regeneration.

REFERENCES:

1. Qiao W, Wong KHM, Shen J, et al. TRPM7 kinase-mediated immunomodulation in macrophage plays a central role in magnesium ion-induced bone regeneration. Nat Commun. 2021;12(1):2885. doi:10.1038/s41467-021-23005-2. PMID: 34001887; PMCID: PMC8128914.
2. Burgess D, Cottrell J, Iverson T. Zinc chloride treatment in ATDC5 cells induces chondrocyte maturation. Int J Regen Med. 2018;1(2):1-11.

IMAGES AND TABLES:

Table 1. Fracture Callus Relative Magnesium Levels (Male & Female)

Group	Male Normalized Ratio (ratio ± SD)	Male % Change	P value	Female Normalized Ratio (ratio ± SD)	Female % Change	P value
Day 0: Pristine	1.0	0.0	—	1.0	0.0	—
Day 3: Pristine	0.717 ± 0.0566	-28.3	**0.013**	0.87 ± 0.265	-13.0	0.283
Day 7: Pristine	0.769 ± 0.141	-23.1	0.105	0.678 ± 0.14	-32.2	**0.002**

Table 2: Fracture Callus Relative Zinc Levels (Male & Female)

Group	Male Normalized Ratio (ratio ± SD)	Male % Change	P value	Female Normalized Ratio (ratio ± SD)	Female % Change	P value
Day 0: Pristine	1.0	0.0	—	1.0	0.0	—
Day 3: Pristine	0.755 ± 0.00897	-24.5	**0.0004**	0.934 ± 0.195	-6.6	0.444
Day 7: Pristine	0.909 ± 0.17	-9.1	0.450	0.816 ± 0.079	-18.4	**0.0008**