

Disturbed arginine-NO metabolism inhibits callus formation resulting in delayed- and nonunion development

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Introduction

• In high-risk patients, up to 45% of all fractures heal inadequately resulting in nonunion development¹.

• Non-union development results in a significant decrease in quality of life and high socio-economic costs.

• Nitric oxide (NO), a signaling molecule and produced solely during the conversion of the amino acid arginine into citrulline by one of the nitric oxide synthase (NOS)-isoforms, has been shown to be of major importance during fracture healing.

• Inducible iNOS is mainly upregulated during the inflammatory phase and endothelial eNOS during callus formation and remodeling².

• Arginine can be converted into ornithine by arginase, which acts as a precursor for collagen synthesis, a second key element in fracture healing (Figure 1).

• **Hypothesis:** inhibition of NO production by absence of NOS-isoforms will delay fracture healing resulting in non-union in an experimental animal model.

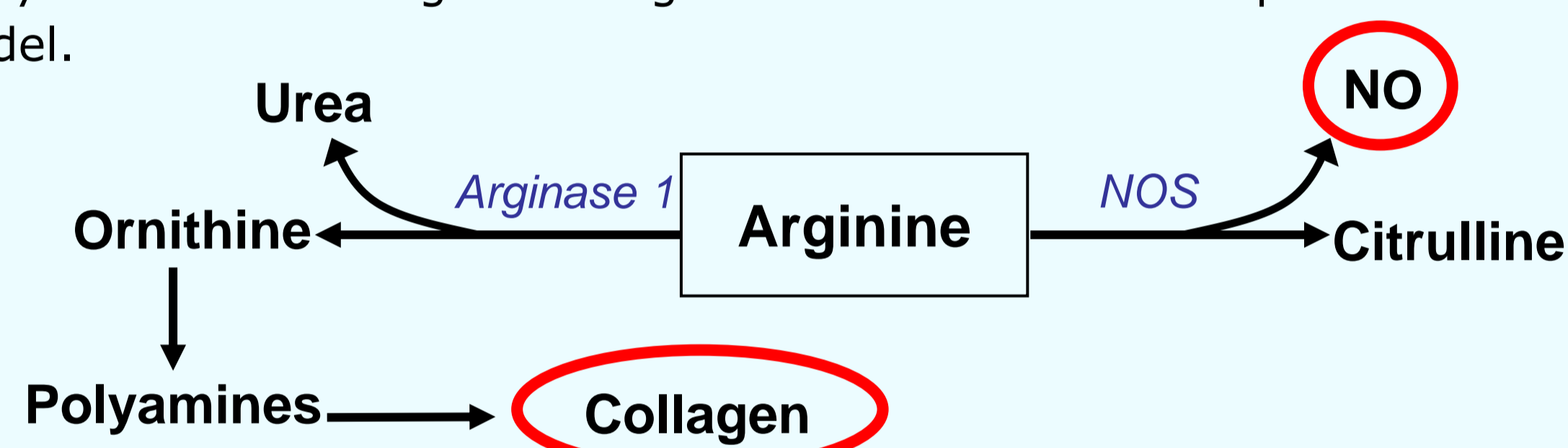


Figure 1: Arginine-NO metabolism in fracture healing. Adapted from Wijnands et al.³

Objective

Investigate the influence of the depletion of different NOS-isoforms in mice on fracture healing and the development of non-union formation.

Methods

Model

• 0.45 mm femur osteotomy with periosteal cauterization followed by plate-screw osteosynthesis.

• 20-24 week old wildtype (WT), iNOS^{-/-} and eNOS^{-/-} mice (N=8/group).

• Mice were sacrificed after 7, 28 and 42 days (contralateral legs served as control)

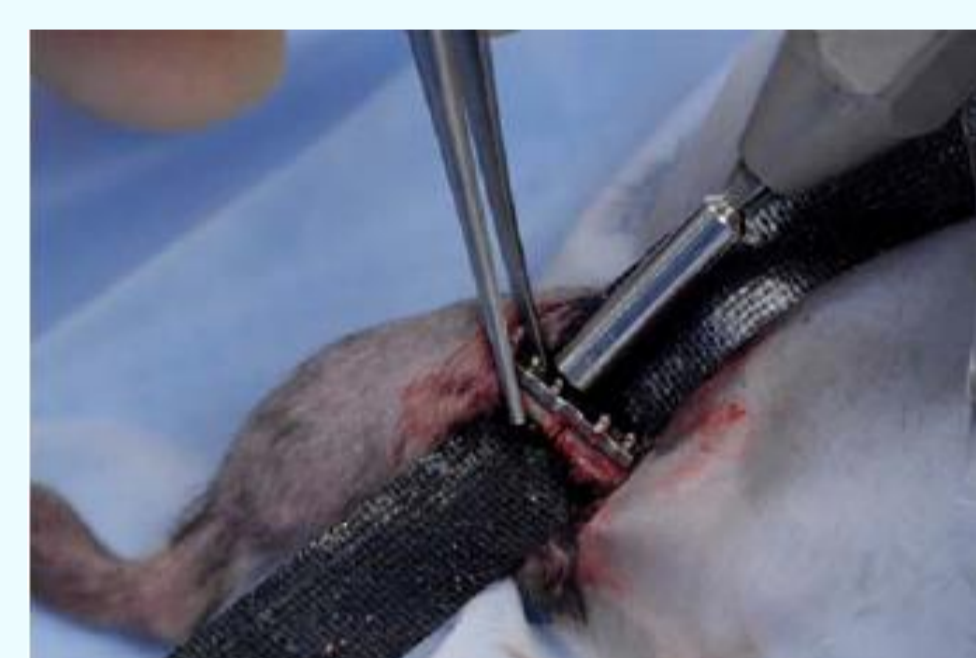


Figure 2: Femur osteotomy and fixation using MouseFix locking plate in non-union mouse model⁴

Analysis

- μ CT: quantification of lamellar and woven bone volumes
- qPCR: eNOS, iNOS and Arg-1 expression in callus tissue
- HPLC-MS: amino acid concentrations in callus tissue and plasma
- IHC: myeloperoxidase staining for neutrophil influx assessment

Results



Figure 3: MPO staining in wildtype, iNOS^{-/-} and eNOS^{-/-} mice after 28 days of repair

References

1. Frölke JP, Patka P. Definition and classification of fracture non-unions. *Injury*. 2007 May;38 Suppl 2:S19-22.
2. Diwan AD, Wang MX, Jang D, Zhu W, Murrell GA. Nitric oxide modulates fracture healing. *J Bone Miner Res*. 2000 Feb;15(2):342-51.
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4. Matthys R, Perren SM. Internal fixator for use in the mouse. *Injury, Int J Care Injured*. 2009 40S4, S103-9

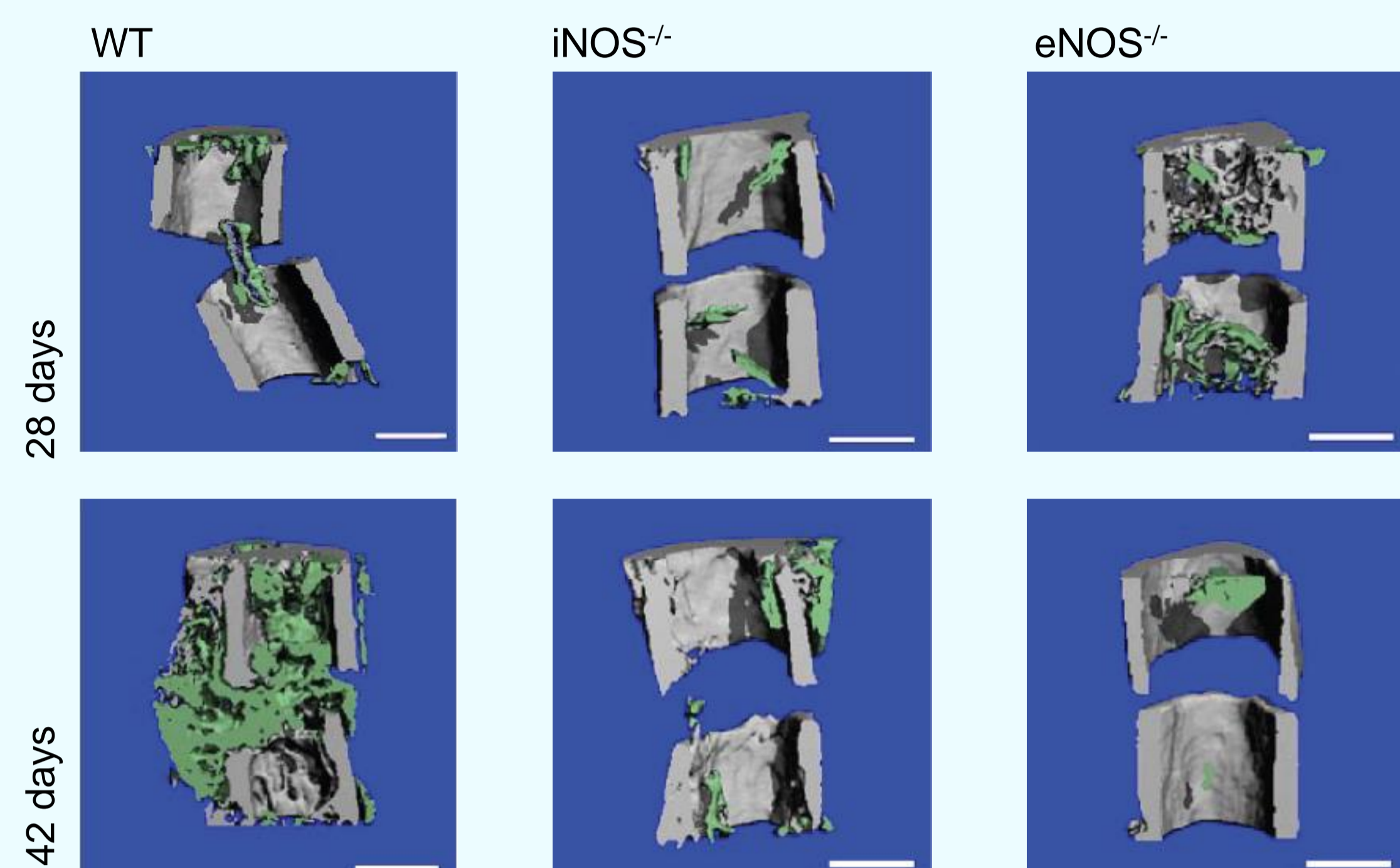


Figure 4: Qualitative μ CT-measurements

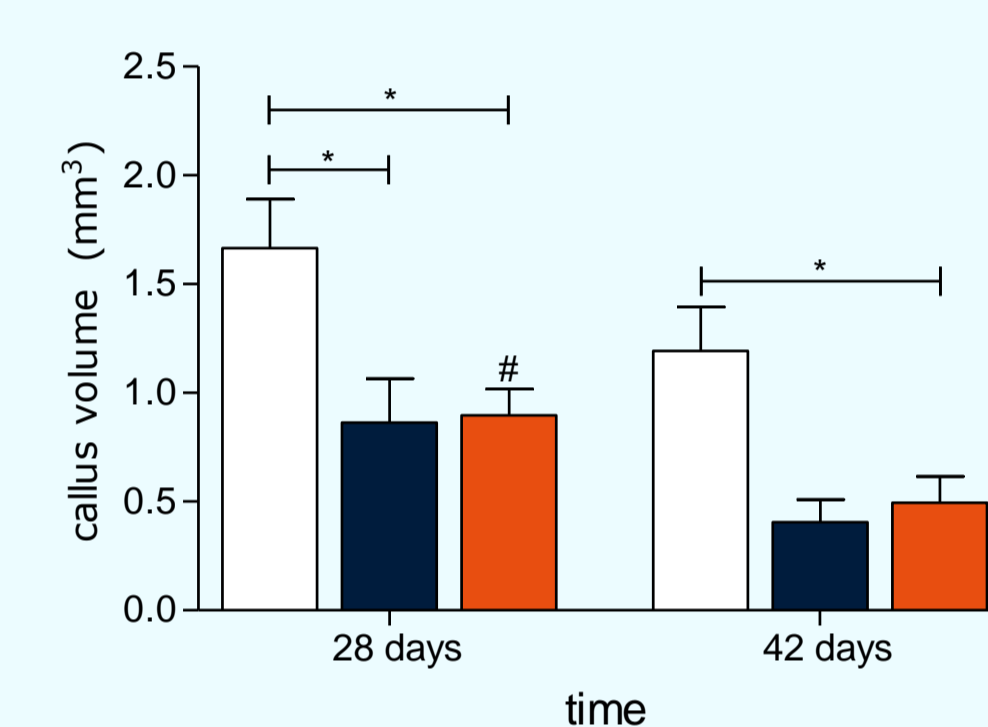


Figure 5: Quantitative μ CT-measurement of callus volume. *: $p < 0.05$, between groups; #: $p < 0.05$ between time points

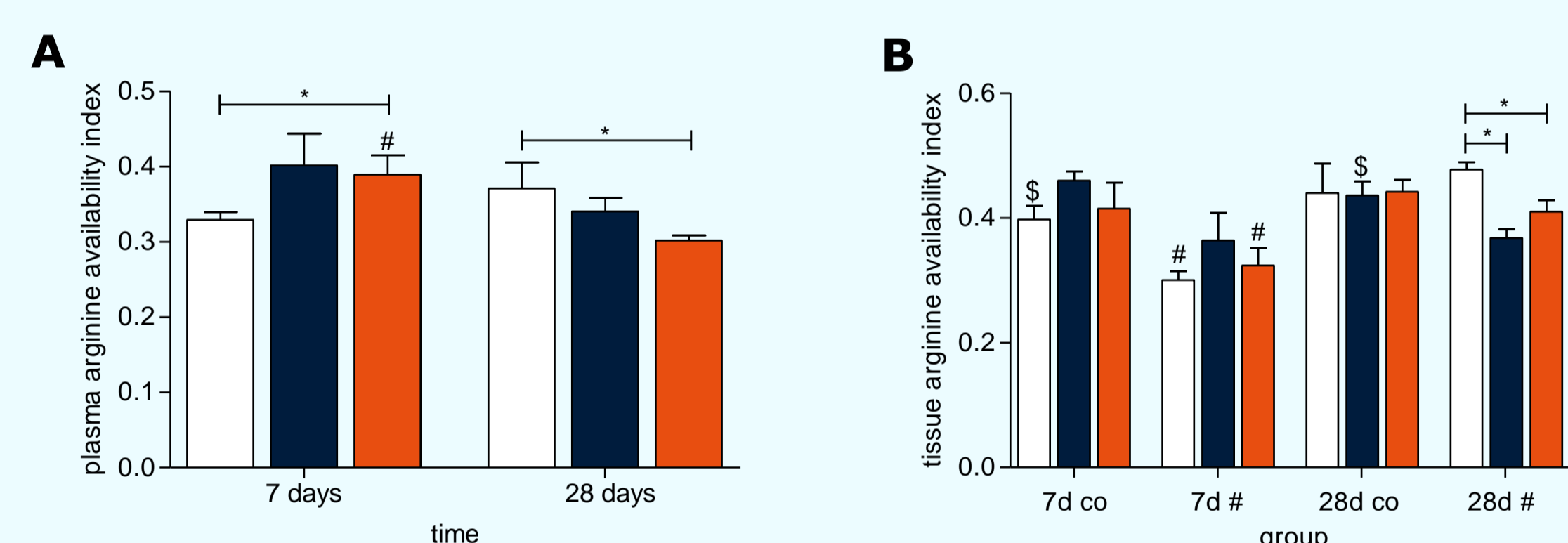


Figure 6: Arginine availability index (AAI) in plasma (A) and callus tissue (B) samples. AAI is calculated as the ratio between the arginine concentration and the combined concentrations of ornithine and lysine. *: $p < 0.05$ between groups; #: $p < 0.05$ between time points; \$: $p < 0.05$ between control and fractured femurs.

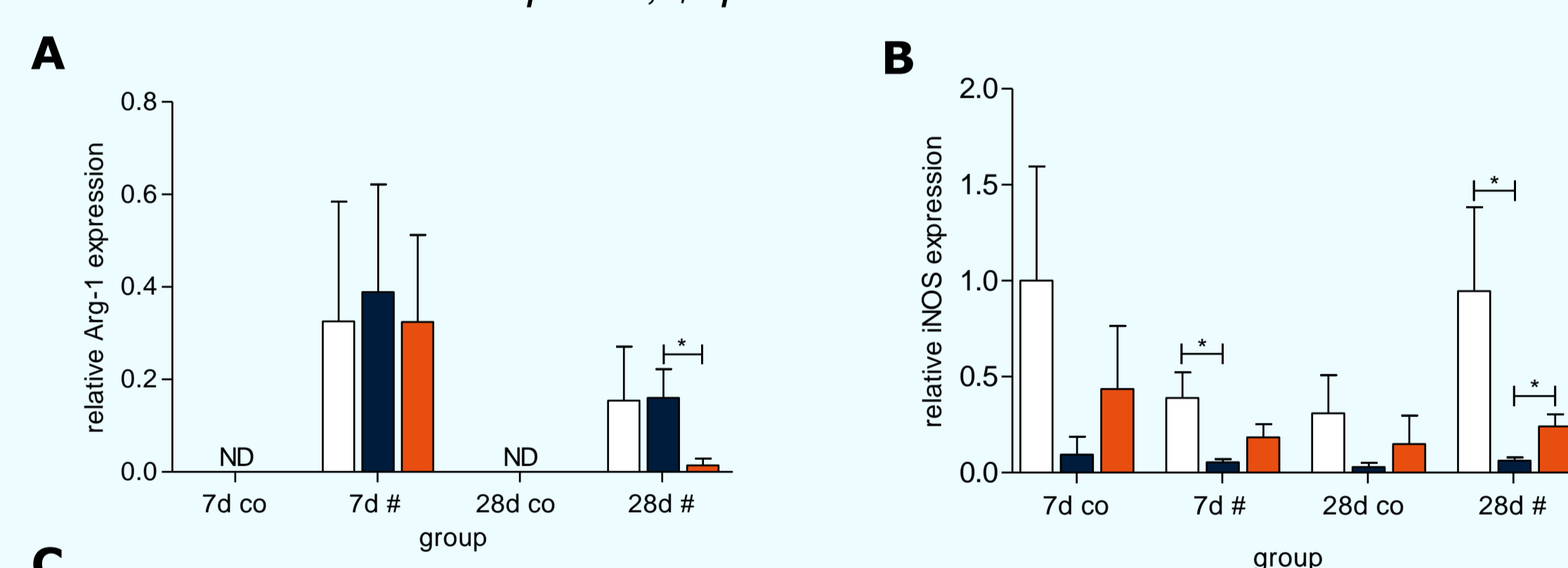


Figure 7: Quantitative PCR analysis in control and fractured bones after 7 and 28 days of fracture healing. (A): Arginase 1, (B): inducible nitric oxide synthase and (C): endothelial nitric oxide synthase. *: $p < 0.05$

Conclusions

Absence of NOS-isoforms disturbs the arginine-NO metabolism and enhances the development of non-union by decreasing callus volume and inducing a prolonged inflammatory state.