

Oral K₃Citrate Supplementation Mitigates Age-Associated Intervertebral Disc Mineralization in LG/J Mice

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INTRODUCTION: Intervertebral disc mineralization is a prevalent subphenotype of intervertebral disc degeneration, with incidence as high as 100% reported in a study of randomized cadaveric samples. Mineralization of the disc can contribute to increased stiffness of the tissue and exacerbate susceptibility to intervertebral disc herniation, conditions which contribute to back pain. One challenge which has limited the study of disc mineralization and development of therapeutics to mitigate it is small animal models which recapitulate the pathology. We have previously shown the LG/J inbred mouse strain exhibits age-associated intervertebral disc mineralization, with notable features including high amounts of free calcium in mineralized discs and transcriptomic signatures relating to endochondral bone and calcium-phosphate homeostasis. In this study, we build on these findings by further characterizing the phenotype and testing the suitability of oral supplementation of K₃Citrate to mitigate disc mineralization.

METHODS: Starting at 17 months of age, LG/J mice received 80mM K₃Citrate continuously supplemented in their drinking water. Prior to euthanasia at 23 months, animals underwent grip strength, open field, and gait analyses to evaluate global impacts of K₃Citrate on animal locomotion and frailty. Postmortem, motion segments were scanned using μ CT, and detailed histological and spectroscopic analyses were performed on the discs. Plasma chemistry was evaluated by quantitative NMR spectroscopy as a measure of general health and by multiplex ELISA to measure the abundance of various cytokines and proinflammatory markers. Based on observations that LG/J disc mineralization may be driven by dysregulation of the cartilaginous endplates, in vitro studies were conducted using the ATDC5 chondrocytic cell line to investigate the effect of K₃Citrate on cell differentiation and cartilage mineralization. Significance was measured using a student's t-test and χ^2 test, where appropriate, at a significance level of $p < 0.05$. Mice were kept according to protocols approved by the Institutional Animal Care and Use Committee (IACUC) of Thomas Jefferson University.

RESULTS SECTION: Consistent with studies of the effects of K₃Citrate supplementation in humans, our plasma analyses and behavioral assessments indicated no adverse effects of K₃Citrate supplementation on LG/J mice. Notably, K₃Citrate-treated mice demonstrated higher grip strength than the control cohort. μ CT imaging showed significant reductions in the incidence and size of disc mineralization with K₃Citrate treatment. Histological analyses of control and K₃Citrate-treated mice revealed that the cartilaginous endplates may be the locus for mineralization in LG/J mice, evidenced by the irregular presence of hypertrophic chondrocytes in the endplates and the apparent spread of these cells into the subchondral bone and NP and AF compartments.

DISCUSSION: Calcification of the cartilaginous endplates of the intervertebral disc is a common feature observed in the aging human population. Such calcifications may also correspond to mineralization of the other disc compartments and the depletion of nutrients in the disc. Here, we show that dysregulation of the endplate chondrocyte differentiation program is a significant contributor to the intervertebral disc mineralization observed in the LG/J mouse model. Importantly, our results provide robust evidence that K₃Citrate supplementation successfully reduces the incidence and severity of disc mineralization in the LG/J model. This work reinforces the safety of K₃Citrate as a supplement and provides mechanistic insight into the means through which mineralization of the disc may be mediated.

SIGNIFICANCE/CLINICAL RELEVANCE: This study for the first time demonstrates the ability of an oral citrate supplement to prevent intervertebral disc calcification, a pathology of high clinical relevance. Importantly, this and other research indicate no adverse effects, providing a promising lead for further research on and application of citrate to mitigate this pathology.

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IMAGES AND TABLES:

