Cartilage -specific knockout of Sirt1 Significantly reduces bone quality and catch-up growth efficiency

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INTRODUCTION

Linear growth usually occurs without any obstacles, but can be impaired by numerous genetic and environmental factors. In most cases, when the restricting factor is resolved, spontaneous catch-up (CU) growth occurs. However, its efficiency is sometimes inadequate and growth deficits remain permanent.

The therapeutic toolbox for short stature is currently very limited, thus, finding new regulatory pathways is important for the development of novel means of treatment.

Sirtuin-1 (Sirt1) is a member of the Sirtuins, a family of proteins that act predominately as nicotinamide adenine dinucleotide-dependent (NAD+) – deacetylases. Our previous studies using a nutrition-induced CU growth model showed that Sirt1 was significantly increased in a growth plate of food-restricted animals and decreased during CU growth. Prompted by our previous finding that Sirt1 is expressed mostly by cells of the proliferative zone, we sought to investigate the role of Sirt1 in nutrition-induced CU growth by knocking out its expression using a collagen-II-specific Cre-Lox system

AIM

This study sought to investigate the role of Sirt1 in modulating the response of the epiphyseal growth plate (EGP) to nutritional manipulation

CONCLUSIONS

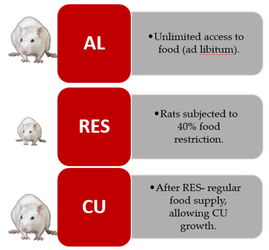
This study shows that Collagen type II-specific knockout of Sirt1 led to:

- Increased proliferative zone and proliferative cells
- Affected EGP height & organization
- Reduced bone mineralization.
- Affects bone structure in both the steady state and in response to nutritional manipulation.
- Less efficient CU growth (body weight, bone length, EGP height, and bone structure)

METHODS

Sirt1-CKO mice **were generated by mating mice with the floxed Sirt1 gene with Col2a1-mice expressing the Cre recombinase under the control of the alpha1 Collagen II promoter.**

Experimental model: **Collagen type II-specific Sirt1 knockout (CKO) or control (CTL) mice were tested for response to our CU growth model consisting of a period of food restriction followed by re-feeding**



Morphological and immunohistochemical staining were performed on 6 µm paraffin sections.

Micro-CT analysis was performed on the entity right humeri. Morphometric parameters were determined by a direct three-dimensional approach in three different pre-selected analysis regions using customized software.

RESULTS

1. Characterization of Col II-Sirt1 knockout mice

Fig.1 IHC with anti-Sirt1 antibodies showing very faint staining in the EGP of the CKO mice compared to the intense staining in the EGP of the CTL mice, especially in the resting and proliferative zones. Faint staining of Sirt1-positive cells is identified in the hypertrophic zones of both CTL and CKO mice.

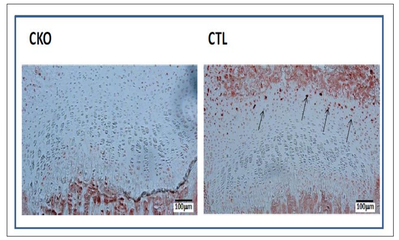
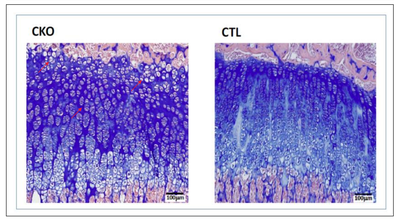


Fig.2 Representative stained sections of the EGP derived from CKO and CTL mice at age 22 days. Hematoxylin–eosin and Alcian blue staining shows that the EGP of the CKO mice is significantly less organized (see arrows).



2. Catch-up growth experiment

CKO and CTL mice were tested for 36 days of food restriction, followed by 28 days of re-feeding. Weight, tibial and humeri length, as well as EGP height were measured . CU growth was less efficient in CKO mice

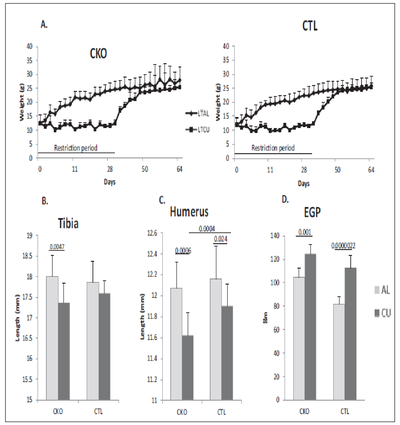
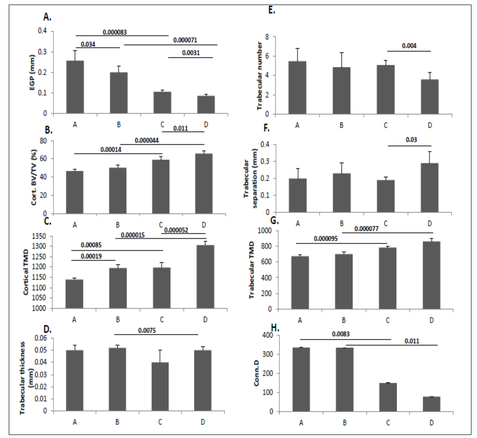


Fig.3 Weight changes over the catch-up growth experiment in CKO (A, left panel) and CTL (A, right panel) mice. Values of tibial length (B), humerus length (C) and EGP height (D).

3. Micro-CT analysis of humeri and EGP

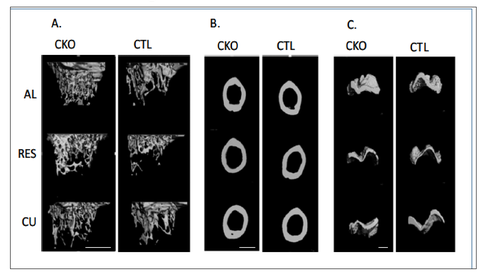
Humeri of CKO and CTL mice were subjected to micro-CT analysis. Both the CTL and CKO mice showed a similar increase In bone mineralization (BV/TV) concomitant with shrinkage of the EGP (by ~60%) with age, as expected. EGP height was still significantly greater in the CKO mice (by 28%). Cortical bone parameters also increased with age in both strains, but were all lower in the CKO mice. At age 88 days, there were also significant differences between the two strains in trabecular volume fraction (Tb.BV/TV), trabecular number (Tb.N), and connectivity density (Conn.D), which were higher in the CKO mice, and in cortical tissue mineral density (Cort.TMD), trabecular thickness (Tb.Th), and trabecular separation (Tb.Sp), which were lower in the CKO mice.

Fig.4 Cortical and trabecular bone parameters at 35 days in CKO (A) and CTL (B) mice; at 88 days in CKO (C) and CTL (D) mice (x-axis).



3D reconstruction of the micro CT data shows the differences in mineralization and EGP height. The response of the CKO mice to food restriction and refeeding was less efficient and differed from that of CTL mice

Fig.5 Representative 3D model of micro-CT findings. Trabecular bone (A), Cortical bone (B), and EGP (C)



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