11β-Hydroxysteroid dehydrogenase type 1 activity contributes to glucocorticoid-dependent musculoskeletal decline in aged mice

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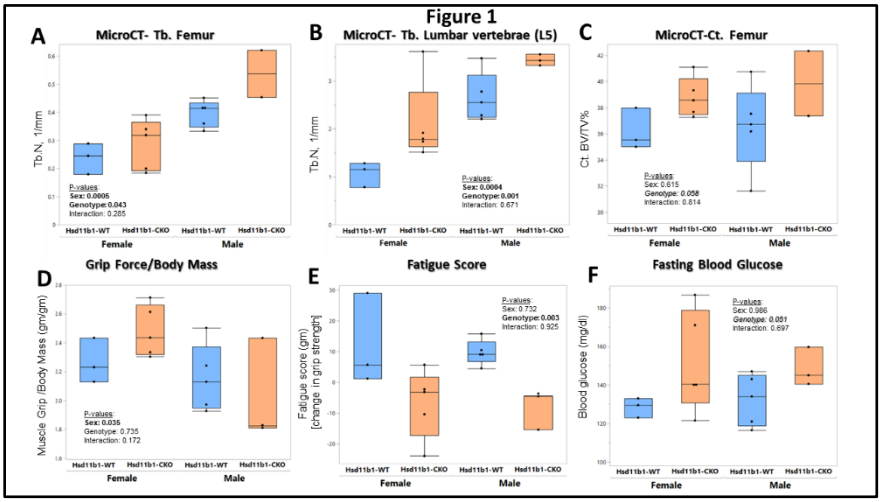
INTRODUCTION: Glucocorticoids (GCs) are hormones that are essential for life, synthesized and released by the adrenal cortex in a circadian manner and in response to stress. It has been previously shown that endogenous GCs increase with age and show disrupted patterns of circadian fluctuation, with older individuals exhibiting higher sustained serum GC levels throughout the day. Endogenous GC levels in mice mimic many of these patterns [1]. GC signaling is unique in that it features an additional layer of local regulation at the tissue level via two enzymes: 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) and type 2 (11β-HSD2). 11β-HSD1 is considered a signaling amplifier in that it re-activates inactive GC, while 11β-HSD2 has an opposite effect. We previously reported that conditional deletion of Hsd11b1 in osteoprogenitor cells using an adult-onset Osx-Cre Hsd11b1$^{f/f}$ mouse model led to higher trabecular bone mass in young adult (6 months old) male and female mice [2]. As we and others have shown that expression of Hsd11b1is upregulated with aging in the bone of male and female mice [2], the aim of this study was to investigate the contribution of osteoprogenitor11β-HSD1 activity to the musculoskeletal system during aging.

METHODS: Hsd11b1 floxed mice were crossed with Osx-Cre+ mice to generate osteoprogenitor-targeted Hsd11b1 conditional knockout (Hsd11b1$^{f/f}$:OsxCre+; Hsd11b1 CKO) and Cre-negative (Hsd11b1$^{f/f}$:OsxCre-) wildtype littermates (WT). Mice were raised on doxycycline until 3 months of age to negate the developmental impact of the Osx Cre as in our previous studies [3], after which they were fed normal chow until 21 months of age. Body composition and bone mineral density (BMD) were measured via dual-energy x-ray absorptiometry (DXA; Kubtec Digimus). Muscle endurance was measured via hang time testing. Muscle strength was measured using a grip strength meter (Bioseb); fatigue score was calculated as per the manufacturer's guidelines by subtracting the average of the first three grip strength measurements from the average of the fourth and fifth readings, where lower fatigue scores indicate higher muscle endurance. Calcein was injected on days 7 and 1 prior to sacrifice to label mineralizing bone surfaces for dynamic histomorphometry studies. Serum, hindlimb bones, spine, skeletal muscles, interscapular brown adipose tissue, and gonadal white adipose tissue were collected at sacrifice. Mice with pathologies (i.e., tumors) discovered at sacrifice were excluded from further study. Femurs and lumbar vertebrae (L4-6) were scanned by micro-computed tomography (Skyscan 1272, 9-micron resolution) to measure cortical and trabecular bone architecture. Groups were compared with 2-way ANOVA to investigate the effects of sex, genotype, and any synergistic interactions on measured properties.

RESULTS: No differences were observed between Hsd11b1 CKO and WT littermates in terms of overall body mass, lean mass, or fat mass in either males or females at 21 months of age ($p_{genotype} > 0.27$). Interestingly, however, whole-body BMD was significantly greater (+7.3%) in aged Hsd11b1-CKO as compared to WT littermate mice in both sexes ($p_{genotype} = 0.045$), suggesting a beneficial impact from the loss of Hsd11b1 activity on the skeleton. Consistent with this high BMD phenotype, and the high trabecular bone mass seen in young adult animals [2], 21-month-old Hsd11b1 CKO mice exhibited increased trabecular bone mass in both the distal femur and L5 lumbar vertebra, driven primarily by an increase in trabecular number (**Figure 1A-B**) and a decrease in trabecular separation in both skeletal locations. Interestingly, whereas cortical bone mass was surprisingly reduced in Hsd11b1-CKO mice at 6 months of age [2], the aged Hsd11b1 CKO mice demonstrated a strong trend for an increase in their femur cortical bone fraction (Ct. BV/TV% $p_{genotype} = 0.058$), although cortical bone area and cortical bone thickness were unaffected ($p_{genotype} > 0.66$) (**Figure 1C**). There was no effect ($p_{genotype} = 0.735$) of Hsd11b1 knockout on muscle strength when normalized to body weight (**Figure 1D**), however, aged Hsd11b1-CKO mice had lower muscle fatigue scores as measured by forelimb muscle grip testing ($p_{genotype} = 0.003$) as compared to WT littermates (**Figure 1E**). While we previously saw no difference in fasting blood glucose levels between WT and CKO mice at 6 months of age, 21-month-old Hsd11b1 CKO mice demonstrated a strong trend for elevated fasting blood glucose levels (p=0.051) (**Figure 1F**). These results suggest a crucial role of local GC activation by Hsd11b1 in bone homeostasis during aging and support the importance of local endogenous GC signaling in mechanisms of skeletal crosstalk with other organ systems.



DISCUSSION: The expression of Hsd11b1 in bone increases with age, likely contributing to the dysregulation of skeletal GC signaling. In the current study, knocking down Hsd11b1 in Osx-expressing cells provided significant benefits to both trabecular and cortical bone compartments in aged mice. We have previously shown that GC signaling through the glucocorticoid receptor (GR) is essential for skeletal homeostasis during aging [3]. It is important to note that GC signaling is not prevented in the Hsd11b1-CKO mice as GC are released in active form via the adrenal glands, and deletion of Hsd11b1 in Osx-expressing cells will not prevent signaling of these already-active GC. Instead, Hsd11b1 deficiency reduces the local amplification of GC signaling, preventing GC inactivated by Hsd11b2 elsewhere from being re-activated by Osx-expressing cells within the skeletal tissue itself. The mechanism of crosstalk between altered skeletal Hsd11b1 activity and changes in muscle fatigue and fasting glucose has not yet been elucidated but is the focus of ongoing studies. Together, these results suggest the presence of a novel and important role for the 11β-HSD1 in the context of the aging skeleton, particularly with respect to bone's role as an endocrine organ. In future studies, we will define the relative contributions of Hsd11b1 activity in discrete cell populations (e.g., osteoblasts, osteoclasts, bone marrow adipocytes) within the bone niche to the overall mechanisms of skeletal aging.

SIGNIFICANCE/CLINICAL RELEVANCE: These findings indicate that 11β-HSD1 is a key regulator of musculoskeletal aging, suggesting that 11β-HSD1 may represent a viable therapeutic target in the treatment of age-associated bone loss.

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REFERENCES: [1] Weinstein et al, Aging Cell (9). 2010 [2] Bensreti et al., ASBMR Annual Meeting 2022. [3] Pierce et al, JBMR 2022 37(2): 285-302.
IMAGES: Figure 1