Crosstalk Between Glucocorticoid Receptor (GR) and Aryl Hydrocarbon Receptor (AhR) Signaling in Bone

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INTRODUCTION: Glucocorticoids (GCs) are steroid hormones that regulate the body's systemic stress response and exert whole-body effects on metabolism, immunity, and skeletal homeostasis [1]. Excess or chronic GC exposure promotes osteoporosis and skeletal fragility, mediated in part through the glucocorticoid receptor (GR), a ligand-activated nuclear receptor that regulates gene transcription in bone cells [1]. In parallel, the aryl hydrocarbon receptor (AhR) is a ligand-dependent transcription factor known for regulating xenobiotic metabolism, but it also plays an emerging role in bone biology, where it influences osteoblast differentiation and bone remodeling [2]. Recent work from our group and others has revealed that metabolites such as kynurenine (KYN), a tryptophan-derived endogenous AhR ligand, accumulate with aging and have been linked to musculoskeletal decline [2]. Nuclear receptors rarely act in isolation, and instead extensively interact through crosstalk mechanisms that can either synergize or antagonize downstream transcriptional responses [3]. Furthermore, GR has been shown to interact with other nuclear receptors in metabolic tissues [1], but whether GR and AhR functionally interact in the skeleton remains poorly defined. Thus, understanding GR–AhR crosstalk in bone could provide new insights into how stress hormones and metabolic intermediates collaboratively influence skeletal aging. **METHODS:** ST2 were sequentially treated with the AhR ligand 6-formylindolo[3,2-b] carbazole (FICZ) or the GR ligand dexamethasone (Dex) followed by dual luciferase reporter (DLR) assays and RT-qPCR analyses. For the DLR assay, cells were transfected with either an AhR (XRE-Luc) reporter plasmid or a GR (MMTV-Luc) reporter plasmid for 24 hours. Cells were then subjected to sequential ligand treatments with a 3-hour interval between the first and second treatment to capture early transcriptional dynamics. Treatments included FICZ, Dex, or vehicle controls (DMSO for FICZ, ethanol for Dex). Luciferase activity was measured using a commercial kit (Dual-Luciferase Reporter Assay, Promega). For gene expression analyses, similar experiments were performed but RNA was collected in TRIzol, and RT-qPCR was used to measure expression of Cyp1a1 mRNA (a canonical AhR target gene [2]) and Hsd11b1 mRNA (a GR target gene encoding 11β-HSD1, an enzyme that amplifies local GC signaling [1]). To study GR and AhR crosstalk in vivo, mice with loxp sites flanking exon 2 of the GR gene were crossed with Osx-Cre^{+} mice for several generations to generate GR-conditional knockout (GR-CKO; GR$^{fl/fl}$: Osx-Cre^{+}) mice along with wild-type (GR-WT; GR$^{fl/fl}$: Osx-Cre^{-}) littermates. Mice were group-housed in an accredited facility and maintained on a 12-hour light/dark cycle, and the research was conducted according to guidelines provided by the National Institutes of Health (NIH) under protocols approved by the Augusta University Institutional Animal Care and Use Committee (IACUC). Mice were raised on doxycycline until 3 months of age to negate developmental impact of the Osx Cre transgene as in our previous studies [4], after which they were fed normal chow. Adult (6-12 mo old) male and female GR-CKO and wild-type mice were treated with daily injections of KYN (20 mg/kg/day) or PBS vehicle for 8 weeks. Bone mineral density (BMD) and body composition were assessed at baseline and at 4 and 8 weeks of treatment using dual-energy X-ray absorptiometry (DXA). Muscle function was evaluated with forelimb grip strength testing and endurance hangtime assays. In addition, fasting blood glucose was measured following a 6-hour fast. After 8 weeks, mice were sacrificed and tissues collected for skeletal and metabolic analyses. *Ex vivo* high-resolution microCT (Skyscan 1272) was performed to analyze trabecular and cortical microarchitecture of the femur and lumbar vertebrae. *In vitro* data were analyzed using Student's t-tests, and the *in vivo* data were analyzed using two-way ANOVA to assess the effects of genotype, treatment, and their interaction in male and female mice. **RESULTS:** Sequential ligand treatments in ST2 cells revealed asymmetric crosstalk between GR and AhR. Activation of GR with Dex significantly suppressed AhR transcriptional activity, as shown by decreased XRE-luciferase reporter activity (p=0.05; Fig. 1A) and reduced expression of the AhR target gene Cyp1a1 (p=0.038; Fig. 1B). In contrast, AhR activation with FICZ did not significantly alter GR reporter activity or Hsd11b1 expression, although a trend toward increased GR reporter transcriptional activity was observed (p=0.083 and p=0.111; Fig. 1C–D). In vivo, KYN treatment induced sex-, genotype-, and site-specific effects on bone. In male WT mice, KYN tended to decrease trabecular number (ANOVA $p_{Interaction}$=0.056; Fig. 1F) and significantly increased trabecular separation (ANOVA $p_{Interaction}$=0.018; Fig. 1H) in the lumbar vertebrae. These detrimental effects were absent in GR-CKO males, and in fact, DXA analysis revealed a significant increase in L4–L6 lumbar vertebral BMD in male GR-CKO mice treated with KYN (ANOVA $p_{Interaction}$=0.02; Fig. 1J). Cortical bone thickness was not significantly affected by KYN, although it tended to be reduced in male WT but not male GR-CKO mice that received KYN injections (ANOVA $p_{Interaction}$=0.056; Fig. 1L), suggesting that deletion of GR in osteoprogenitor cells may provide a protective effect against KYN in cortical bone. In female mice, no synergistic trends between GR expression and KYN treatment were detected for most trabecular or cortical bone parameters (ANOVA $p_{Interaction}$>0.05; Fig. 1E, I, K). However, trabecular separation was increased in female GR-CKO mice treated with KYN (ANOVA $p_{Interaction}$=0.007; Fig. 1G), indicating a site- and sex-dependent response. Body composition analyses did not show any change in lean mass and fat mass between experimental groups. Muscle functional assays revealed genotype-dependent differences in males (ANOVA $p_{Interaction}$=0.044; Fig. 1N), where KYN treatment enhanced grip strength in GR-CKO mice, although this effect was absent in female mice (Fig. 1M). Fasting blood glucose was not significantly altered by KYN treatment in either genotype (*not shown*). **DISCUSSION:** Our studies revealed that GR and AhR engage in asymmetric crosstalk in osteoblast-lineage cells. GR activation with Dex significantly suppressed AhR reporter activity and Cyp1a1 expression, whereas AhR activation with FICZ did not significantly affect GR reporter output or Hsd11b1 transcription, suggesting that GR exerts dominant control over AhR signaling in osteoblast-lineage cells. Consistent with this idea, KYN treatment produced different site- and sex-specific skeletal responses between bone-targeted GR knockout mice and their WT littermates. In WT males, KYN negatively affected vertebral microarchitecture as we have previously reported [5], whereas these effects were absent in GR-CKO males. Strikingly, GR-CKO males instead exhibited increased lumbar vertebral BMD following KYN treatment, suggesting that deletion of GR in osteoprogenitors may provide protection against KYN-induced bone loss and could even promote compensatory effects in specific skeletal sites. In females, most parameters were unaffected by KYN in this study, although trabecular separation was increased by KYN in GR-CKO animals, highlighting a distinct and potentially adverse site-dependent response. Cortical bone parameters were largely unchanged across groups. Outside of bone, KYN enhanced grip strength in GR-CKO males, pointing to possible systemic musculoskeletal effects of AhR ligands that depend on GR expression in bone. Fasting glucose was unaffected, suggesting that short-term GR–AhR interactions in bone may not directly influence systemic glucose metabolism. Overall, these findings establish GR as a critical regulator that suppresses AhR transcriptional activity in bone and reveal that the skeletal effects of KYN are both sex- and site-specific, as well as GR-dependent. The results highlight a previously unrecognized role for GR–AhR crosstalk in shaping skeletal responses to metabolic intermediates that accumulate with aging and inflammation. **SIGNIFICANCE/CLINICAL RELEVANCE:** Crosstalk between GR and AhR signaling pathways may contribute to skeletal responses to stress and environmental metabolites. Identifying GR-dependent modulation of AhR activity provides insight into osteoporosis pathogenesis and may inform therapeutic strategies targeting nuclear receptor crosstalk in bone. **ACKNOWLEDGEMENTS:** Supported by NIA P01-AG036675 (Project 4) and R01AG067510. **REFERENCES: [1]** Bensreti et al, COR. 2023;21(1):32-44 **[2]** Alhamd et al,. JME. 2022;69(3) **[3]** Jin et al., IntJE. 2017;2017:5679517 **[4]** Pierce et al., JBMR 2022 37(2): 285-302 **[5]** El Refaey JBMR 2017; 32(11): 2182-2193. **IMAGES**: Figure 1

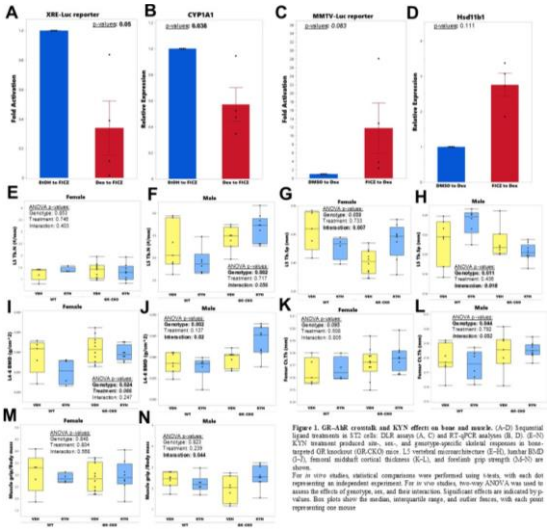

Figure 1. GR-AhR crosstalk and KYN effects on bone and muscle. (A-D) Sequential ligand treatments in ST2 cells: DLR assays (A, C) and RT-qPCR analyses (B, D). (E-N) KYN treatment produced site-, sex-, and genotype-specific skeletal responses in bone-targeted GR knockout (GR-CKO) mice. L5 vertebral microarchitecture (E–H), lumbar BMD (I–J), femoral midshaft cortical thickness (K–L), and forelimb grip strength (M–N) are shown. For in vitro studies, statistical comparisons were performed using t-tests, with each dot representing an independent experiment. For in vivo studies, two-way ANOVA was used to assess the effects of genotype, sex, and their interaction. Significant effects are indicated by p-values. Box plots show the median, interquartile range, and outlier fences, with each point representing one mouse