Glucocorticoids reduce bone strength through reduction in vascularity and hydration, while concurrent treatment with PTH increases bone mass and preserves angiogenic and nitric oxide gene expression in glucocorticoid-treated mice

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Introduction

Glucocorticoids (GC) induce osteonecrosis (ON) and osteoporosis (OP); however, the mechanism is complicated. While GCs may increase the risk of ON by reducing angiogenesis and vasoactivity, the reduction in bone strength that accompanies GC use is greater than can be explained by the loss of bone mass alone. To try to understand this discrepancy, we evaluated GC's effects on novel bone quality measures, including bone bone hydration, bone blood flow, and bone angiogenesis gene expression. We performed two experiments. The first was to understand the role of GC on bone hydration, bone blood flow, and strength, and whether this is altered by anti-vascular endothelial growth factor (VEGF). In the second study we evaluated GC effects on bone vascularity by evaluating gene expression in bone, and if PTH, a known vasculoactive agent, influences this.

Methods

- **Part 1. NOVEL MEASURES OF BONE STRENGTH: HYDRATION, BLOOD FLOW (SUV), & STRENGTH**
  - 9-week-old male BALB/cj mice (n=8 per group) were randomized into groups receiving Vehicle (VEH), GC (4 mg/km/d methylprednisolone) for 120 days), GC for 60 days followed by anti-VEGF for 60 days, or GC for 60 days followed by no treatment for 60 days. Mice were sacrificed on day 60 or 120
  - Outcome measures: bone strength, PET/CT NaF for blood flow (SUV), bone hydration volume fractions of bound water (BW) using ^{1}H-NMR relaxometry were measured on the intact right femurs
  - IHC of distal femur blood vessels with endomucin and CD31.
- **Part 2. GC EFFECTS ON BONE ANGIOGENESIS GENE EXPRESSION & BONE HEALTH**
  - 12-week-old male BALB/cJ mice were randomized into groups receiving VEH, GC (4 mg/kg/d methylprednisolone by pellet), or GC+PTH 40 ug/kg/d for 45 days (n=12-24 per group). Mice were sacrificed on day 45.
  - Outcome measures: trabecular bone volume (BV/TV), trabecular thickness (Tb.Th), trabecular number (Tb.N), and structure model index (SMI) of lumbar vertebral body (LVB) 5 trabecular bone was determined by MicroCT
  - RNA was extracted from LVB4 to perform 3'-Tag RNA-Sequencing (RNA-Seq) (n=4/grp).
  - Differentially-expressed genes were determined followed by hierarchical clustering and functional annotation enrichment analyses with the ToppFun tool.

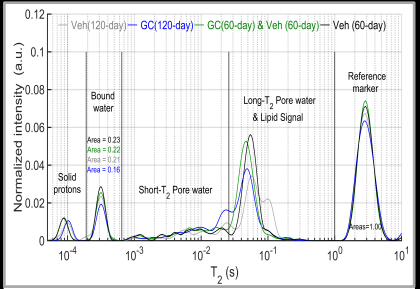

Figure 1. Hydration of bone in GC, GC-vehicle and control groups. Area under the curve for bound water is notably reduced in GC-120 day group compared to vehicle and recovery groups.

Results

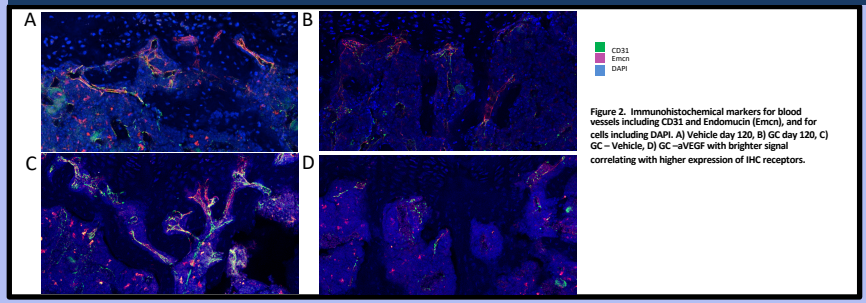

Figure 2. Immunohistochemical markers for blood vessels including CD31 and Endomucin (Emcn), and for cells including DAPI. A) Vehicle day 120, B) GC day 120, C) GC – Vehicle, D) GC –aVEGF with brighter signal correlating with higher expression of IHC receptors.

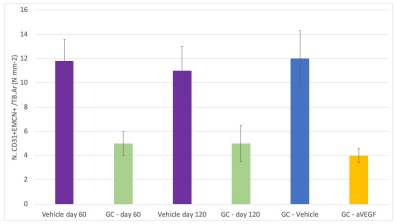

Figure 3. CD31 signal was significantly higher in the vehicle group compared to the GC group at 60 days. The signal from the CD31 IHC was also significantly higher in the vehicle group compared to the GC-120 day group, the GC-vehicle group compared to the GC – aVEGF group, and the control group compared to the GC-aVEGF group.

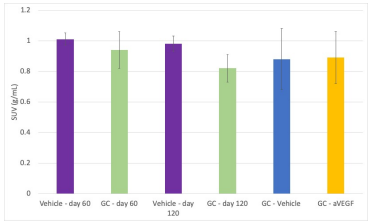

Figure 4. SUV as a indirect measure of bone blood flow measured at 30 minutes

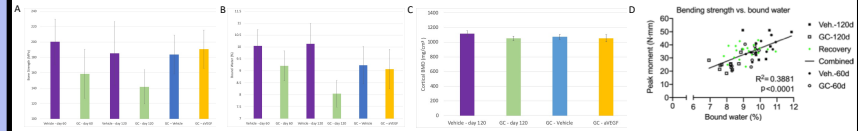

Figure 5. A) Bone strength, measured as bending strength, was significantly different between vehicle day 60 and GC groups, vehicle day 120 and GC-120, GC-vehicle and GC-120, and GC-aVEGF and GC groups. B) Bound water was significantly different between all groups. C) Bone mineral density (BMD) was stable across all groups. D) Bending strength, a reflection of bone strength, and bound water were significantly correlated.

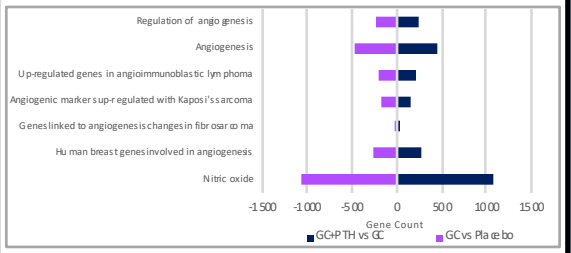

Figure 6. Gene expression associated with the angiogenic and Nitric Oxide (NO) pathways differed between GC-only and VEH mice, and GC-only and GC+PTH treated mice.

Table

Group	VEH (n=10)	GC only (n=24)	GC+PTH (n=23)
Variable	Mean±SD	Mean±SD	Mean±SD
BV/TV (%)	22.9±2.2	18.8±3.4^{v}	30.8±3.5^{v§}
Tb.Th (μm)	47.9±2.1	42.4±3.4^{v}	57.7±4.3^{v§}
Tb.N (1/mm)	4.80±0.35	4.80±0.36	4.71±0.38
SMI	0.79±0.25	1.23±0.25^{v}	-024±0.41^{v§}
Incidence of Osteonecrosis (%)	0 (0/8)	28 (6/21)	6 (1/17)

^{v}P<0.0005 compared to VEH; §P<0.0005 compared to GC-only

Table 1. Measurements of bone strength, microarchitecture, and osteonecrosis.

Summary and Conclusion

GCs reduce bone strength through reduction in bone vascularity and hydration with less change in bone mass. Interestingly, GCs reduces nitric oxide and angiogeneic gene expression while hPTH(1-34) can reverse it. Future studies should address if GC+PTH can prevent GC induced bone fragility through maintenance of vascularity and hydration.

Acknowledgement

This work was supported by National Institutes of Health/National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIH/NIAMS); Grant numbers: P50AR060752, P50AR063043, R01 AR043052; the California Institute of Regenerative Medicine (CIRM); and the endowment for the Center for Musculoskeletal Health and the Aging Endowment to NEL.