Inhibition of Vascular Endothelial Growth Factor Causes Low Bone Blood Flow, Bone Strength, and Bone Hydration with no Effect on Bone Mass

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Avastin™ Background

- Humanized monoclonal antibody to VEGFA (bevacizumab [BVZ]) that slows cancer growth by inhibiting blood vessel proliferation and normalizing blood vessel structure within solid tumors.
- First approved 15yrs ago as *adjuvant* chemotherapy for colorectal, lung (non-small cell), ovarian, cervical, and renal cancer.

Avastin™ Adverse Events

- Disrupts bone elongation
- Clinical adverse events include:
  - Hypertension (20–40%)
  - Delayed wound healing (>70%)
  - Heart attack
  - Stroke
  - Periodontitis/osteonecrosis of jaw (?)

Avastin™ Teaching Moment

- *Vascular Integrity Maintenance*
  - As blood vessels in mature tissues cease to function, new blood vessels sprout into tissue regions where existing vessels have failed.
    - **collateral circulation**
  - *Vascular Integrity Maintenance* is mediated by VEGF and related signaling pathways.

- Murakami M, Simons M. Regulation of vascular integrity. *J Mol Med* 2009; 87:571–582.

Hypothesis

- Inhibition of VEGF in adult mice decreases bone blood flow in trabecular bone regions, with no effect on bone mass or bone strength.

Experimental Design

- 10 week old BALB/cJ male mice
- Two groups (N=12 each)
  - Vehicle (0.9% saline, SC)
  - Anti-VEGFA rodent antibody (B20-4.1.1; 5mg/kg 2X/wk SC; Genentech; South San Francisco, CA)
- Necropsy after 6 wks. Obtain:
  - LV6 and right femur wrapped in gauze at -20 ˚C
  - Right proximal humerus in 70% EtOH

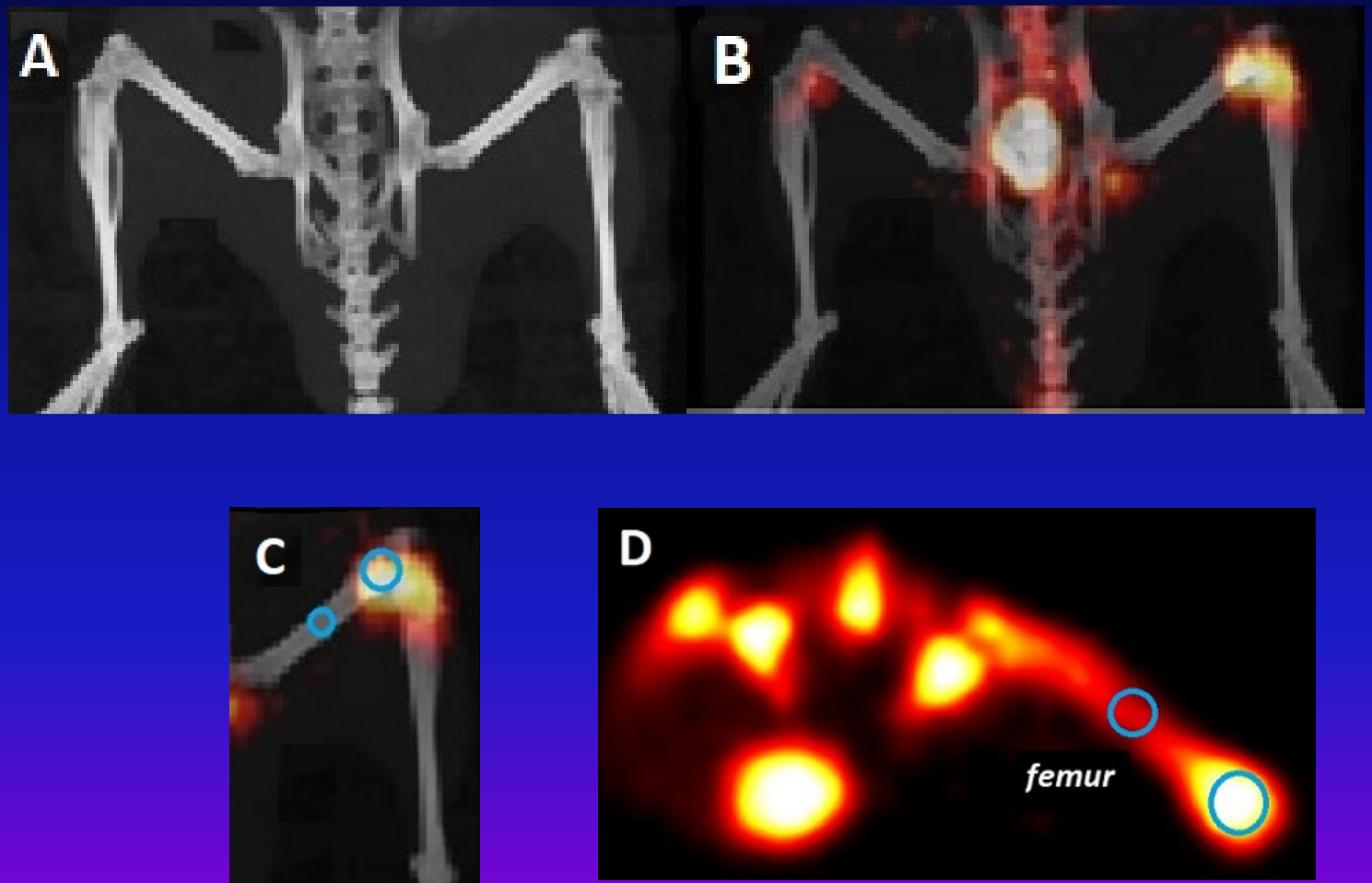
Endpoints

- Bone Blood Flow (Distal Femur)
  - *In Vivo* ^{18}F–PET/CT, a morphometric method; K1– rate of blood flow to bone
- Bone Strength (Lumbar Vertebral Body 6)
  - Ultimate Load (Compression Test)
- Bone Mass (trabecular region of Proximal Humeral Metaphysis)
  - BMD (pQCT)
- Bone Hydration (^{1}H NMR) (Whole Femur)
  - Volume Fraction of Bound Water (%)

^{18}F-NaF-PET/CT

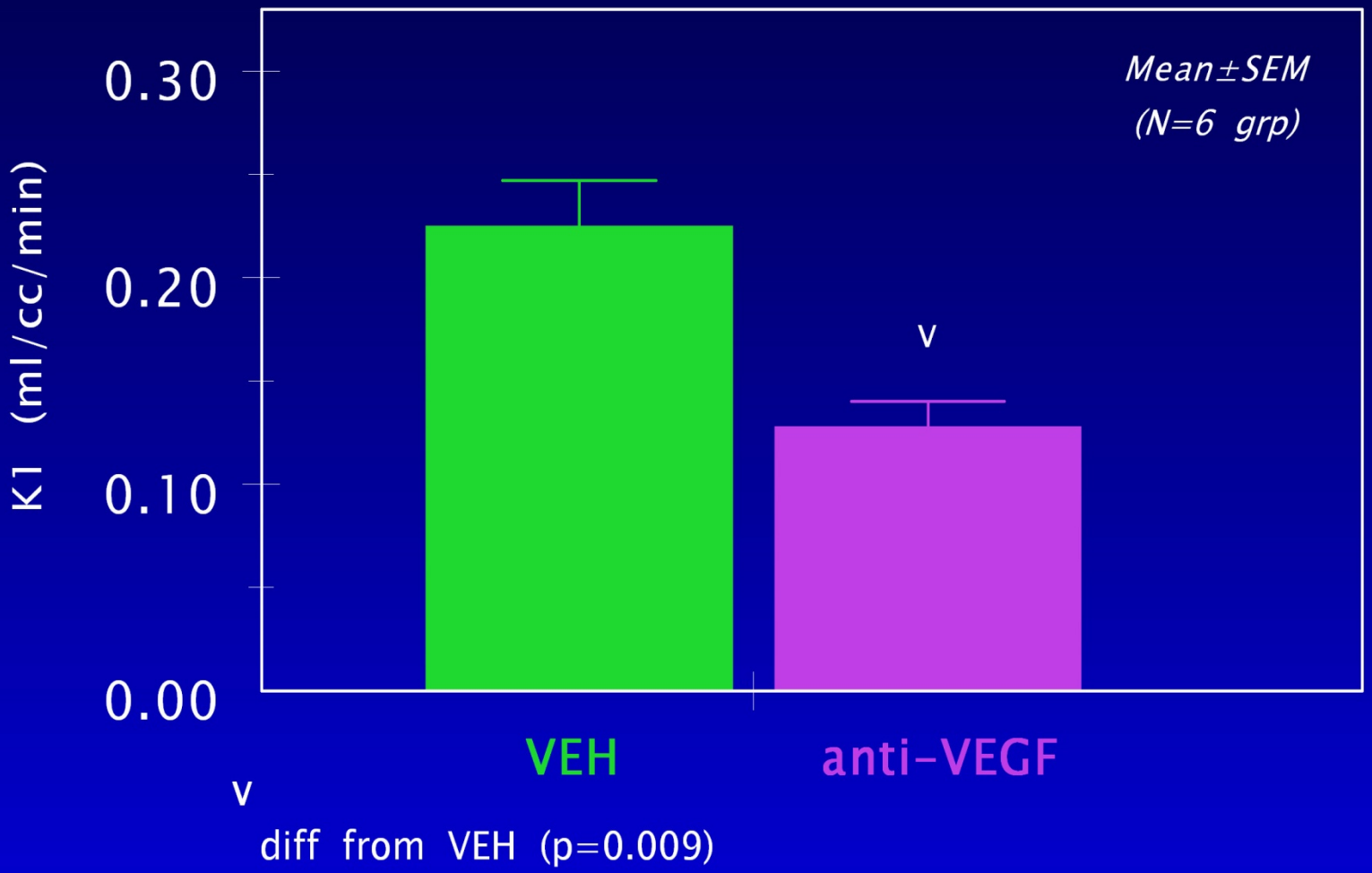
- Position anesthetized mouse on scanning bed with fiducial markers
- Start PET Scan, then inject ^{18}F IV. Scan continuously for 30min (0.8mm voxel)
- Do CT scan (0.15mm voxel)
- Reconstruct scans (PET Scan in selected timeframes w/r to ^{18}F location); superimpose PET & CT scans
- Place VOIs in left ventricle at 0-1min and trabecular & cortical regions at 15-20min post-^{18}F injxn
- Quantitate ^{18}F in each VOI; calculate K1 (ml/cc/min), rate of ^{18}F flow from blood to bone ECF

^{18}F–NaF–PET/CT

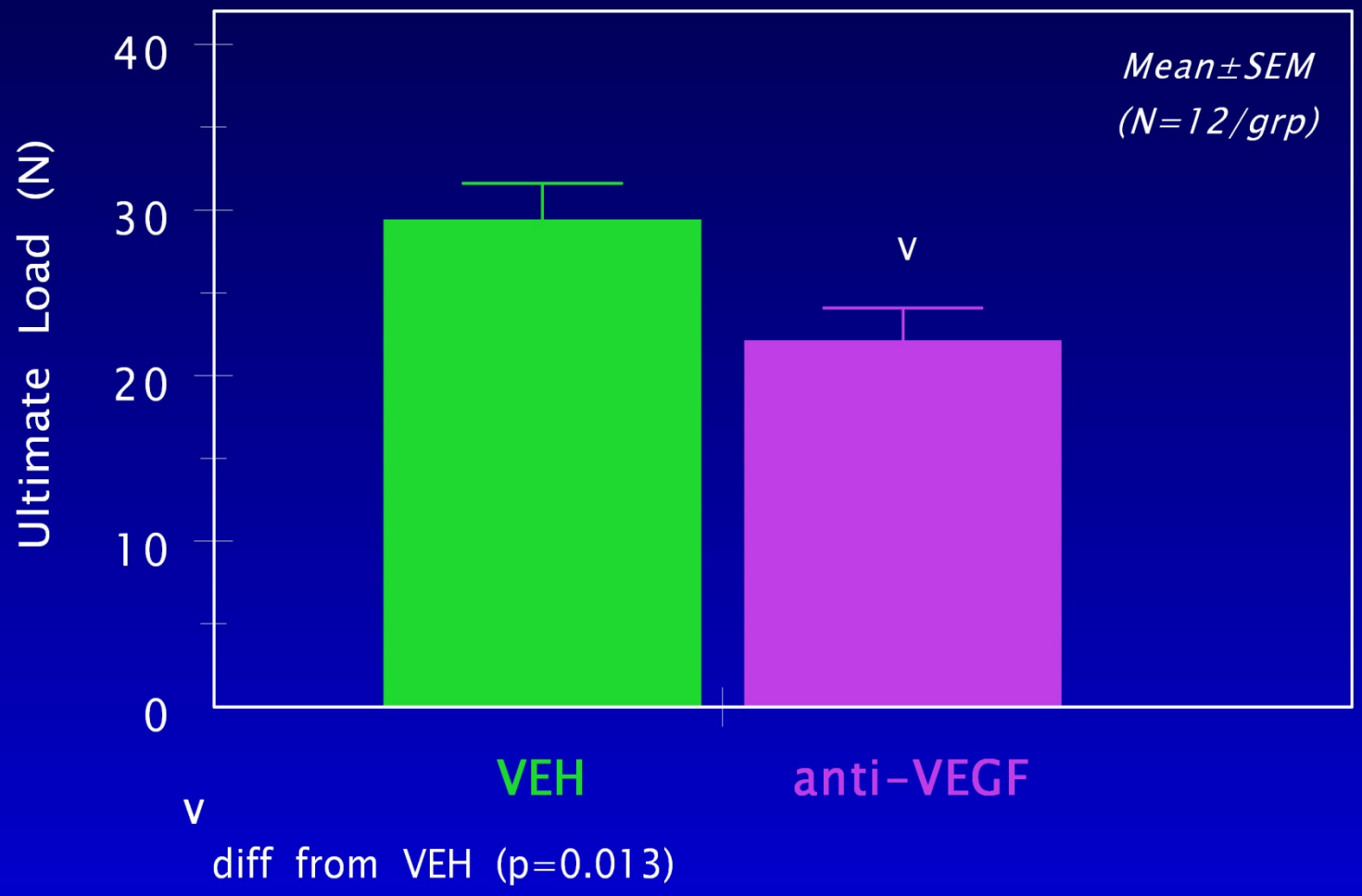


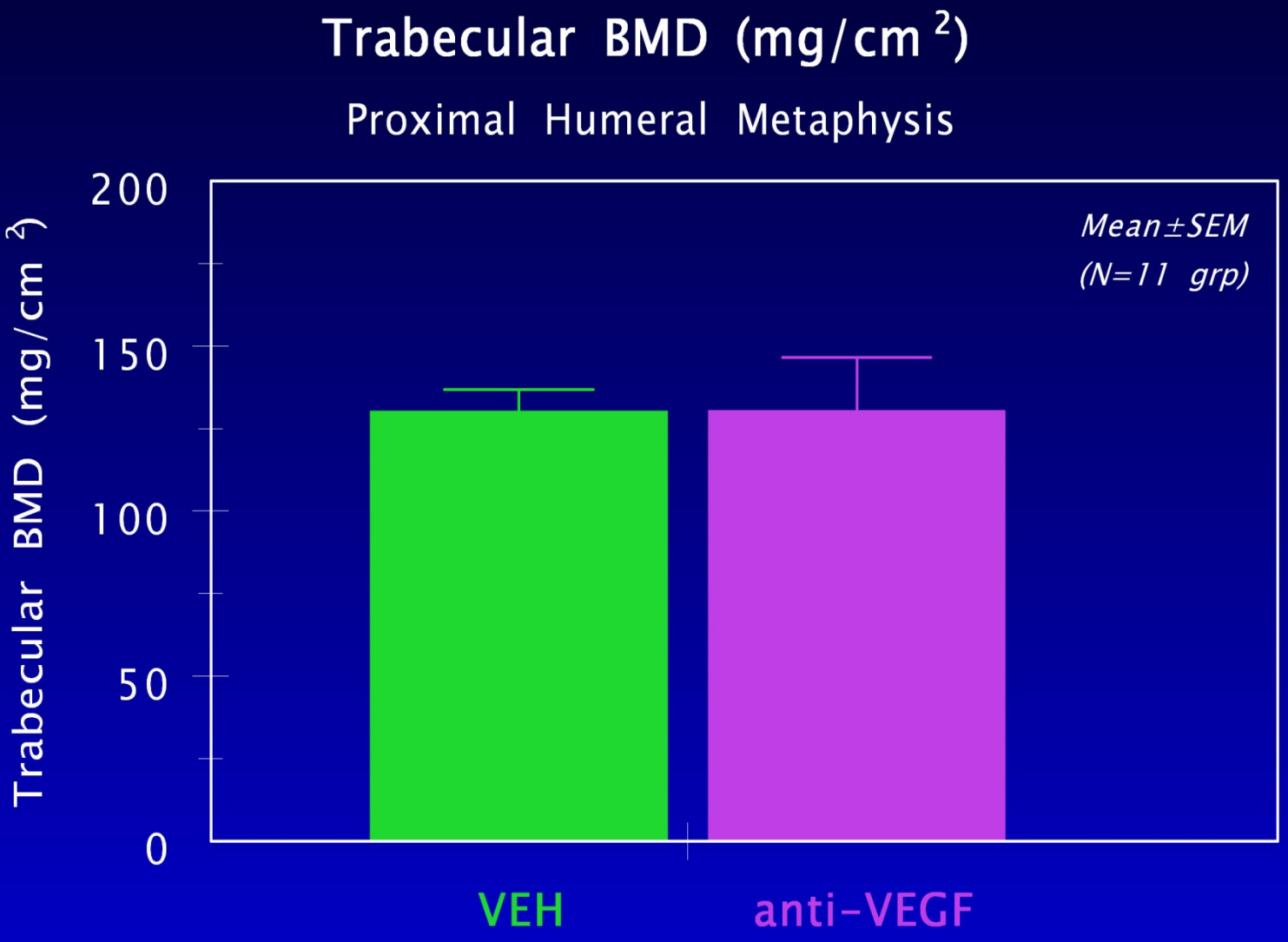
Blood Flow (K1)

Right Distal Femur

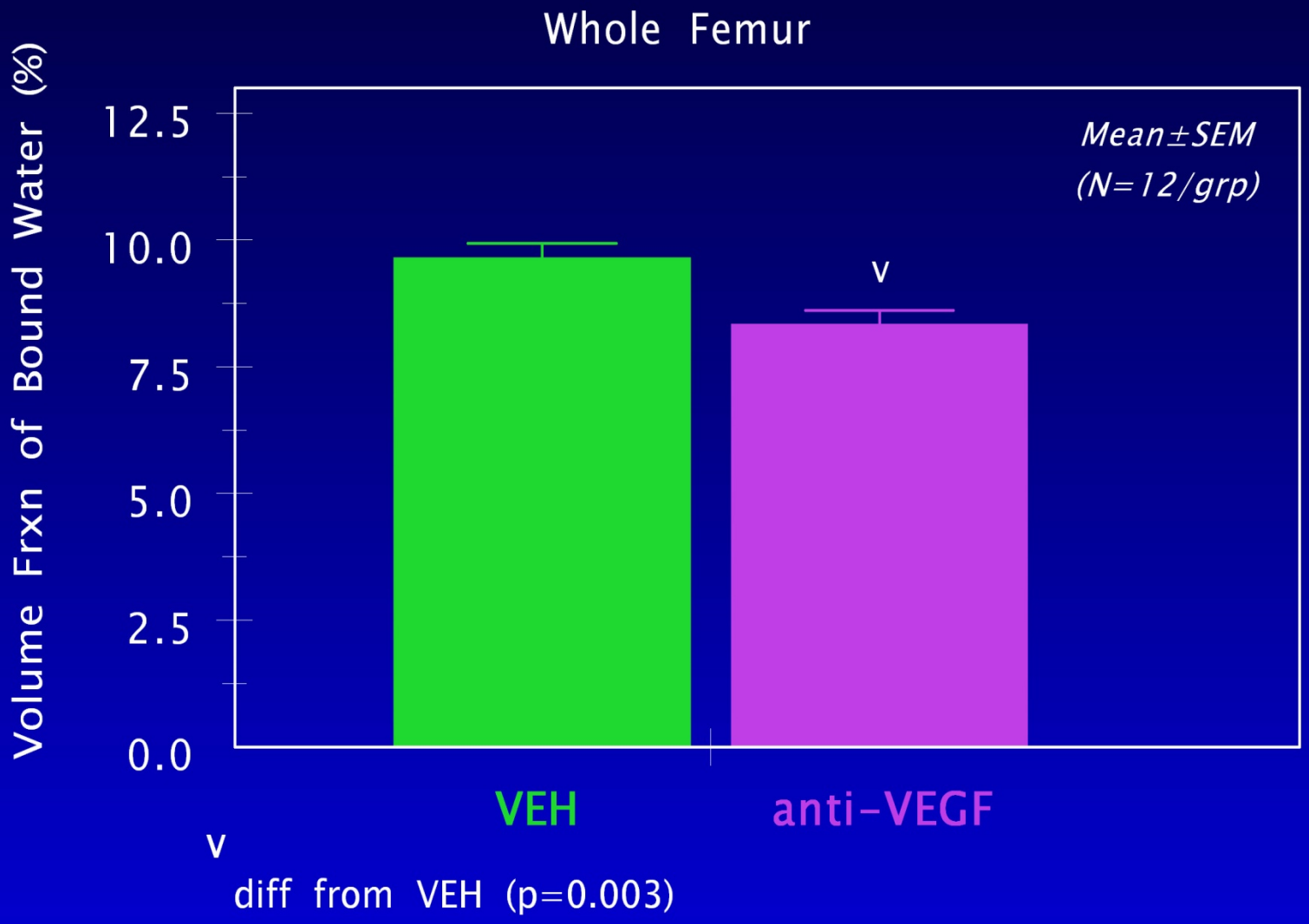


Ultimate Load (N)




Trabecular BMD (mg/cm 2)
Proximal Humeral Metaphysis
Trabecular BMD (mg/cm 2)
200
150
100
50
0
Mean ± SEM
(N=11 grp)
VEH
anti-VEGF

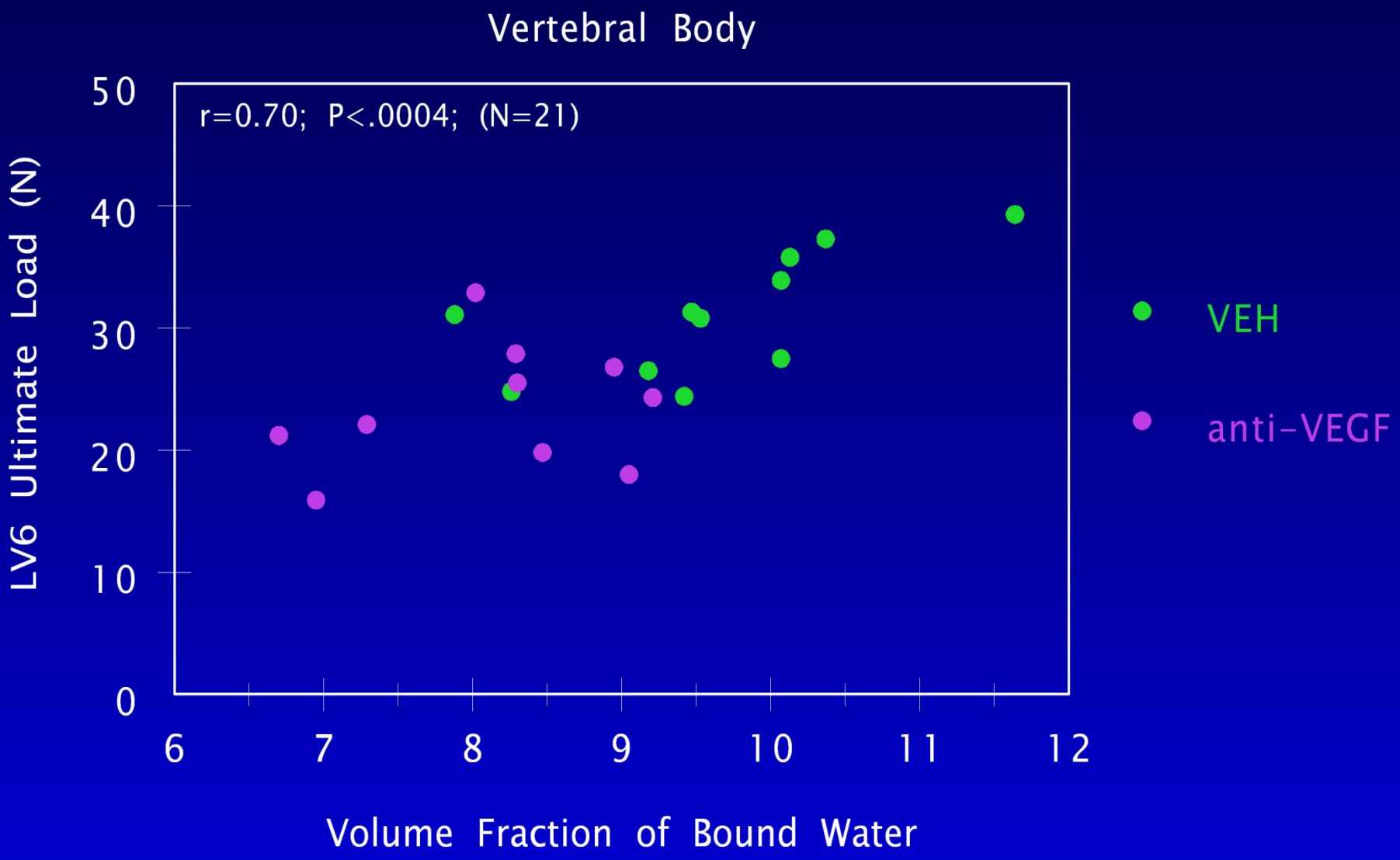
Volume Fraction of Bound Water (%)



Data Summary

- In trabecular bone regions, anti-VEGFA causes:
  - 41% lower bone blood flow (distal femur)
  - *23% lower ultimate load (LV6 body)*
  - no effect on BMD (proximal humeral metaphysis)
  - 10% lower bone hydration (whole femur)

Ultimate Load vs. Volume Fraction of Bound Water



Conclusion

- Anti-VEGFA reduces bone blood flow in trabecular bone of young adult mice.
- Anti-VEGFA reduces bone strength *without* affecting bone mass.
- Anti-VEGFA reduces bone hydration.
- Bone strength is well-correlated to volume fraction of bound water.
- Hypothesis not validated, but…!

Weaknesses

- Studied three *different* trabecular bone rich regions:
  - could have measured both blood flow and BMD in distal femur
  - could have measured both BMD and bone strength in LV6 vertebral body
- Should have used IgG antibody as VEH
- No histomorphometric analyses completed
- Expand analyses to cortical bone
- Recommend larger N

Acknowledgment

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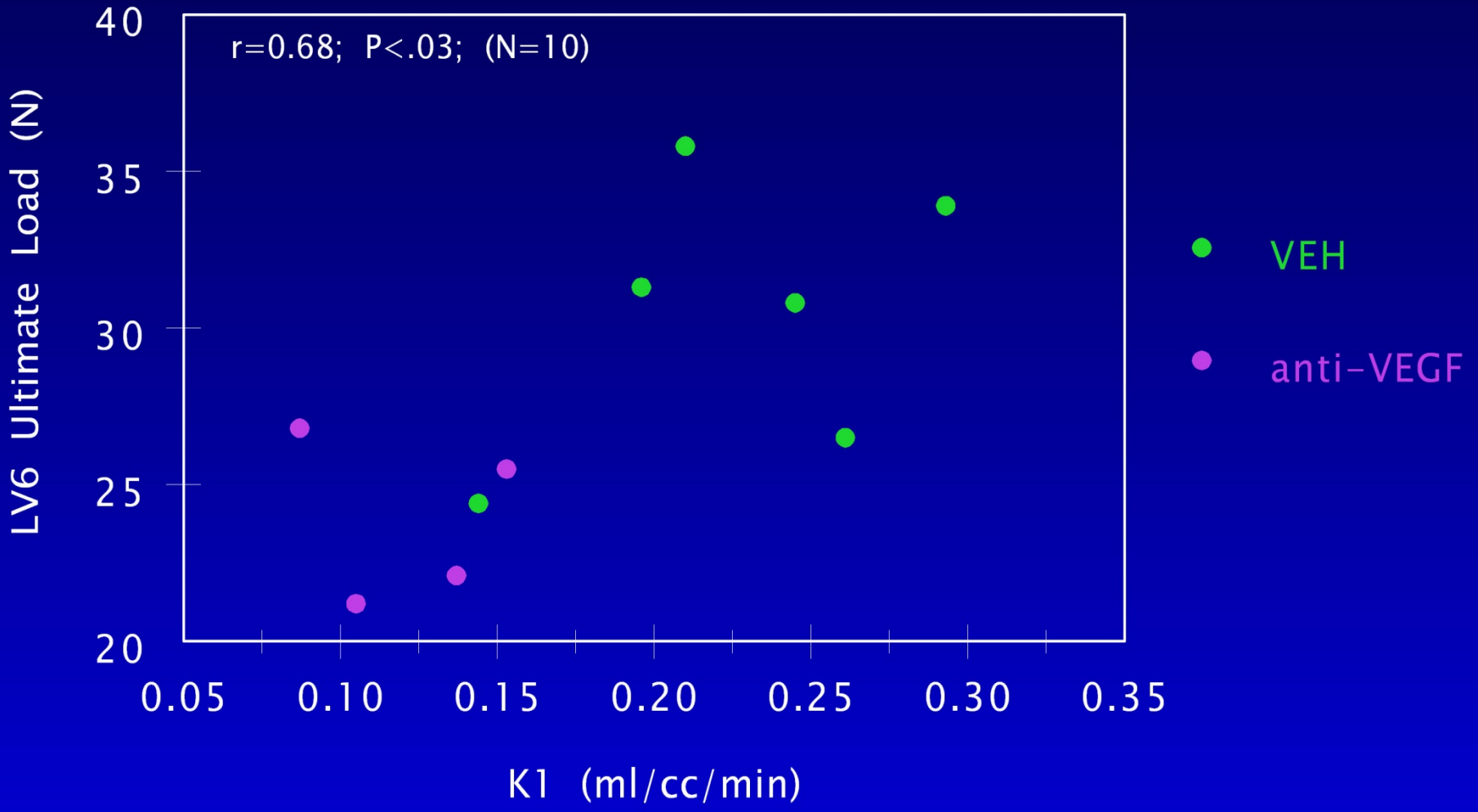
Measuring Bone Blood Flow

- Past techniques include:
  - Necropsy day perfusion with India ink or MicroFil followed by morphometric evaluation of vessel area/volume
  - Necropsy day in vivo ^{95}Nb or ^{103}Ru-labeled microspheres
  - Laser Doppler flowmetry
- Technology for measuring bone blood flow has improved over the past decade (^{18}F-NaF-PET/CT)

Ultimate Load vs. Blood Flow

Vertebral Body



Blood Flow vs. Volume Frxn of Bound Water

Vertebral Body