Using Bone Imaging and Mechanical Testing for Skeletal Phenotyping

WU Musculoskeletal Research Center
Musculoskeletal Structure & Strength Core

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July 13, 2018

Washington University in St. Louis

MUSCULOSKELETAL RESEARCH CENTER
at Washington University

???

- I am studying a genetic mutation. Does my mouse have a skeletal phenotype?
- I treated rats with drug XYZ. What was the effect on their bones?
  - any evidence of increased bone formation?
  - … increased resorption?
  - are the bones stronger?
- What outcomes do I need to measure?
- How do I integrate microCT and mechanical testing outcomes?

Structure & Strength

- Imaging → Structure
  - how much bone is there? how is it distributed?
  - density, morphology (geometry)
  - whole-bone, cortical, cancellous

- Mechanical Testing → Strength
  - stiffness, strength, toughness
  - length scale
    - large: ‘structural’ (whole-bone, organ)
    - small: ‘material’ (tissue, e.g., cortical bone)

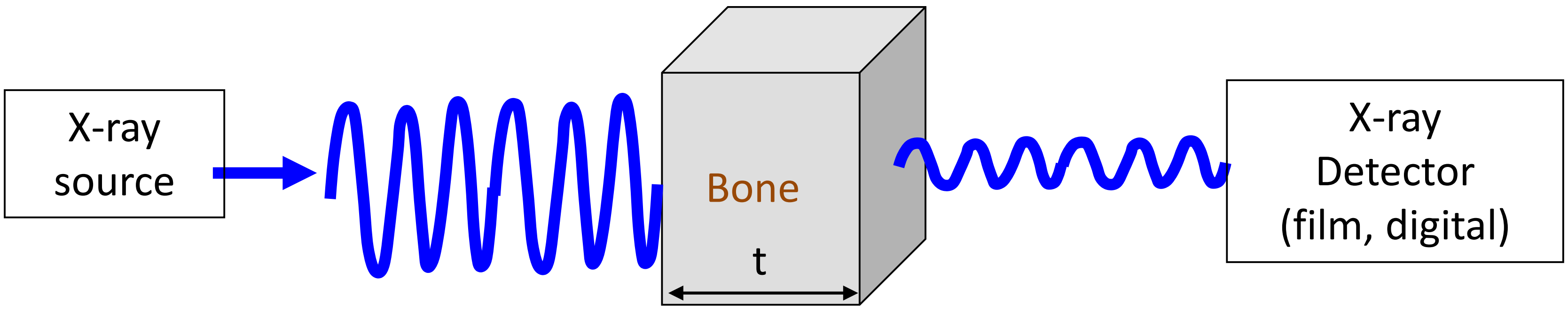
Imaging → Structure

X-Ray Based Imaging

https://theconversation.com/curious-kids-is-x-ray-vision-possible-90393

X-Ray Based Imaging

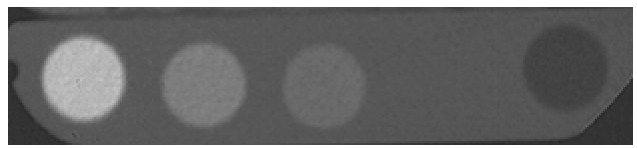
- Based on x-ray absorption/attenuation

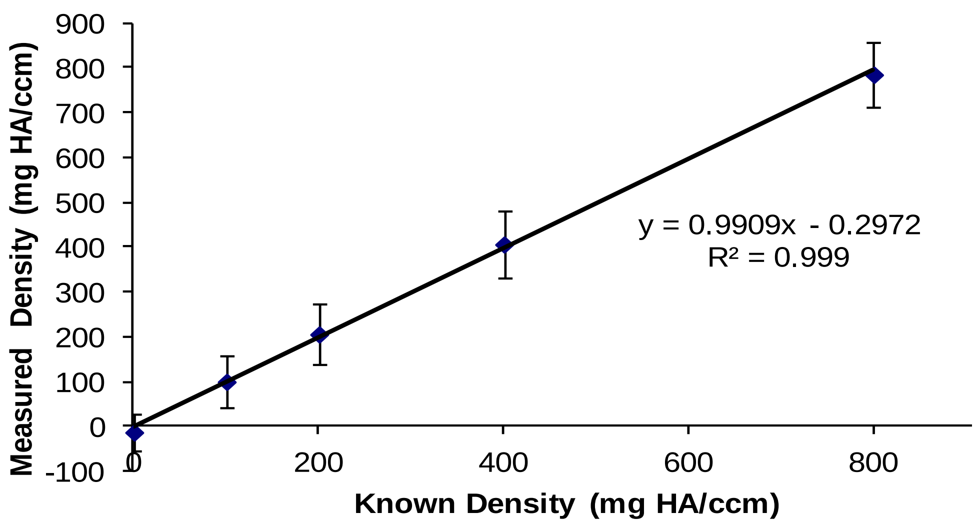


- Attenuation depends on thickness (t), mineral density

X-Ray Based Imaging

- Can calibrate x-ray absorption to equivalent density of hydroxyapatite (HA) phantom





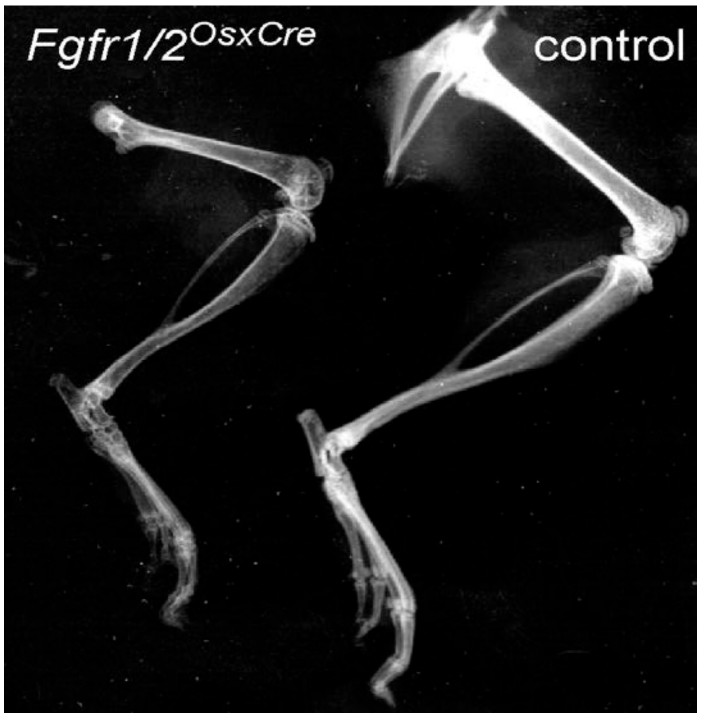
MicroCT density calibration

Radiography

- <u>Outcomes</u>: qualitative phenotype, spontaneous fractures, fracture callus

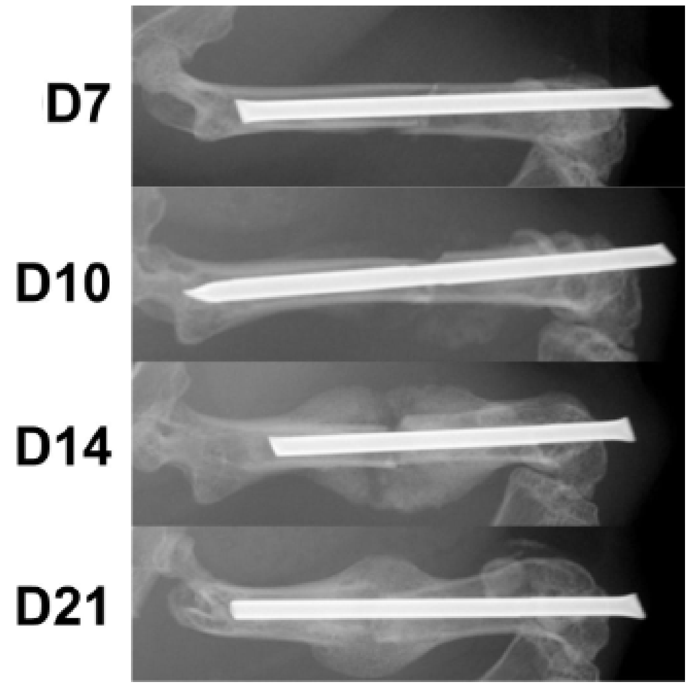

Faxitron

Mouse hindlimbs



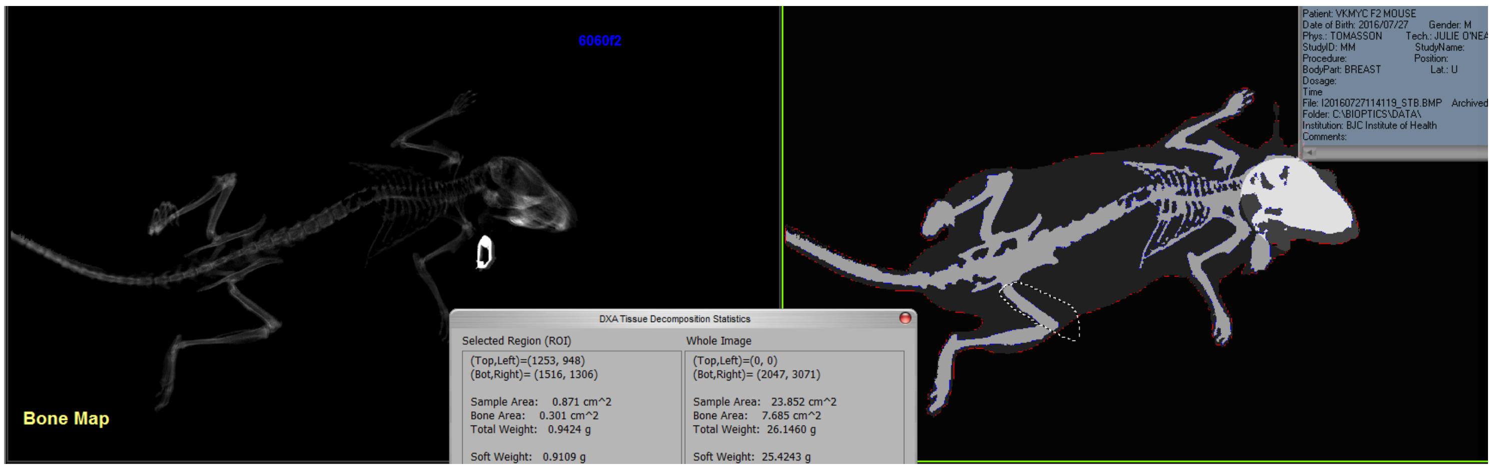
Karuppaiah, Ornitz et al (2016)

Mouse femur fracture



Liu, McKenzie, Silva et al. (2017)

DXA, Dual-Energy X-ray Absorptiometry



Faxitron

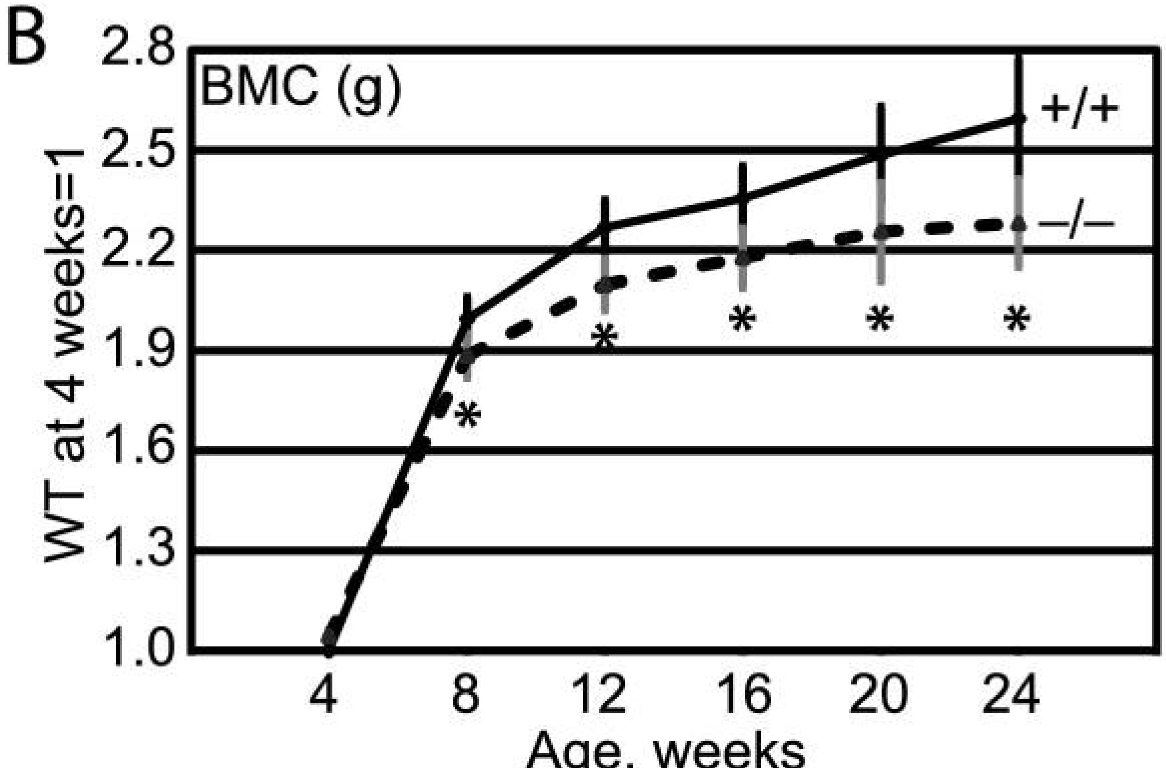
Outcomes:

- BMC (g), aBMD (g/cm^2), %fat (caveat)

Uses:

- Whole animal
- Phenotyping
- Systemic intervention
- Longitudinal studies

DXA shows decreased post-natal accrual of bone mass (BMC) in MAGP-deficient mice



- Phenotyping
- Longitudinal
- Whole animal

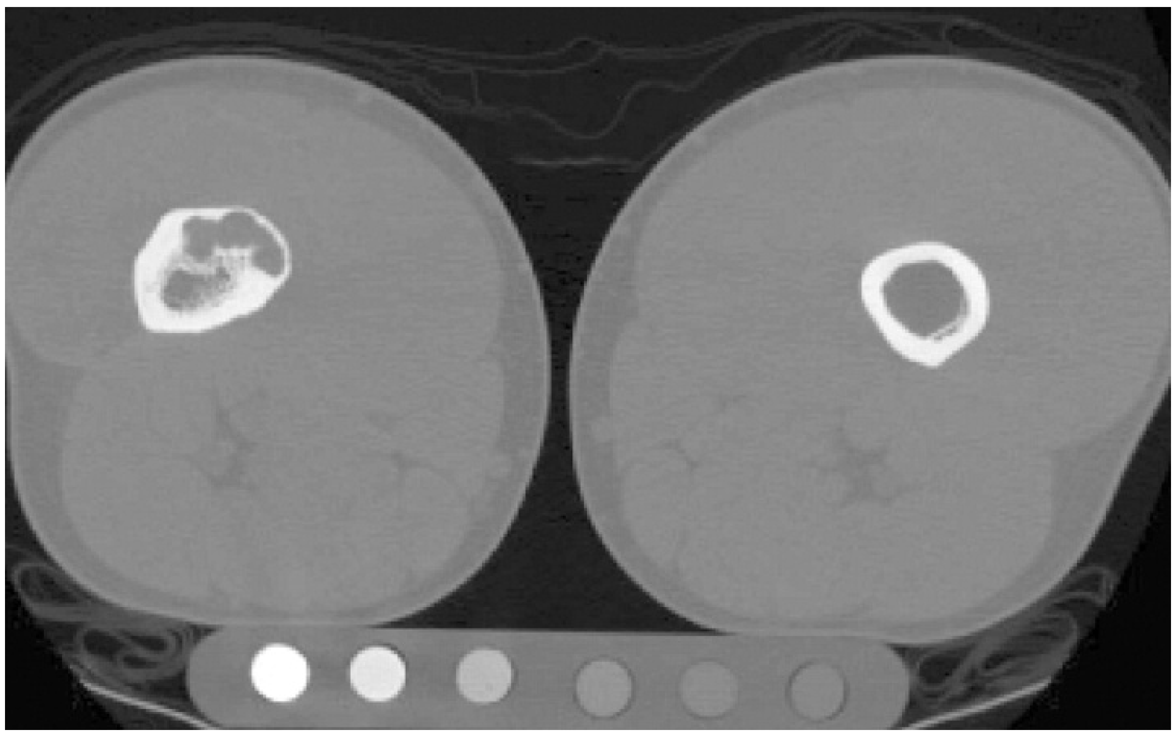
Craft, Mecham et al., 2010

X-Ray Computed Tomography (CT)

Cat Scan

QCT – Quantitative Computed Tomography

- grayscale image reflects local attenuation, i.e., "mineral density map" [whiter = denser]
- morphology & density



microCT

REVIEW

JBMR

Guidelines for Assessment of Bone Microstructure in Rodents Using Micro–Computed Tomography

Mary L Bouxsein,[1] Stephen K Boyd,[2] Blaine A Christiansen,[1] Robert E Guldberg,[3]
Karl J Jepsen,[4] and Ralph Müller[5]

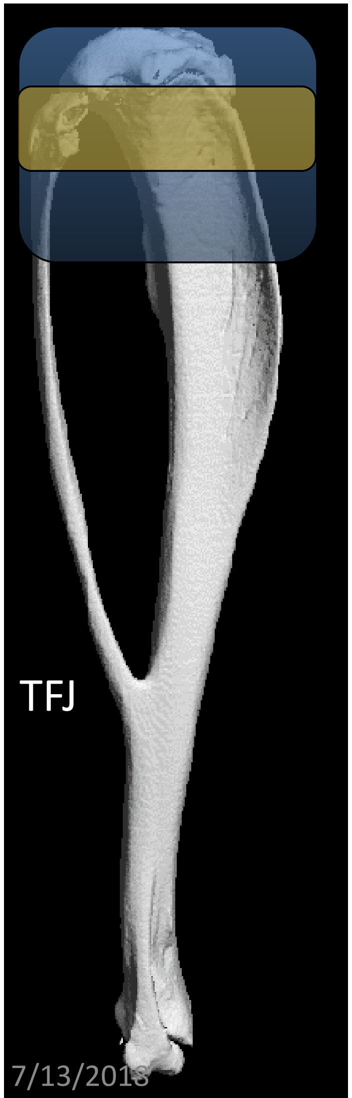
Journal of Bone and Mineral Research, Vol. 25, No. 7, July 2010, pp 1468–1486

Specimens ('microCT')

Specimens or Animals ('VivaCT')

microCT – regions of interest

Mouse tibia



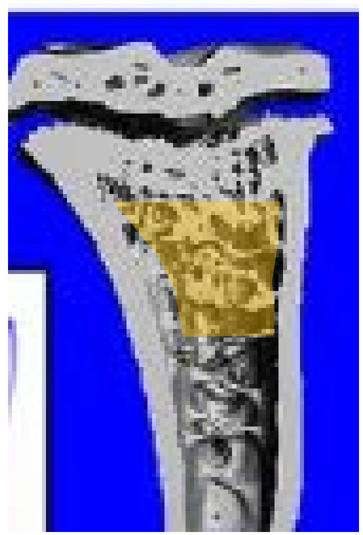
<u>Cancellous/Trabecular/Metaphyseal</u>

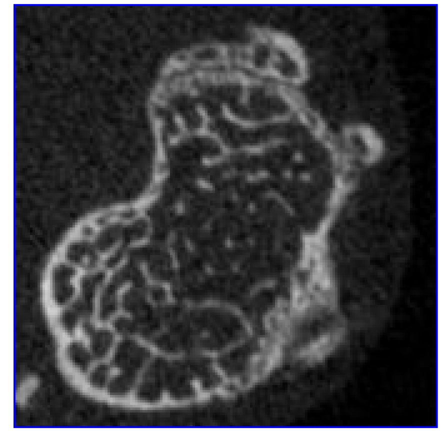
Scan region

- e.g., 300 slices = 4.8 mm*

Analysis region

- landmark (eg, growth plate)
- e.g., 100 slices = 1.6 mm*





*assumes 16 um voxel size

microCT – regions of interest

Cancellous/Trabecular/Metaphyseal

Scan region
- e.g., 300 slices = 4.8 mm*

Analysis region
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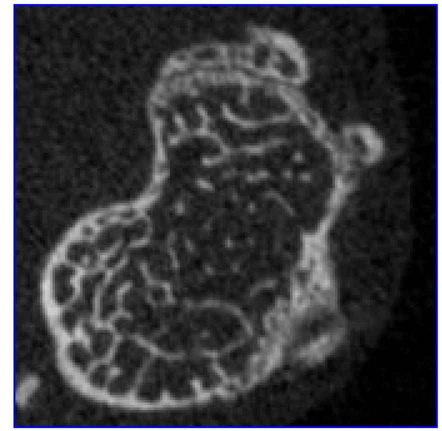
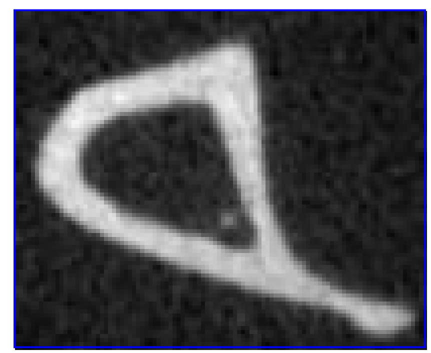
- e.g., 100 slices = 1.6 mm*



Cortical/Diaphyseal

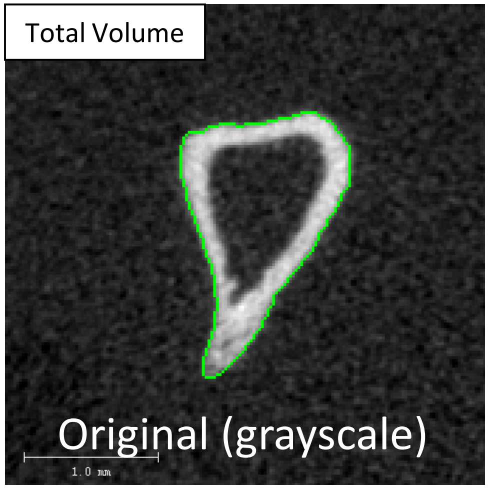
Scan region = Analysis region
- Mid-point (50% of length), or X mm from TFJ
- e.g., 50 slices = 0.8 mm*



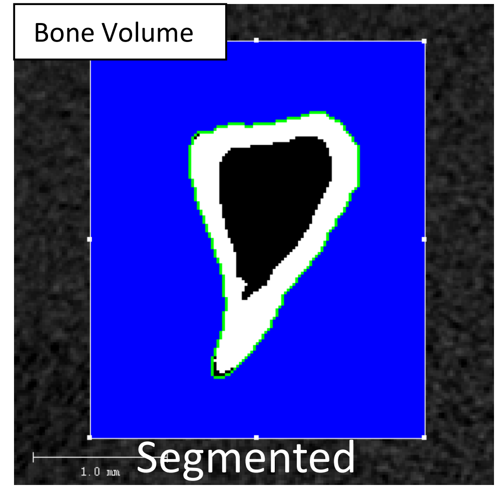
*assumes 16 um voxel size

microCT – cortical analysis

Contour



Threshold/
Segment



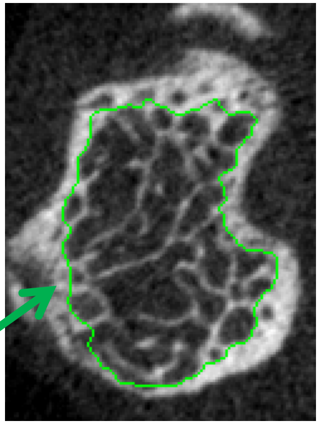
Binary: white=bone
black=not bone

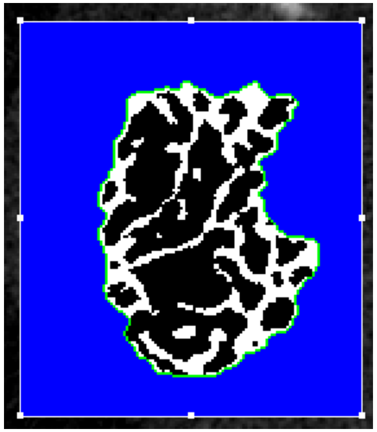
<u>Cortical Outcomes</u>

- Tt.Ar, Total area (or volume) – How big is the bone? → periosteal apposition
- Ma.Ar, Marrow area → resorption
- Ct.Ar, Cortical bone area – How much bone is there? → formation & resorption
- Ct.Th, Cortical thickness → formation & resorption
- J, Area moment of inertia → How is bone distributed? Resists bending loads.
- TMD, Tissue mineral density ("mean2") → ~mineralization

microCT – cancellous analysis

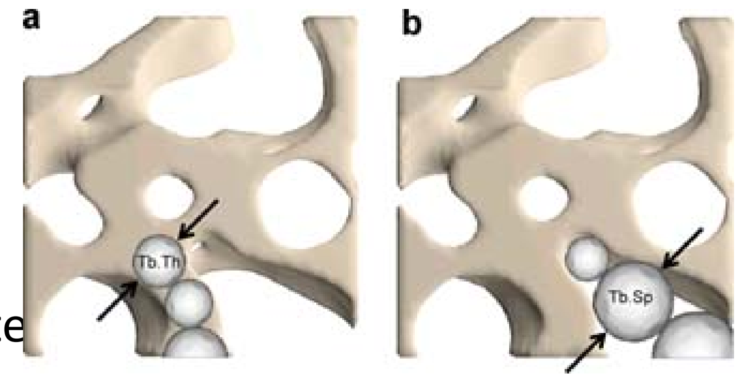
Total volume (TV)





Bone volume (BV) = white

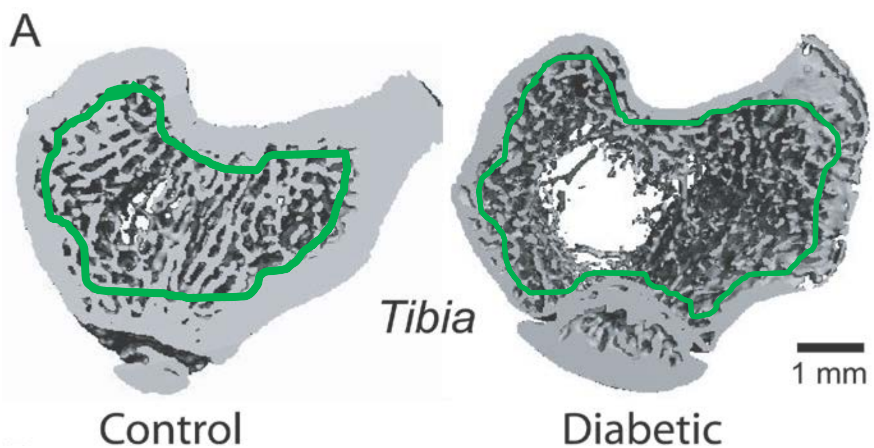
- <u>Cancellous Outcomes</u>*
  - Bone volume fraction (BV/TV) – What fraction of marrow cavity is filled with bone?
  - Trabecular thickness (Tb.Th)
  - Separation (Tb.Sp)
  - Trabecular Number (Tb.N)
  - Density, vBMD (“mean1”) – Should correlate



Bouxsein et al. 2010

* Use Direct Method values; prefix “VOX”; do not use Plate Model values; prefix “TRI”

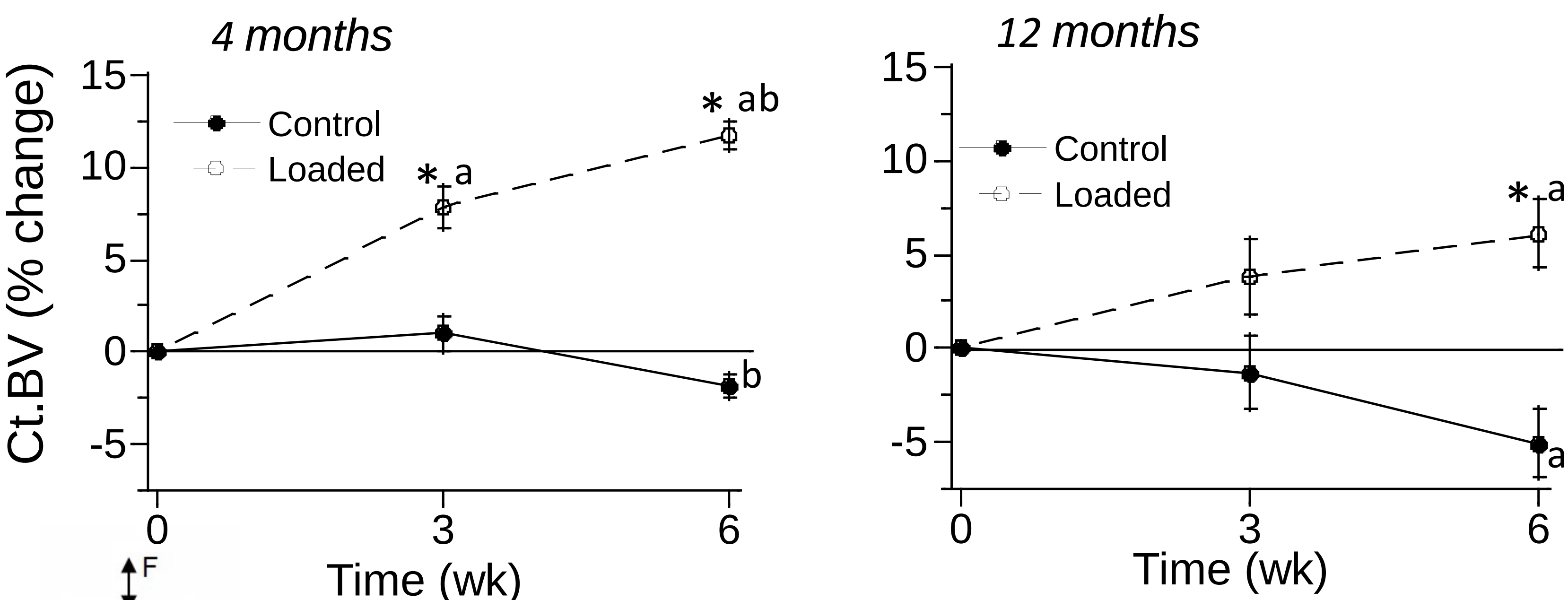
Specimen microCT reveals osteopenia in T1D diabetic rats

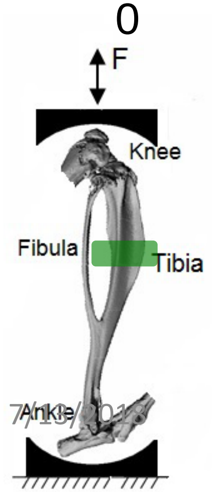


	Parameter	Control (n = 9)	Diabetic (n = 8)
Trabecular	Tb.BV/TV (mm^3/mm^3)	0.37 ± 0.04	0.12 * ± 0.04
	Tb.Th (mm)	0.11 ± 0.01	0.07 * ± 0.01
	Tb.N (1/mm)	4.4 ± 0.2	2.6 * ± 0.5

Silva et al. 2009

In Vivo CT: Mechanical Loading Stimulates Cortical Bone Accrual (in Age-Dependent Manner)



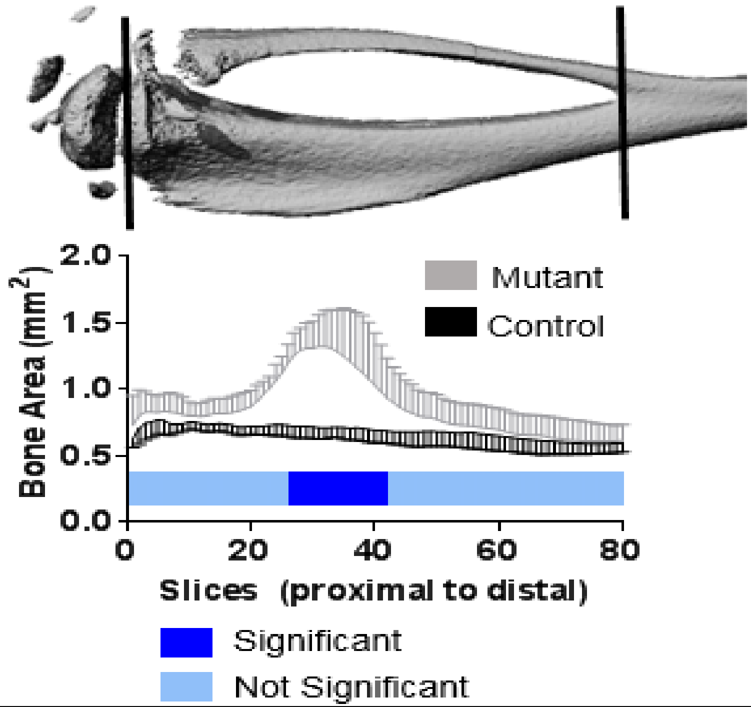


BALB/c
female mice

* $p < 0.05$, Control vs. Loaded
a,b: $p < 0.05$ vs. 0, 3 wks

Silva et al., 2012

New Method: Comparing morphology along bone length



Jenny McKenzie,
Dan Leib, Dave Ornitz

Figure 5 – Prelim Data. Bone area analysis along the length of the tibia revealed a spatially-dependent difference between mutant and control mice. Curves depict mean ± SD from n=3-5/group. Statistical significance ($p < 0.05$) is depicted in color bar, and was assessed by t-test with multiple comparison correction.

Mechanical Testing → Strength

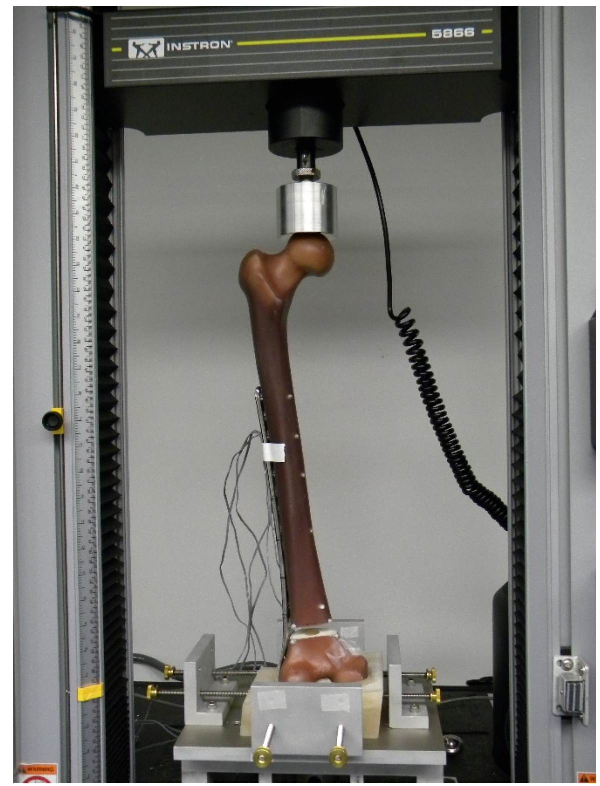
MJ Silva, Washington University
Musculoskeletal Research Center

How to measure bone mechanical properties?

- Anderson “The Spider” Silva measures bone strength

www.mmamania.com

- MRC Core measures bone strength



Instron mechanical testing machine

REVIEW

JBMR®

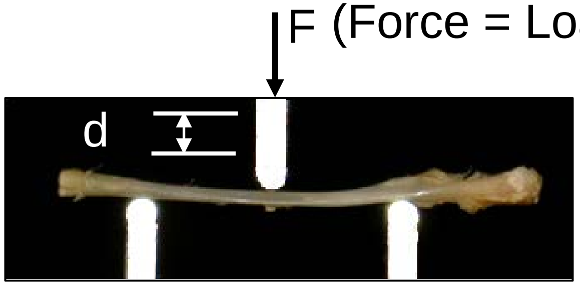
Establishing Biomechanical Mechanisms in Mouse Models: Practical Guidelines for Systematically Evaluating Phenotypic Changes in the Diaphyses of Long Bones

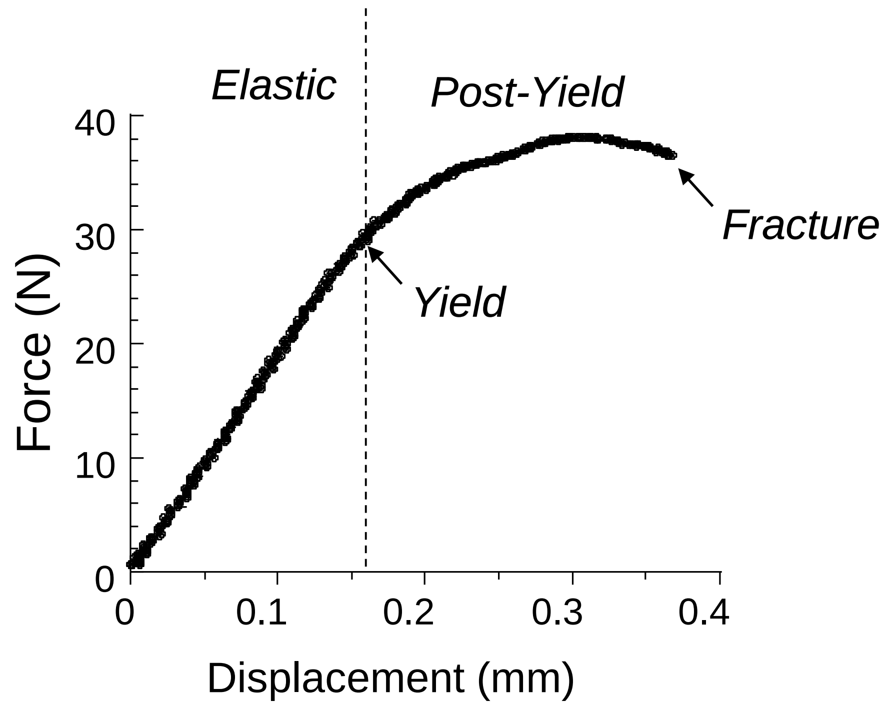
Karl J Jepsen,[1] Matthew J Silva,[2] Deepak Vashishth,[3] X Edward Guo,[4] and Marjolein CH van der Meulen[5]

Journal of Bone and Mineral Research, Vol. 30, No. 6, June 2015, pp 951–966

Mechanical Testing: Force-Displacement Plot

3-pt bending, Rat ulna

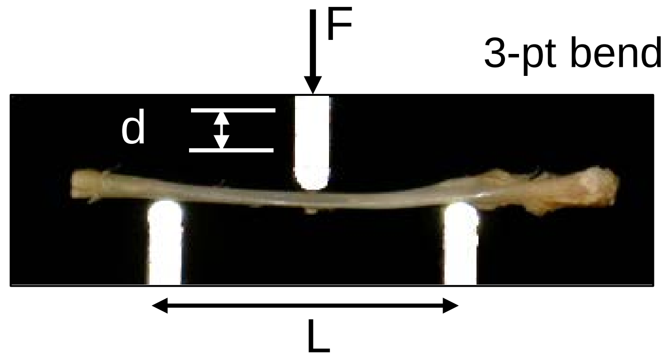


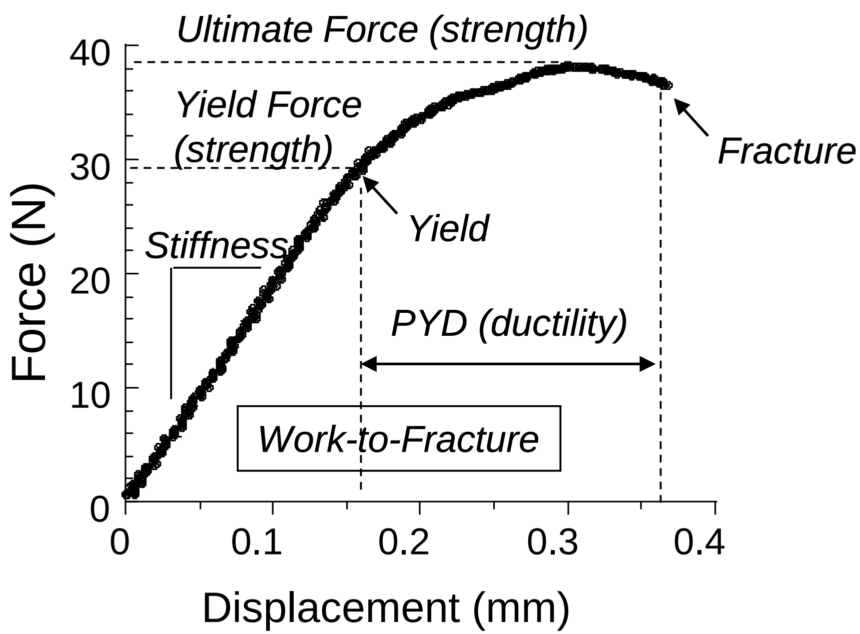


Mechanical Properties

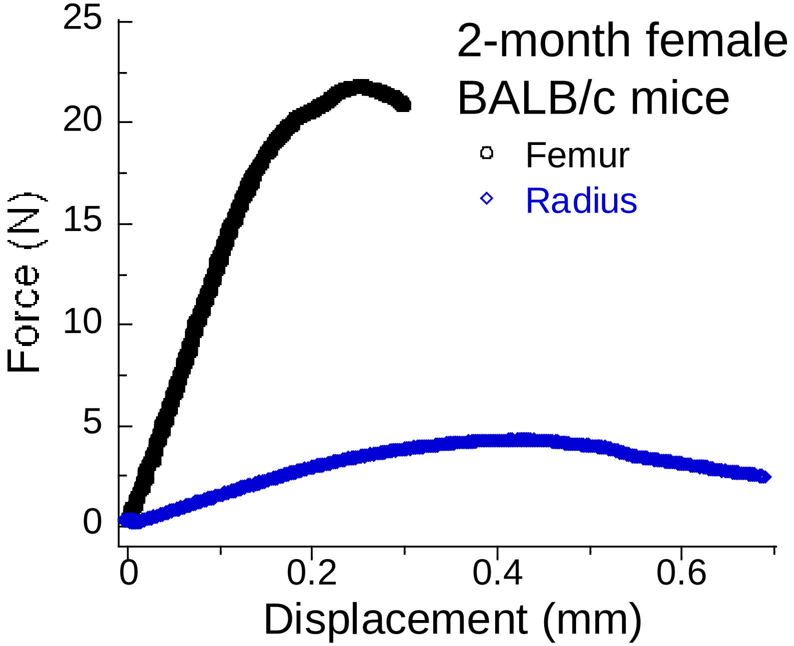
- Stiffness (N/mm)
- Strength
  - Yield Force (N)
  - Ultimate (Max.) Force (N)
- Post-yield displacement (ductility) (mm)
- Work-to-fracture (N*mm)

3-pt bending, Rat ulna





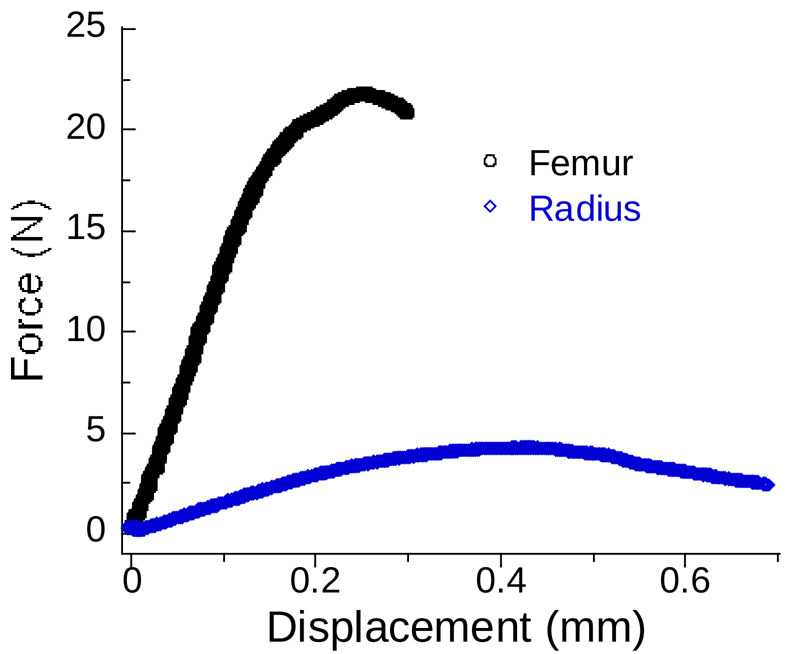
Mechanical properties

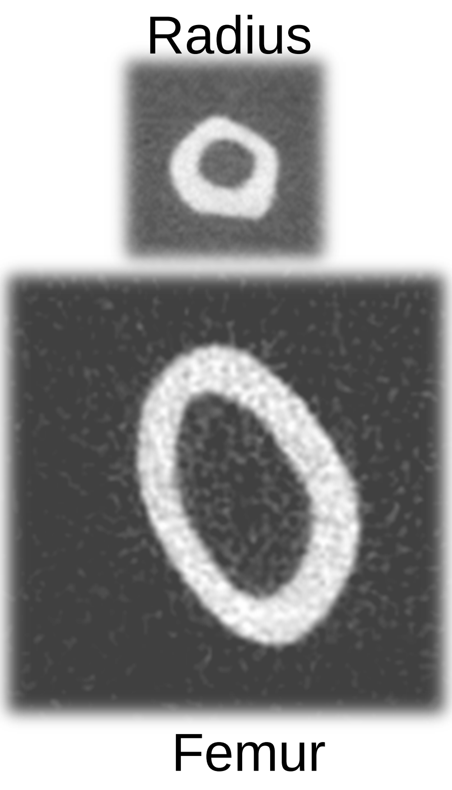


Femur vs. Radius:

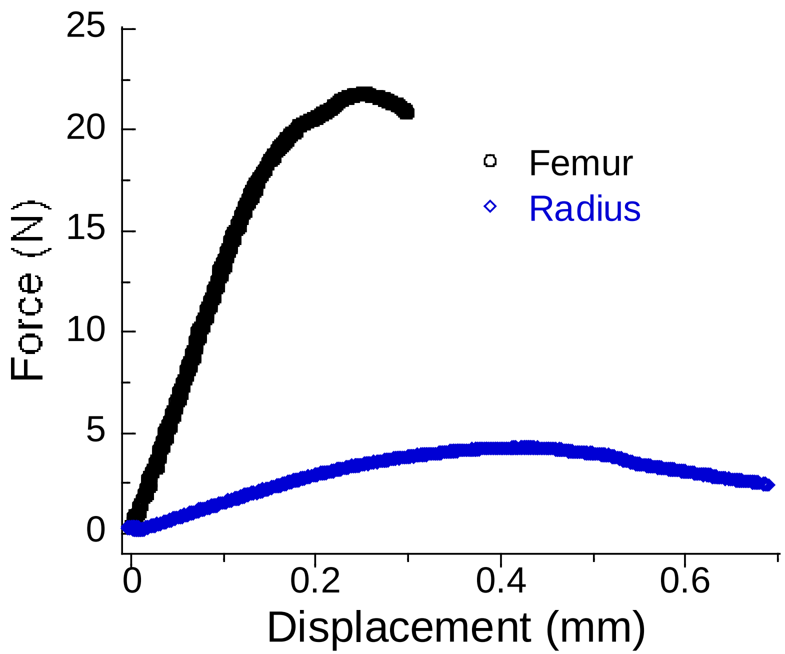
- stiffer
- stronger
- less ductile
- requires more energy to fracture

Why is femur stiffer and stronger than radius?

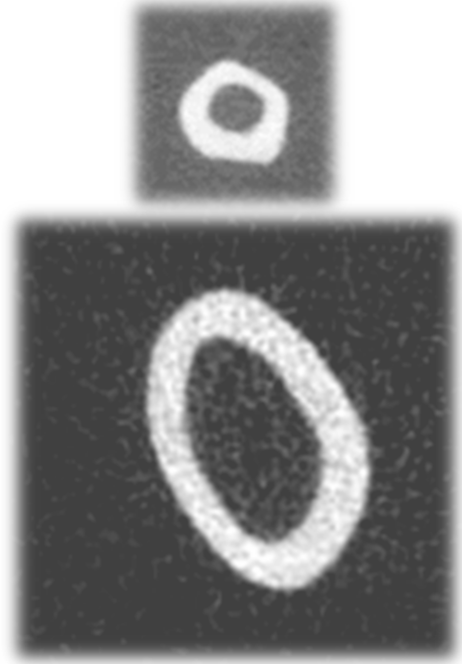




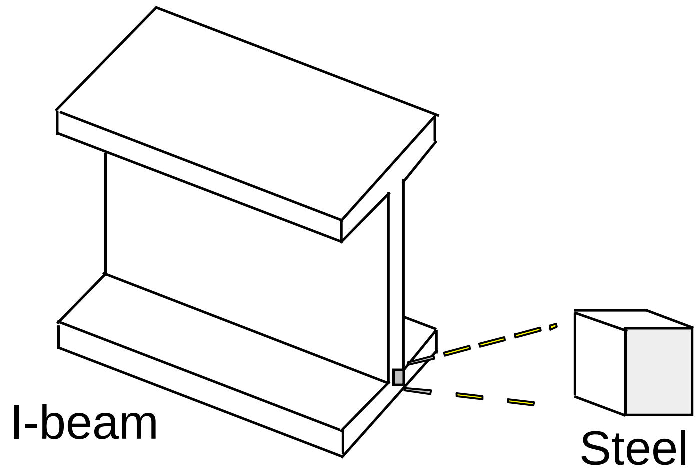
Whole-bone properties depend on morphology & material



Radius

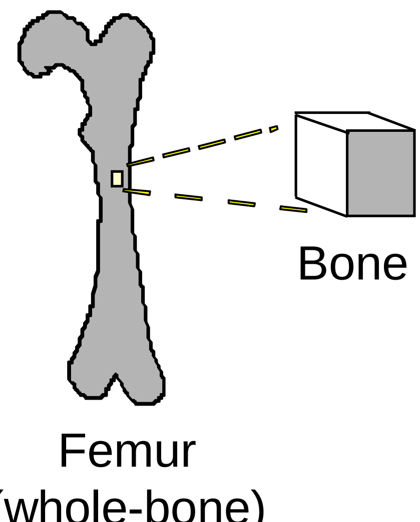


Femur



Larger:
- Structure
- Whole-Bone

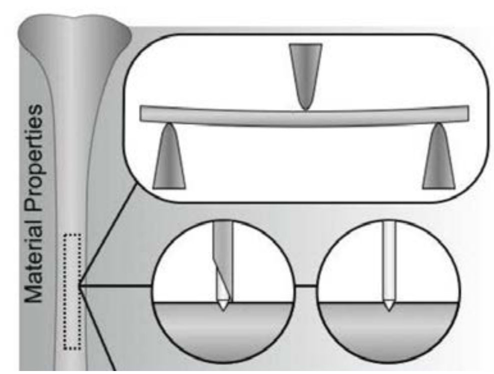
Smaller:
- Material
- Tissue



Material Properties

- Estimate from whole-bone mechanical test and bone size (microCT)
  - Whole-bone strength: Ultimate (Max.) Force (N)
  - Material strength: Ultimate Stress (N/mm^2)

- Measure with tissue-level test
  - Bending a small plank of bone
  - Microindentation



Estimating Material Properties

<u>Measured</u> Whole-bone behavior

Depends on bone size, material and test geometry

<u>Estimated</u> Material behavior

Depends only on material

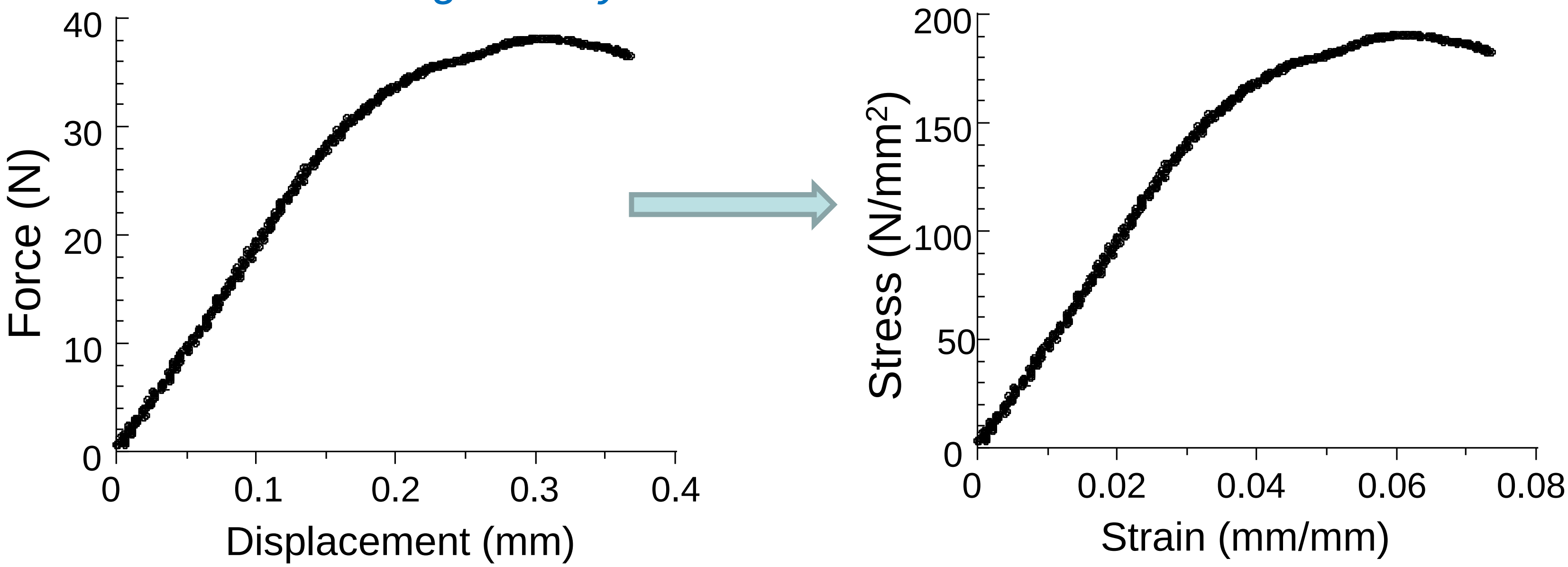


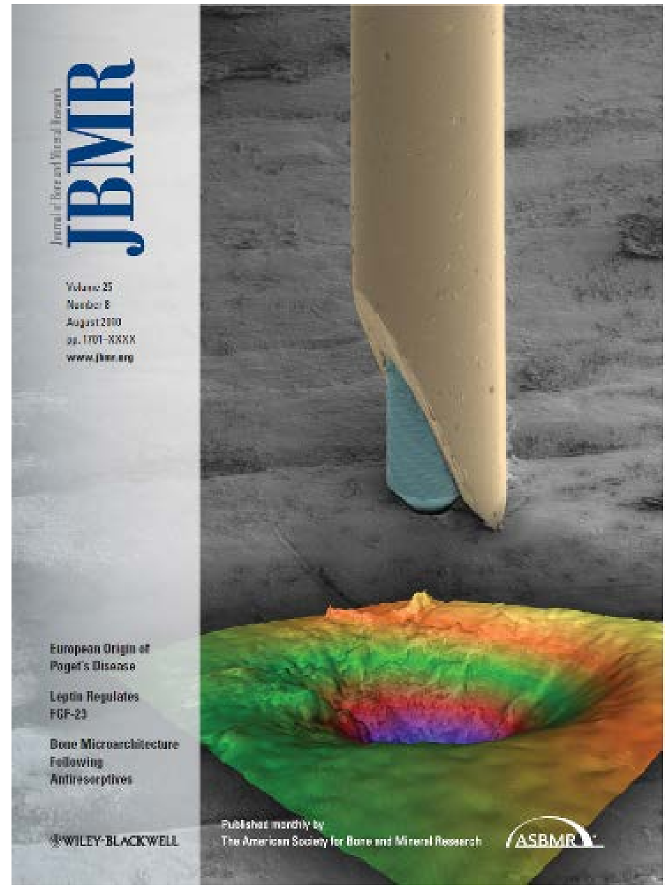
Table 1. Terminology Used for Whole-Bone and Tissue-Level Mechanical Properties

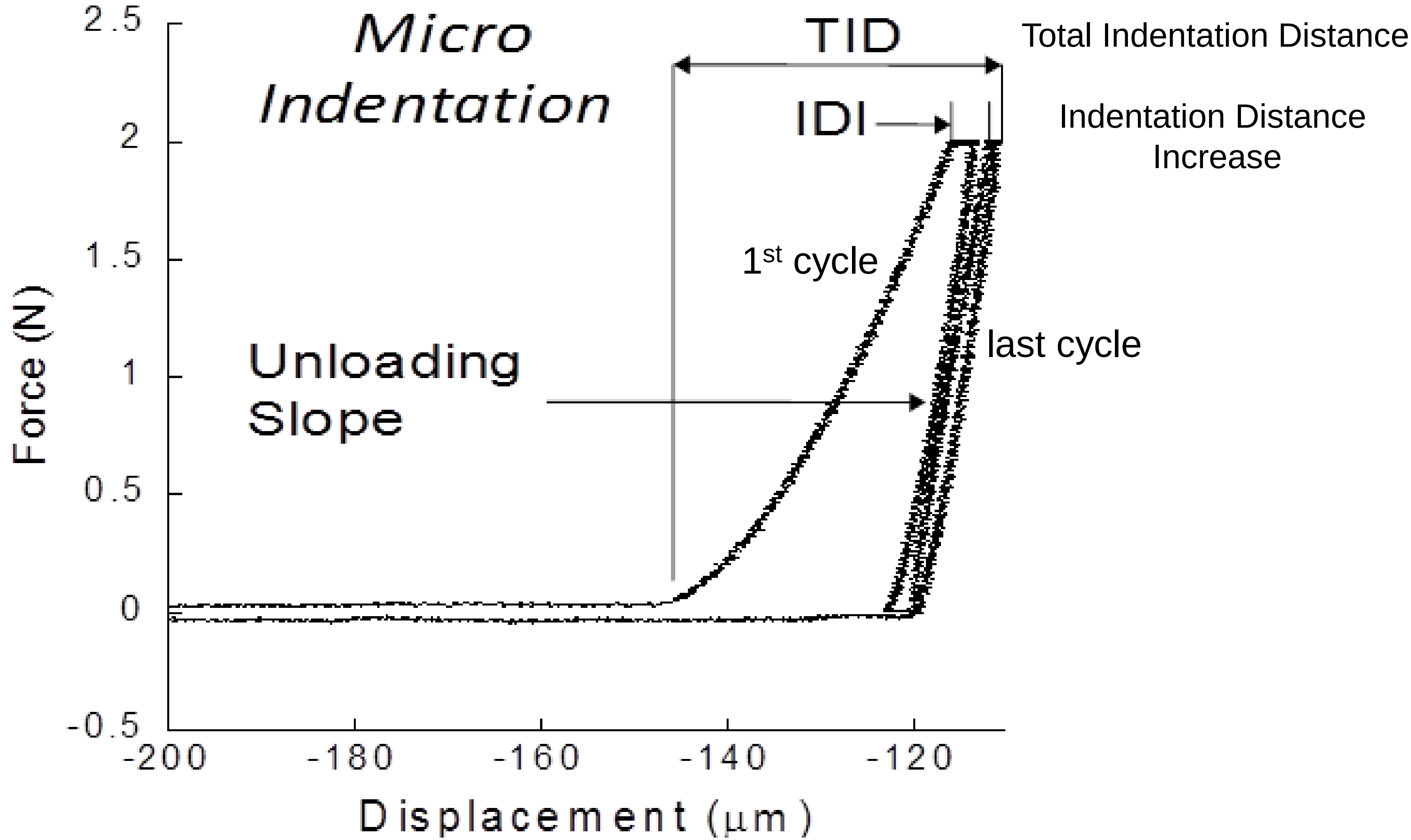
Whole-bone mechanical properties [units]	Tissue-level mechanical properties [units]
Stiffness [N/mm]	Elastic modulus (or tissue-level stiffness) [$N/mm^2 = MPa$]
Maximum load (or whole-bone strength)[a] [N]	Ultimate stress (or tissue-level strength) [$N/mm^2 = MPa$]
Postyield displacement [mm]	Postyield strain [mm/mm (a dimensionless ratio)]
Work-to-fracture [Nmm]	Toughness (or modulus of toughness) [$N/mm^2 = MPa$]

[a] "Load" and "force" can often be used interchangeably; to be precise, "load" is the more general term and may refer to "force" (push or pull) or "moment" (bend or torque).

Jepsen et al., JBMR 2015

Microindentation (BioDent)

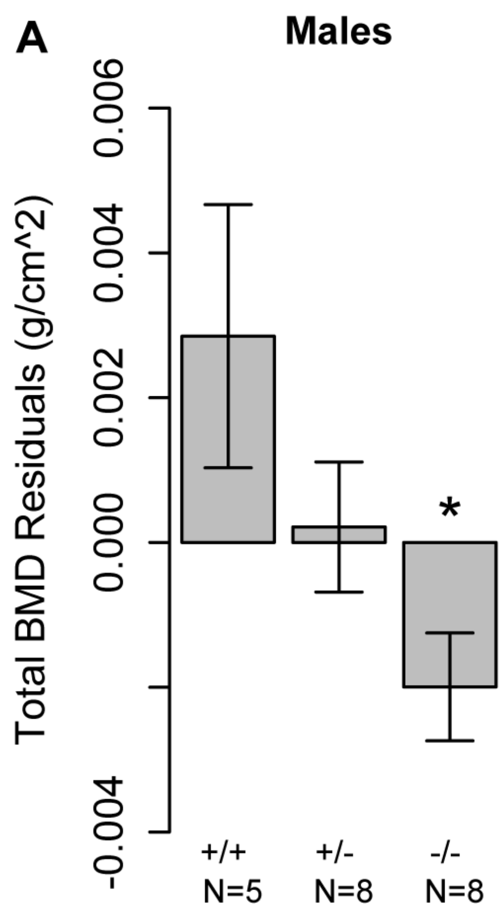



Micro Indentation
TID
Total Indentation Distance
IDI
Indentation Distance Increase
1st cycle
last cycle
Unloading Slope
Force (N)
2.5
2
1.5
1
0.5
0
-0.5
-200
-180
-160
-140
-120
Displacement (μm)

Case Study 1: Asxl2 null mice

Wei Zou, Steve Teitelbaum

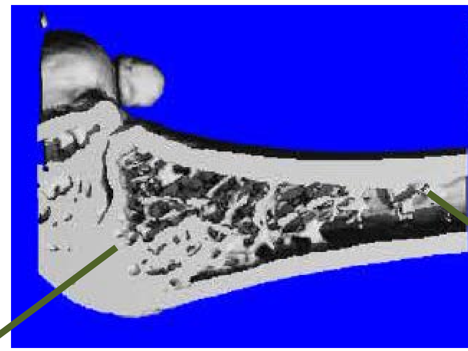
Asxl2-null mice have low BMD



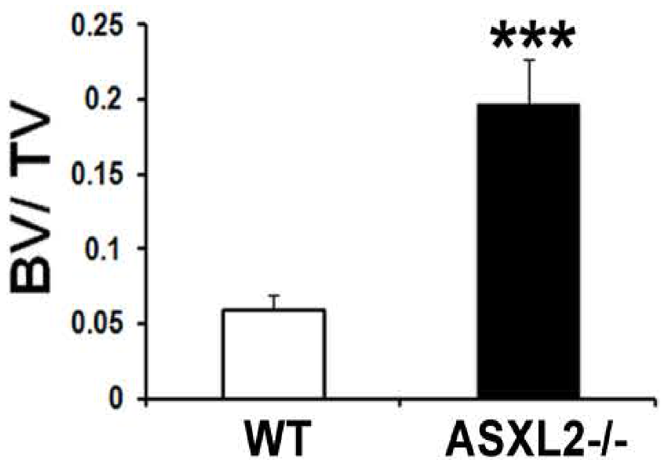
age- and body weight-adjusted

Farber et al, 2011

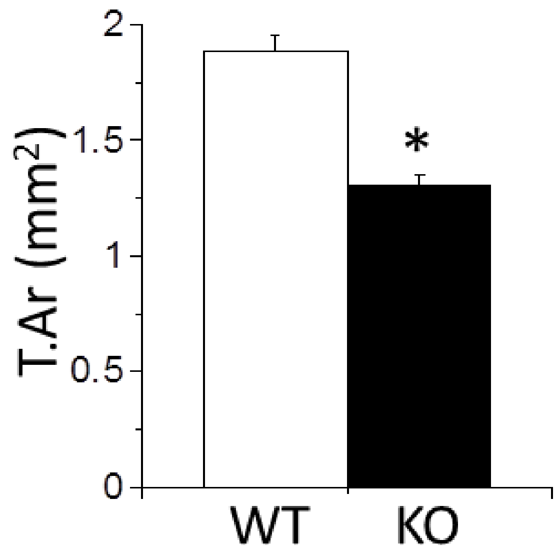
Asxl2-null mice have more cancellous bone, <u>but</u> less cortical bone



Cancellous



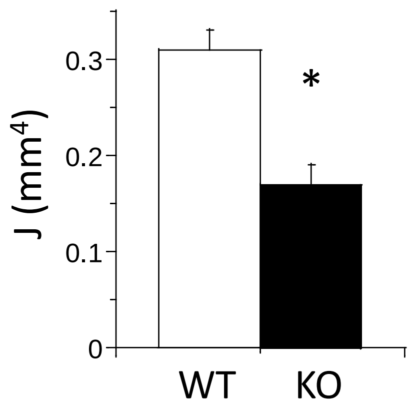
Cortical



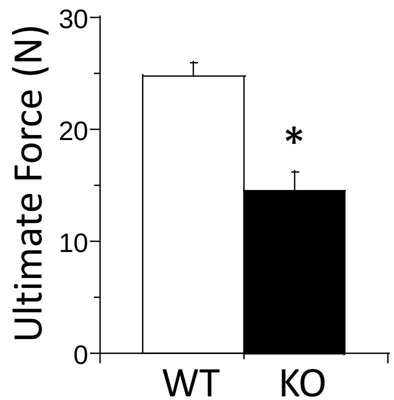
Izawa, et al., Cell Rep, 2015

Asxl2-null femurs: small & weak bones, normal material strength

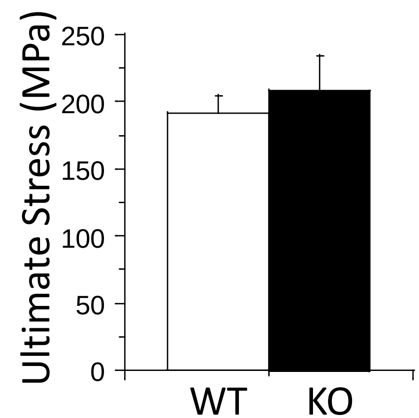
Moment of Inertia

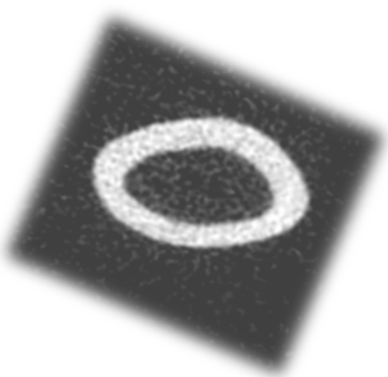


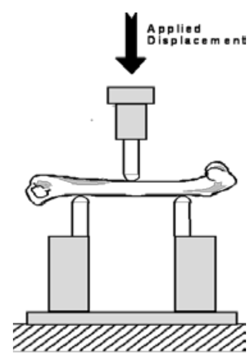
Whole-bone strength



Material strength







MJ Silva, Washington University
Musculoskeletal Research Center

Asxl2-null mice - Summary

- Increased trabecular bone (osteoclast defect)
- Decreased bone size & whole-body BMD
- Bones are weaker in proportion to their smaller size
- Normal material properties

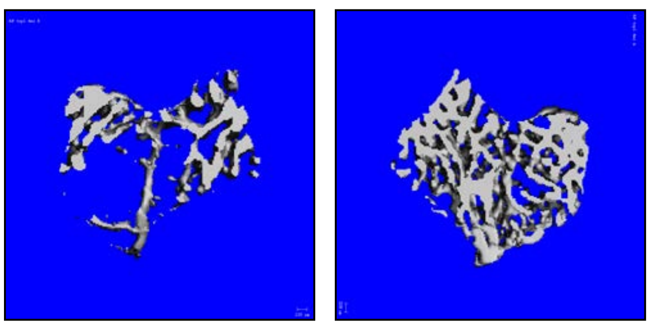
Case Study 2: Tsp1 null mice

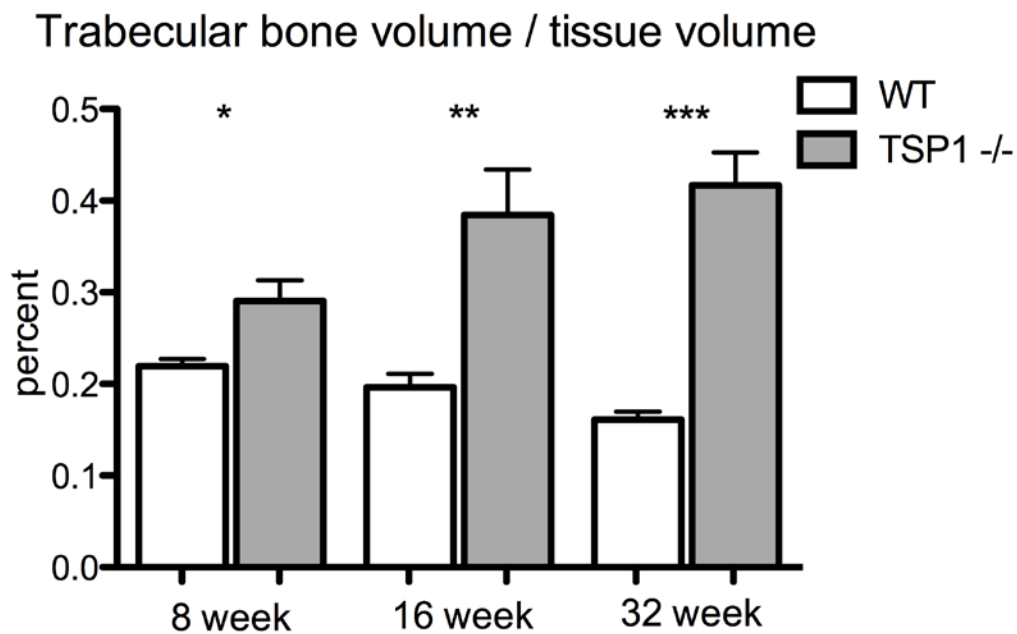
Sarah Amend, Kathy Weilbaecher

Tsp1-/- mice have increased trabecular bone

wild type TSP1-/-

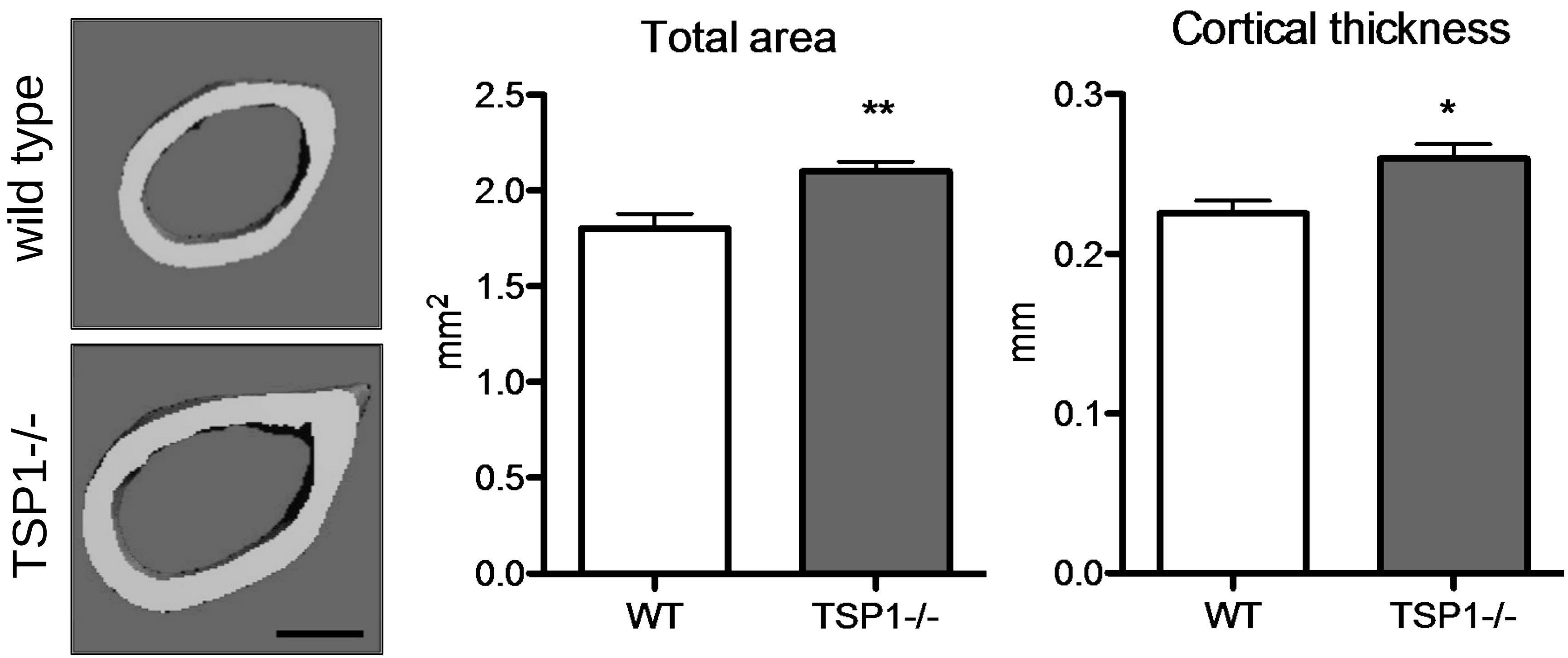
16 week





Amend et al., JBMR, 2015

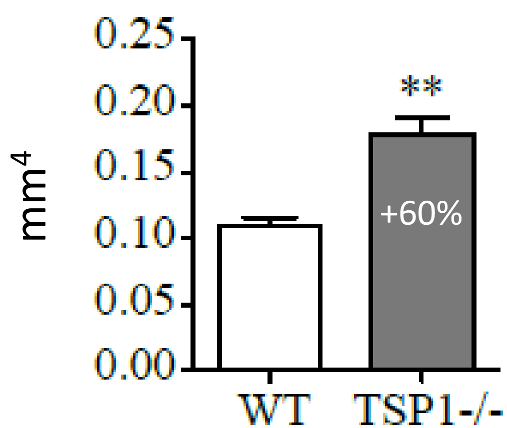
Tsp1-/- mice have larger bones



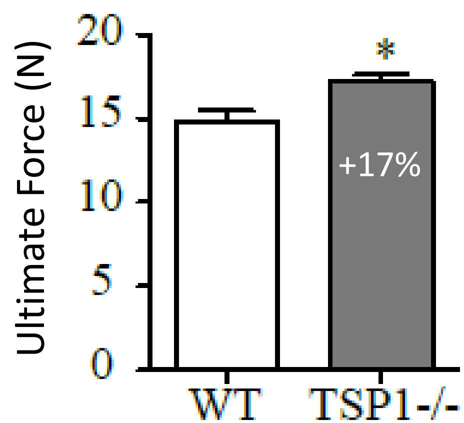
Amend et al., JBMR, 2015

Tsp1-null femurs: larger, moderately stronger, …weaker material

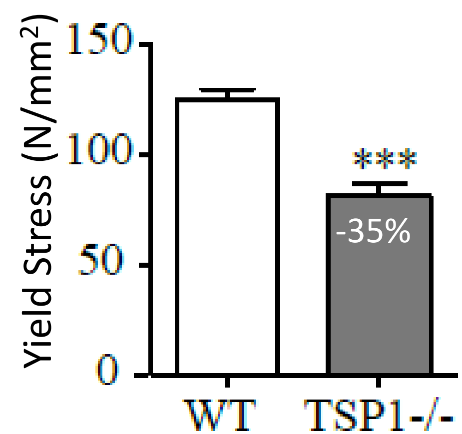
Moment of Inertia

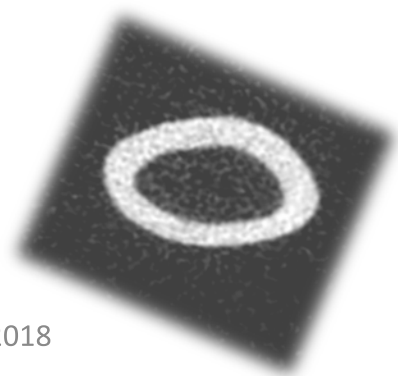


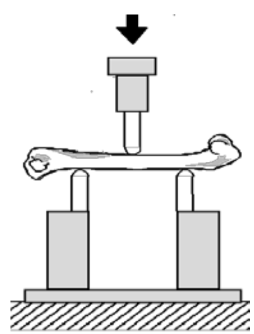
Whole-bone strength



Material strength

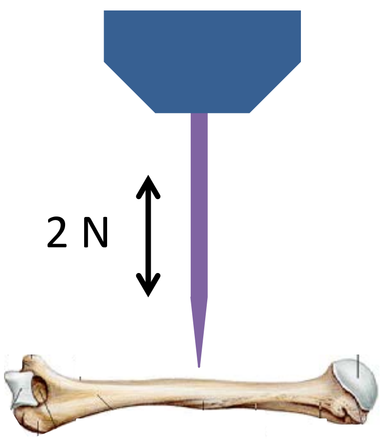






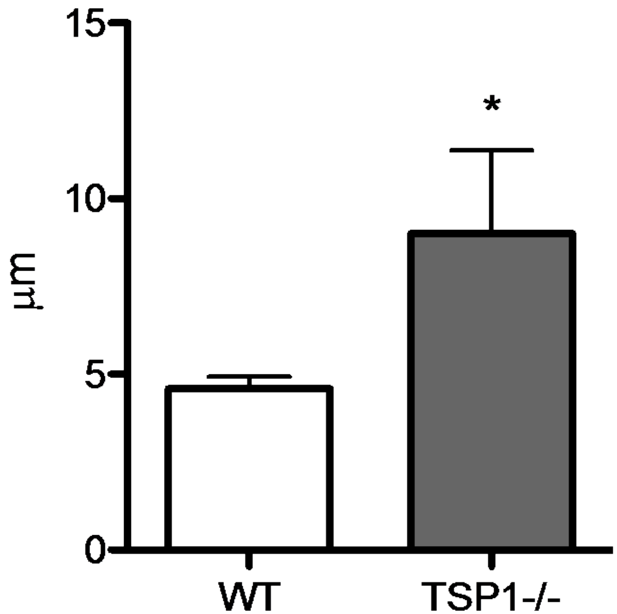
Amend et al., JBMR, 2015

Tsp1-null bone: damages more easily at material level



microindentation

Indentation distance increase



Amend et al., JBMR, 2015

MJ Silva, Washington University
Musculoskeletal Research Center

TSP1-null mice: Summary

- Increased trabecular bone
- Increased cortical bone size
- Bones are moderately stronger – disproportionate to size
- Impaired material properties

musculoskeletalcore.wustl.edu

MUSCULOSKELETAL RESEARCH CENTER
DEPARTMENT OF ORTHOPAEDIC SURGERY
DEPARTMENT OF INTERNAL MEDICINE
DIVISION OF BONE AND MINERAL DISEASES

Washington University in St.Louis
School of Medicine

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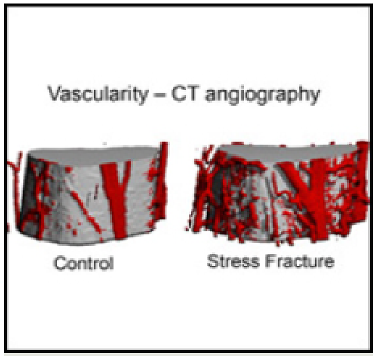
Structure and Strength Core



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Overview

NEW The MRC is partnering with the Wash U Center for Cellular Imaging (WUCCI) to fund a Just-in-time/Microgrant program to support new imaging projects related to musculoskeletal research. Read more...

Structure and strength are perhaps the most relevant properties when assessing functional outcomes in animal models related to musculoskeletal biology and medicine. The **objectives** of the **Musculoskeletal Structure and Strength Core** are: 1) to increase access to existing resources for densitometry, imaging, and mechanical testing; 2) to enhance expert oversight and quality control; 3) to provide training and enrichment opportunities related to core services; and 4) to foster new interactions and enhance existing interactions between members of the Research Base of the Center for Musculoskeletal Research at Washington University.

Consultation: Contact the Core Director or any of our Associate Directors or Technical Staff to discuss your project. We can advise you on outcomes you may consider for a musculoskeletal structure / biomechanics experiment, and what we can do to help you obtain these outcomes. If you are in the planning stages or submitting a proposal, we can help with study design a priori. If you are starting your experiment, we can provide training or do the work for you. If you have collected your data, we can help with data analysis and interpretation.

Imaging: We support the use of x-ray based imaging of musculoskeletal structures and tissues from animal models (mouse to canine) generated by Research Base investigators. Available techniques include plane radiography, dual-energy x-ray absorptiometry (DXA), peripheral quantitative computed tomography (pQCT), and micro-computed tomography (microCT). Importantly, each of these techniques is available for in vivo as well as post mortem imaging.

Mechanical Testing: We perform mechanical testing to assess the functional properties of musculoskeletal tissues and structures from animal models (mouse to canine) generated by Research Base investigators. Available "standard" testing

SERVICES

- Just in Time Funding
- Overview
- Fee Schedule and Forms
- Core B News
- MRC-WUCCI JIT/Microgrant Program
- Technical Notes and Presentations
- Project Gallery
- Publications
- Image Library

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NIAMS National Institute of Arthritis and Musculoskeletal and Skin Diseases