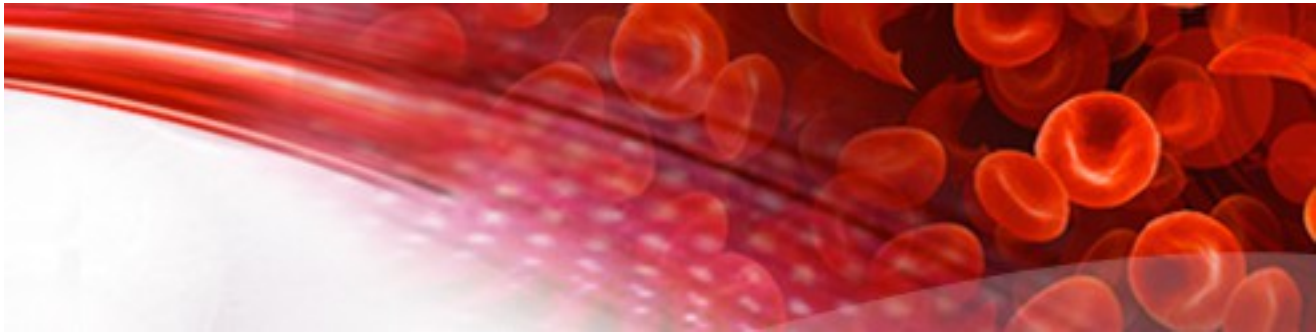

Bone Biomechanics and Pathology in Sickle Cell Disease

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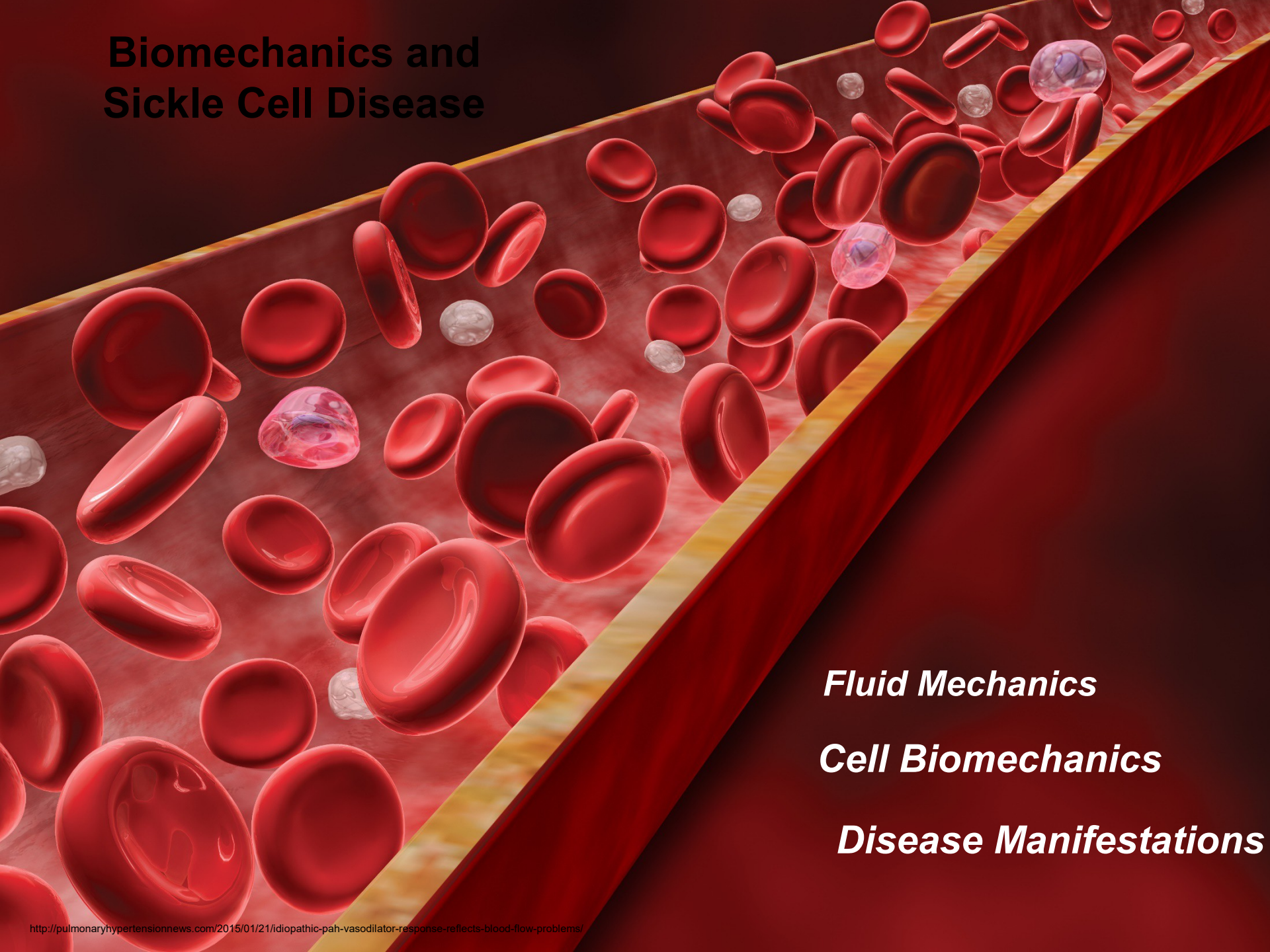
The City College of New York

Barabino laboratory
Vascular and Orthopedic Tissue Engineering Research

Conflicts and Disclosure

I have no conflicts of interest to disclose

Biomechanics and Sickle Cell Disease



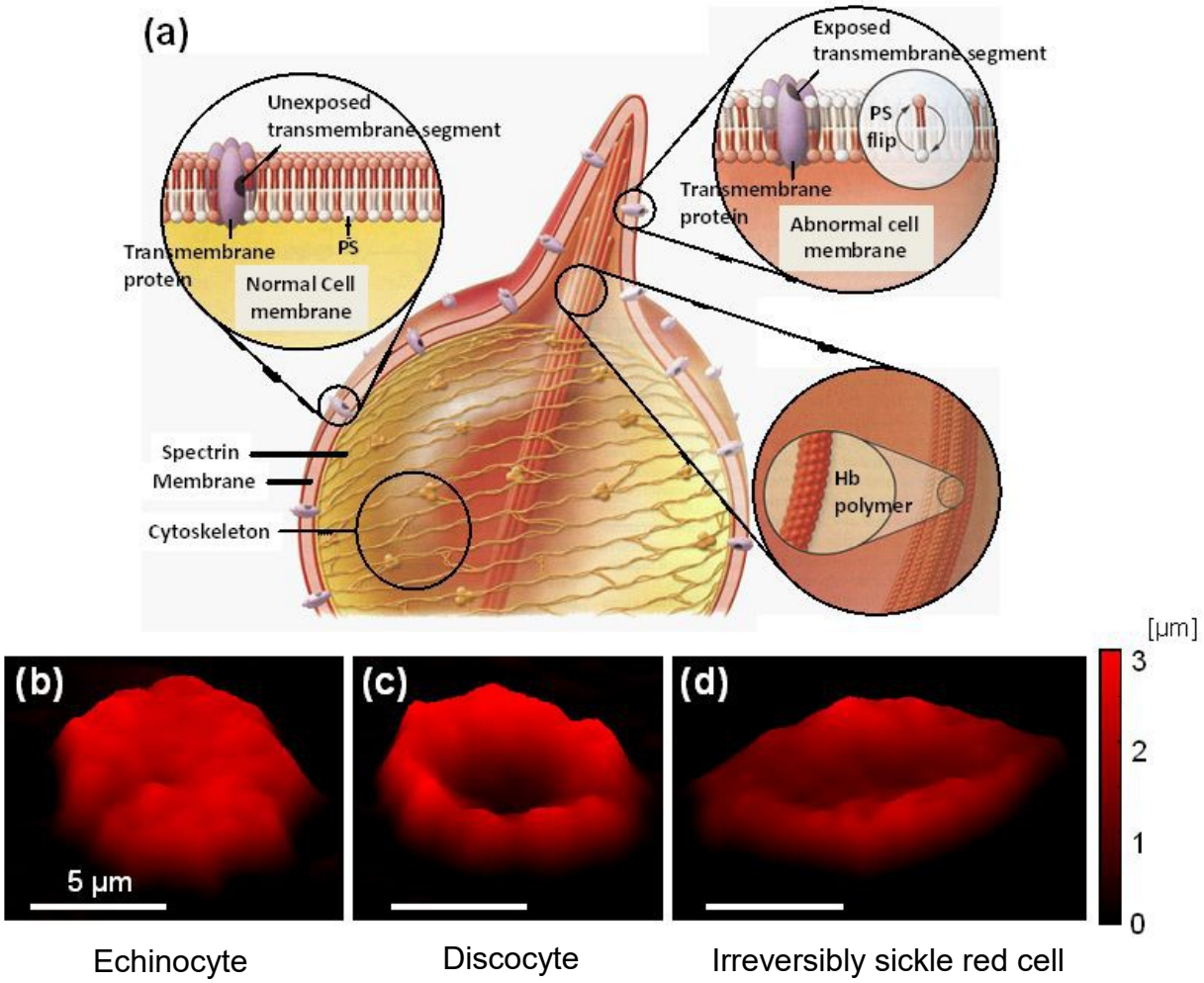
Fluid Mechanics

Cell Biomechanics

Disease Manifestations

http://pulmonaryhypertensionnews.com/2015/01/21/idiopathic-pah-vasodilator-response-reflects-blood-flow-problems/

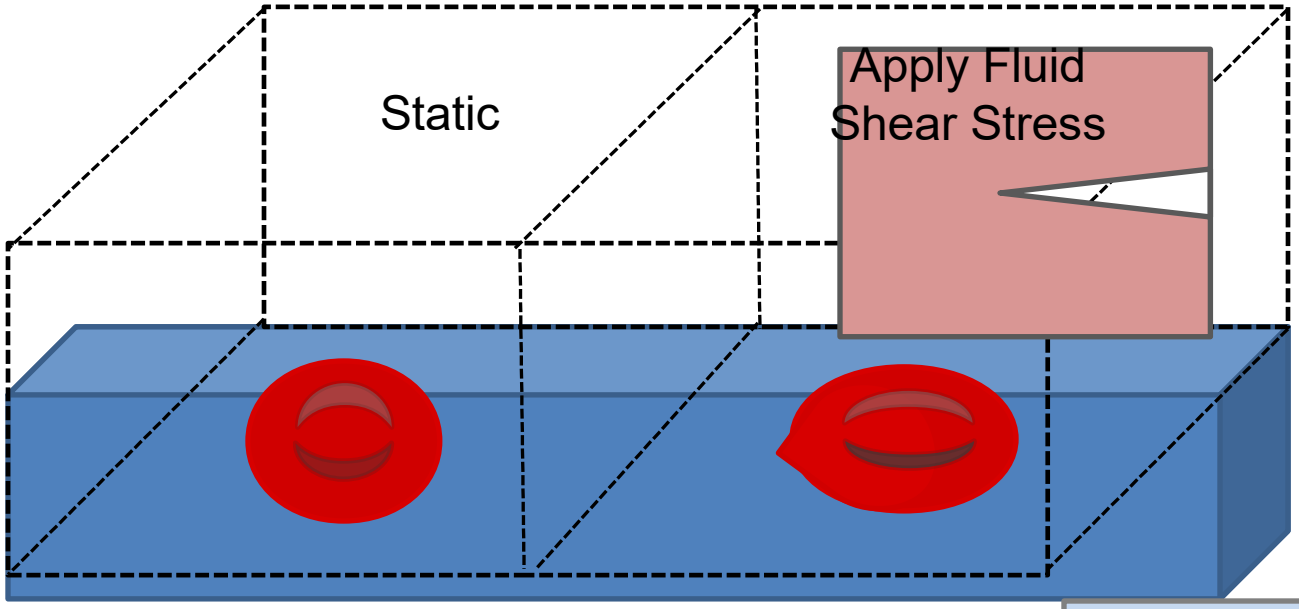
Sickle cell altered membrane properties

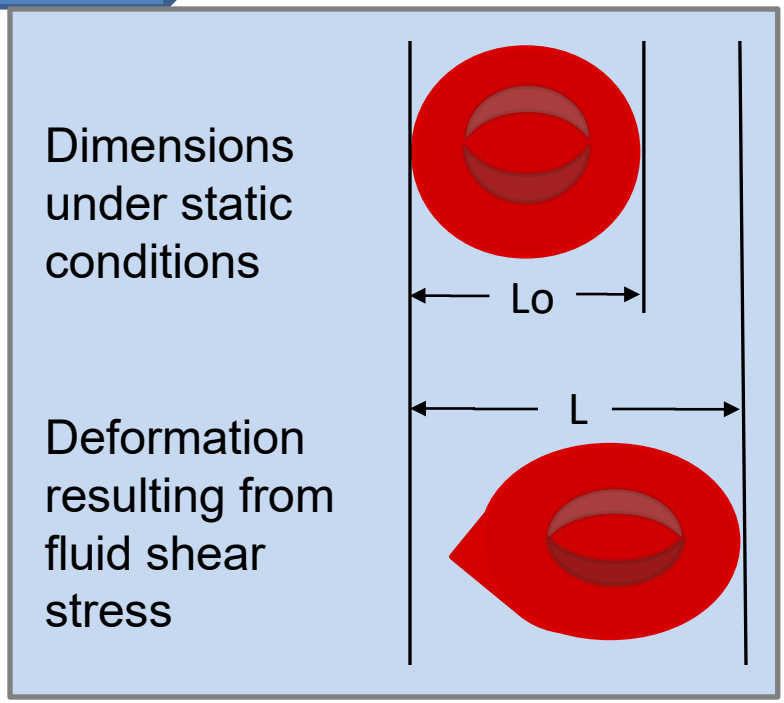


Echinocyte Discocyte Irreversibly sickle red cell

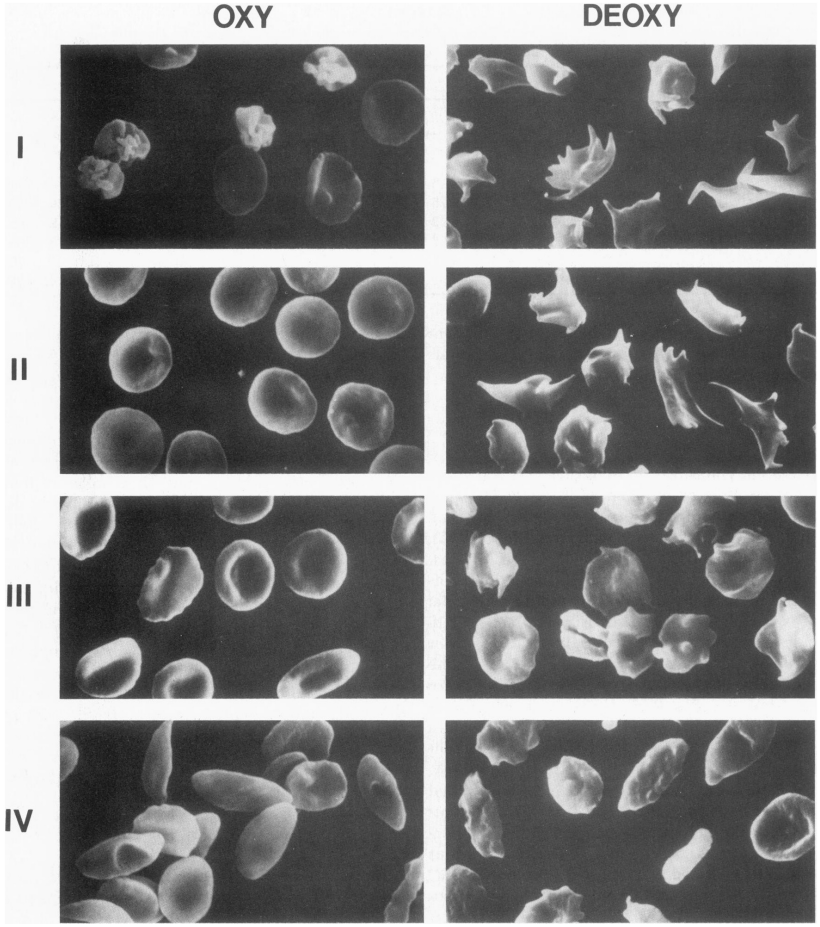
In sickle cell disease beta hemoglobin polymerization (sickling) in low O2 conditions alters the red blood cell's morphology and mechanical properties

Image sources:
(a) Barabino et al., *Annu. Rev. Biomed. Eng.*, 2010. 12:345–67
(b-d) Kim, Youngchan, et al. *Optics express* 20.9 (2012): 9948-9955.


Static
Apply Fluid
Shear Stress


Dimensions
under static
conditions
Lo
L
Deformation
resulting from
fluid shear
stress

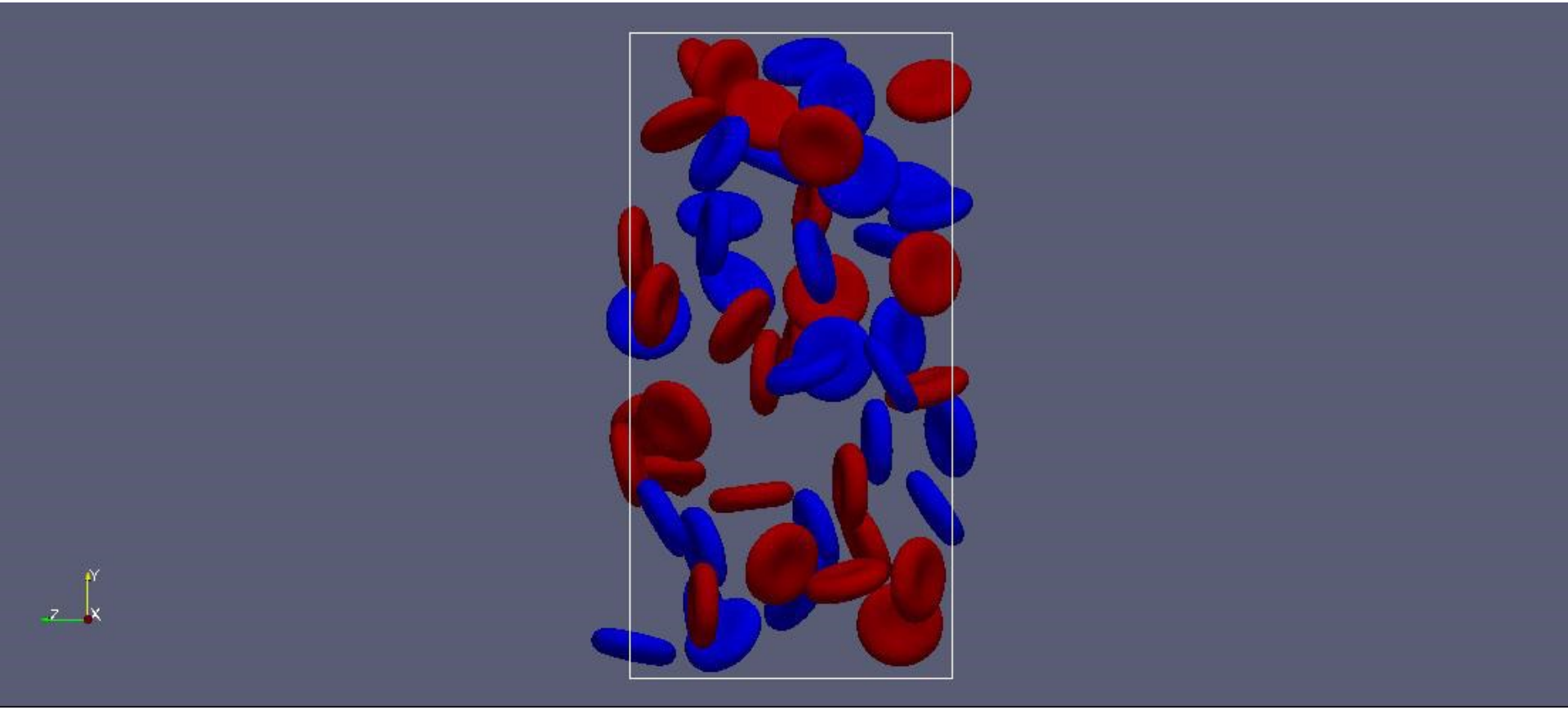
Sickle RBC subpopulations



Kaul et al, J Clin Invest 72: 22, 1983

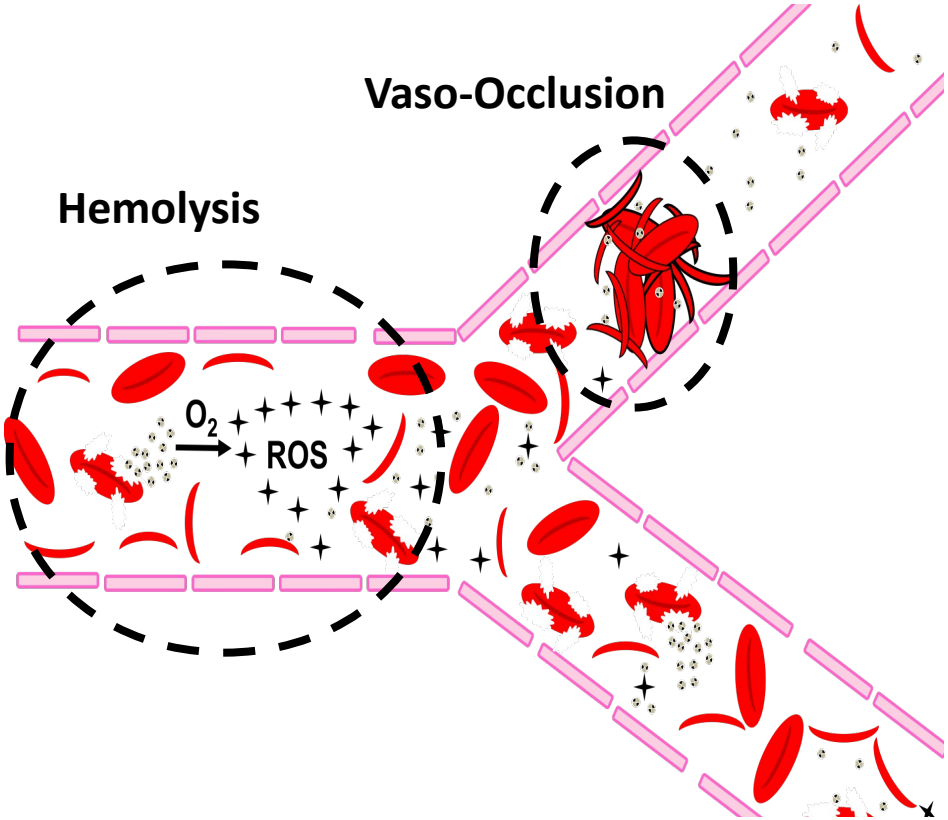
- Four classes based on rheological and hemodynamic characteristics (density, shape and deformability):
  - I Reticulocytes
  - II Discocytes
  - III Dense discocytes
  - IV ISCs

Margination of stiff RBC



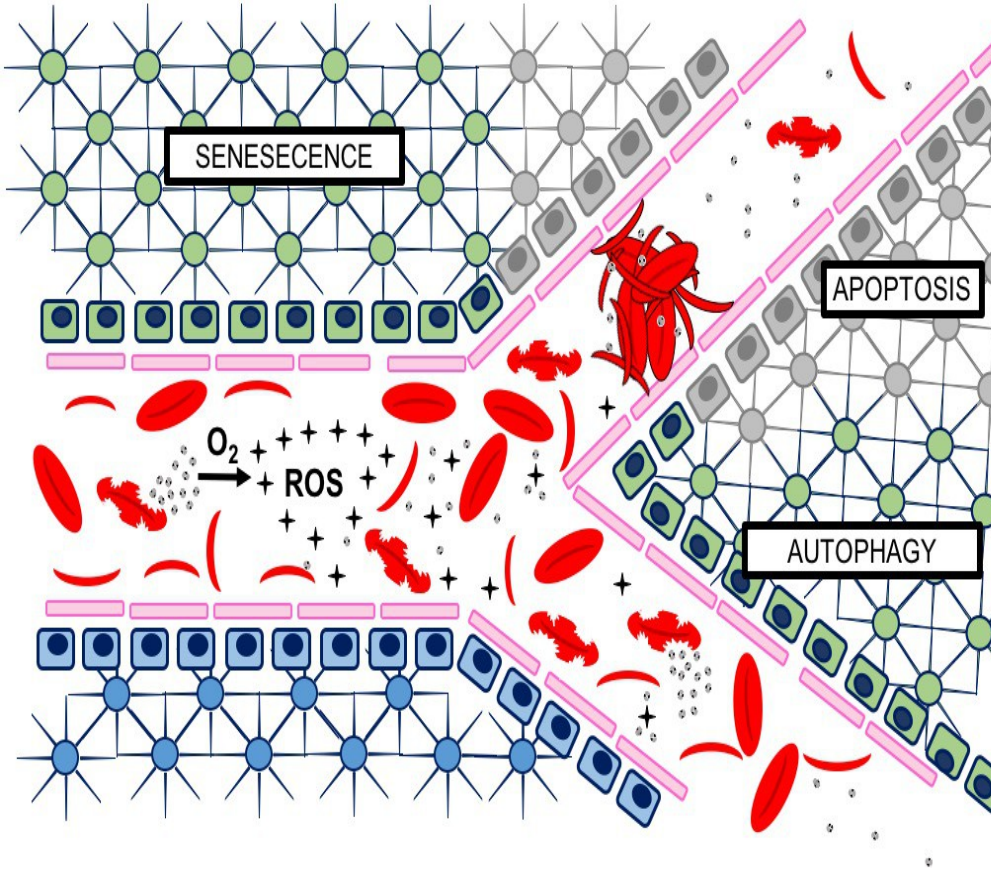
Ahmed et al, (2018) *J Biomech Eng*, 140 (6)

Altered sickle RBC biomechanics contribute to hemolysis and vaso-occlusion



- Stiff undeformable cells are rapidly destroyed and have impaired passage in vessels
- Chronic hemolysis leads to anemia, inflammation and elevated reactive oxygen species (ROS)
- Resolution of vaso-occlusion results in ischemic-reperfusion injury of the vasculature
- Pathologic processes resulting from hemolysis and vaso-occlusion lead to organ damage and disease complications

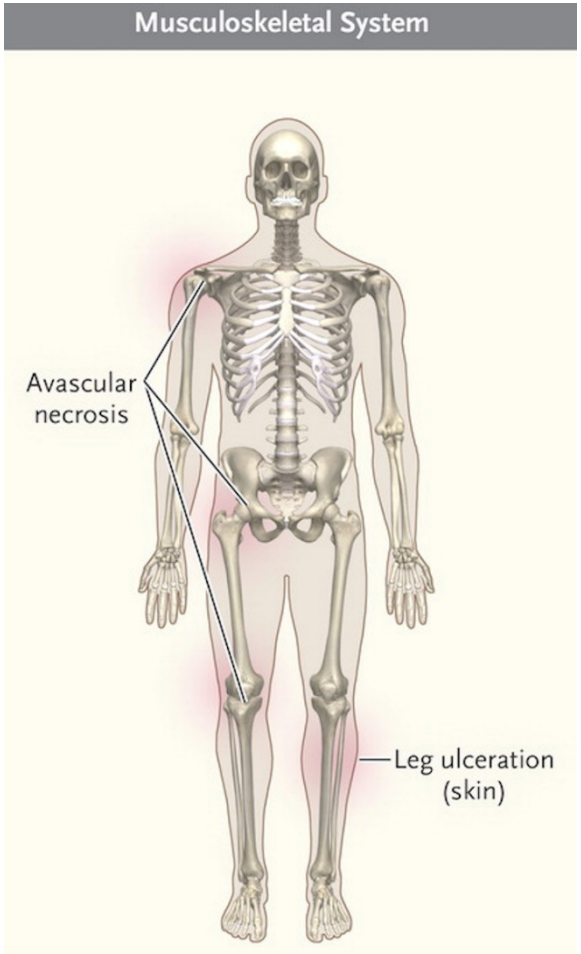
Pathological processes and sickle cell disease complications

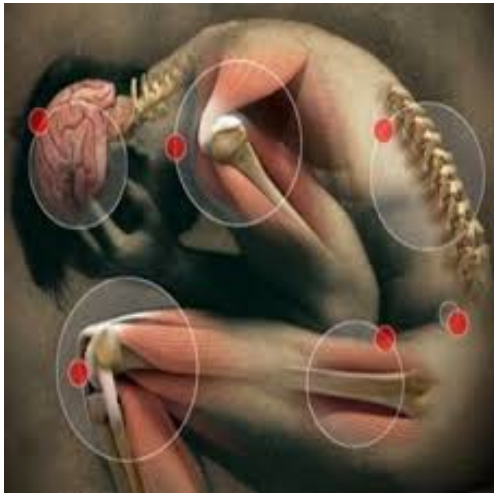


- Pathological processes (vascular dysfunction, NO deficiency, oxidative stress, reperfusion injury, inflammation) lead to chronic and progressive multi-organ damage
- The spleen (responsible for immune defense and control of senescent or altered cells) is typically the first organ injured in both humans and transgenic sickle mouse models due to chronic hemolysis and oxidative stress
- In bone, these processes may impact bone remodeling

Autophagy: self-destructive mechanism to rid cell of unnecessary or dysfunctional components
Senescence: permanent growth arrest without cell death
Apoptosis: cell death

Bone involvement in SCD





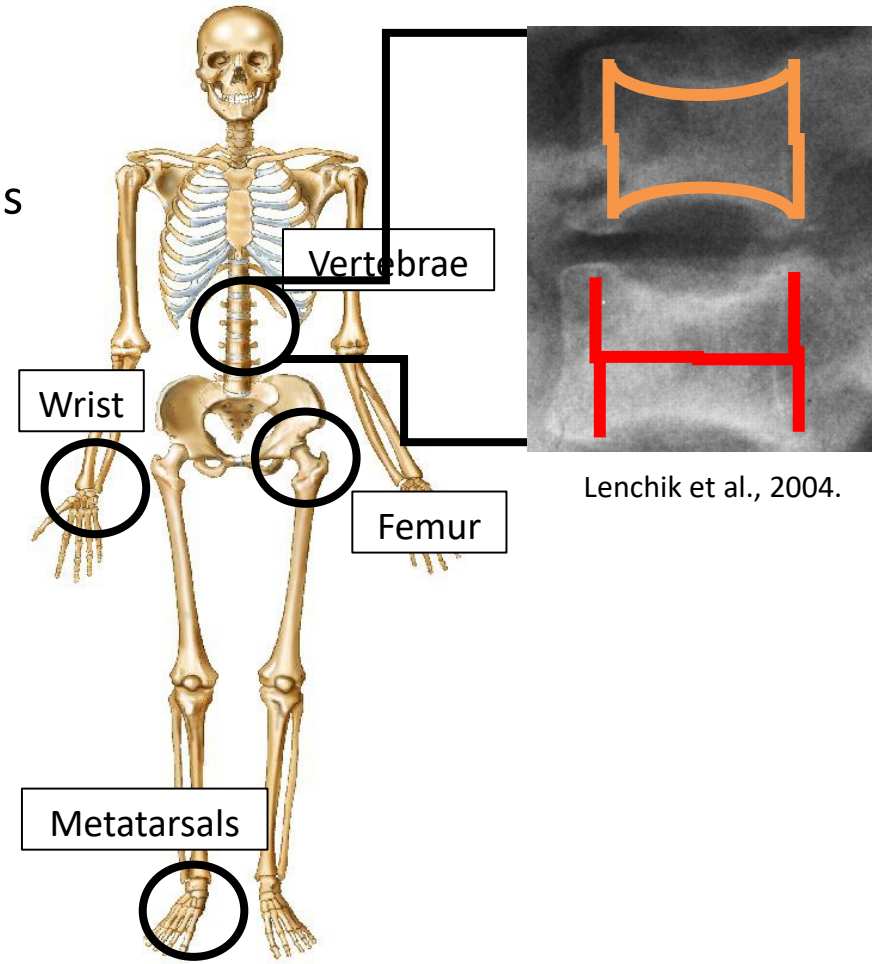
The pathology of sickle bone is not well understood

Clinically, sickle bone resembles osteoporosis

- Reduced mineral content
- Reduced cortical bone thickness
- Expanded marrow cavity

Current mechanistic paradigms

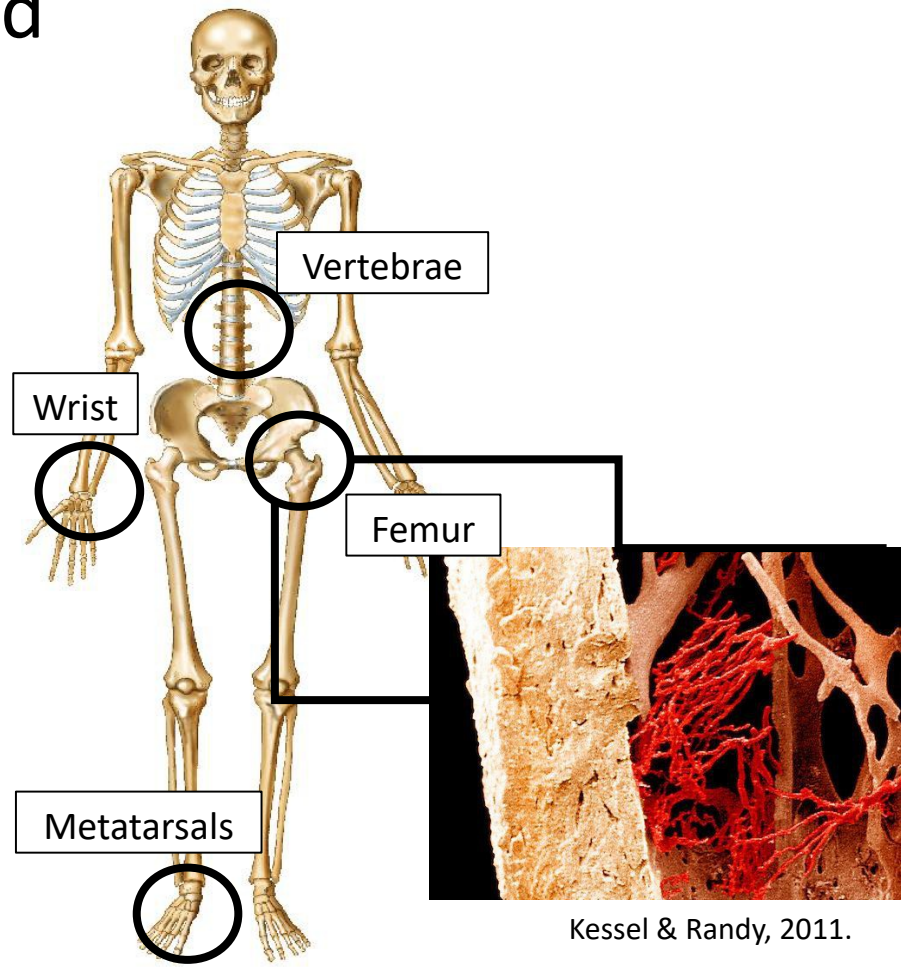
- Infarcts are considered asymptomatic and incidentally discovered (Ware et al., 1991)
- Hypoxic marrow microenvironment promotes sickling (Smith, 1996)
- Erythropoietic hyperplasia leads to retention of red marrow and expansion of marrow cavity (Rao et al., 1989)



Lenchik et al., 2004.

The pathology of sickle bone is not well understood

- Bone is a highly vascularized tissue
  - ➢Especially the trabecular regions
- Densely populated marrow creates oxygen gradient from feeding capillaries
- Vasculature of bone endosteal lining allows for more oxygen perfusion (Spencer et al. 2014)

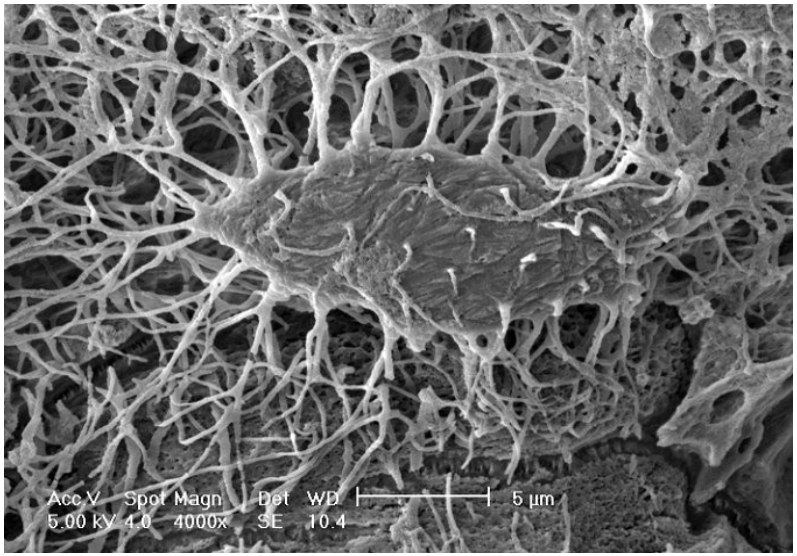


Kessel & Randy, 2011.

Sickle bone damage may not be a direct consequence of vaso-occlusion.

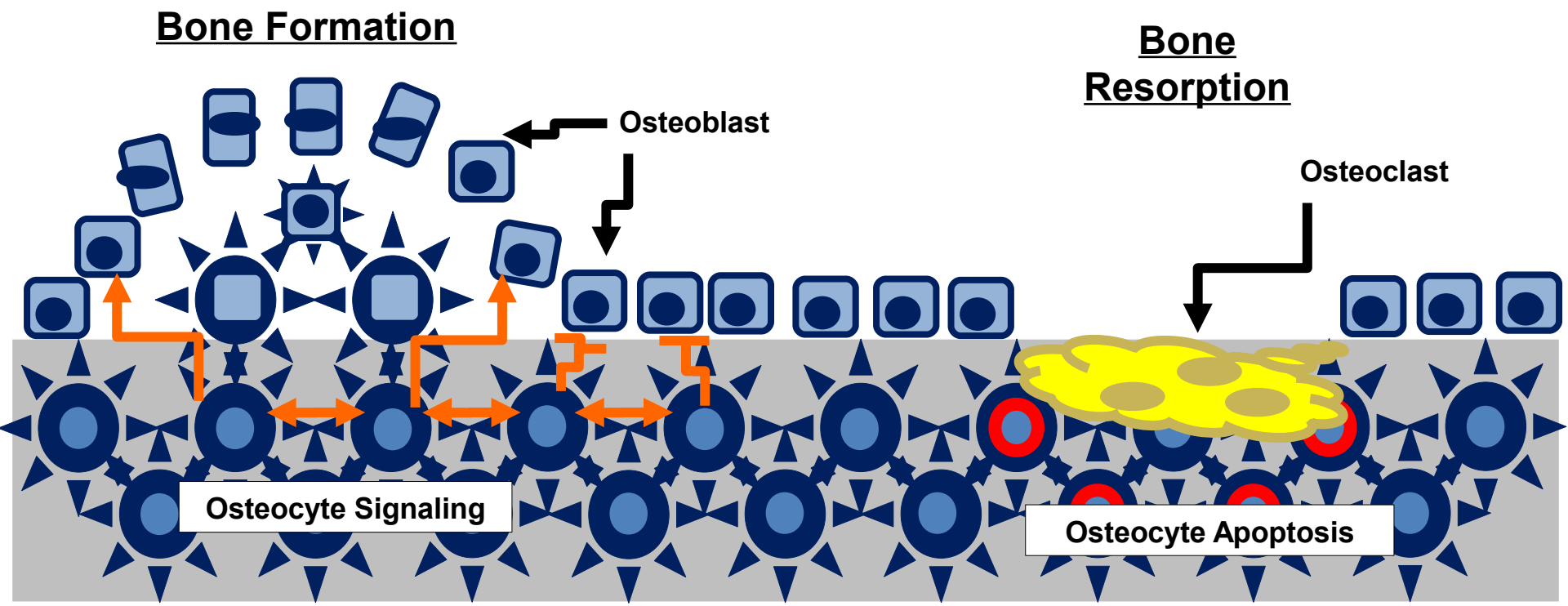
Osteocytes “The Master Orchestrator of Bone”

- Compose 90% of cell population
- Highly interconnected via gap junctions and hemichannels
- Sense mechanical loading
- Inhibit bone formation (osteoblast)
- Initiate bone resorption (osteoclast)
- Regulate systemic and local mineral concentrations
- Able to have long lifespans

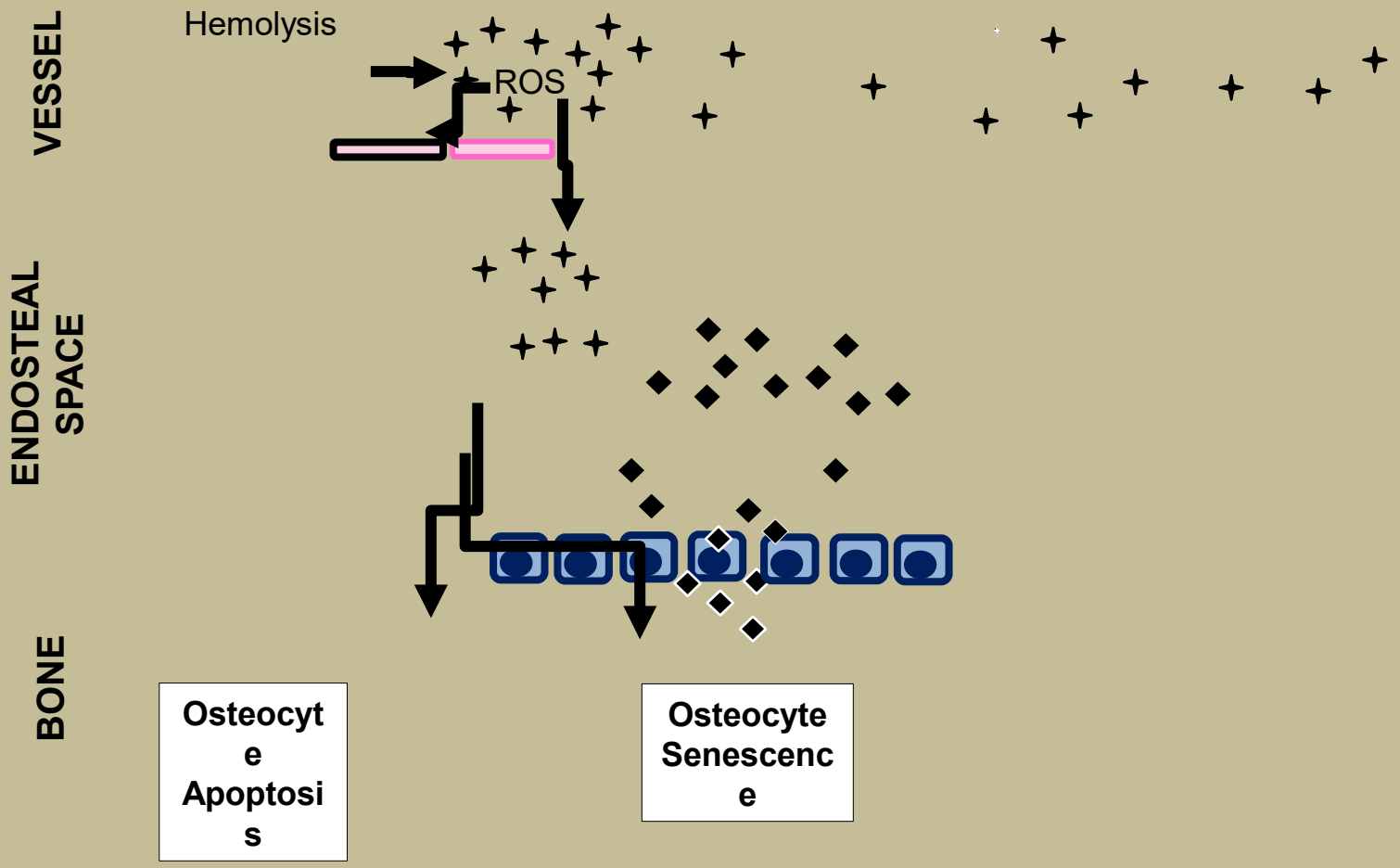


Bonewald, 2010.

Osteocytes and bone remodeling



Conceptual Mechanism of Sickle Bone Pathology



Transgenic Mouse Model of Sickle Cell Disease

- 10 week mice
- 21 week mice

- Expresses human hemoglobin
- Develops hemolytic anemia
- Exhibits severe organ pathology

Bone Imaging with micro-CT

- Micro-computed tomography (micro-CT) allows 3-D quantification of bone microstructure and ultrastructure and establishment of relationship between bone quality and disease

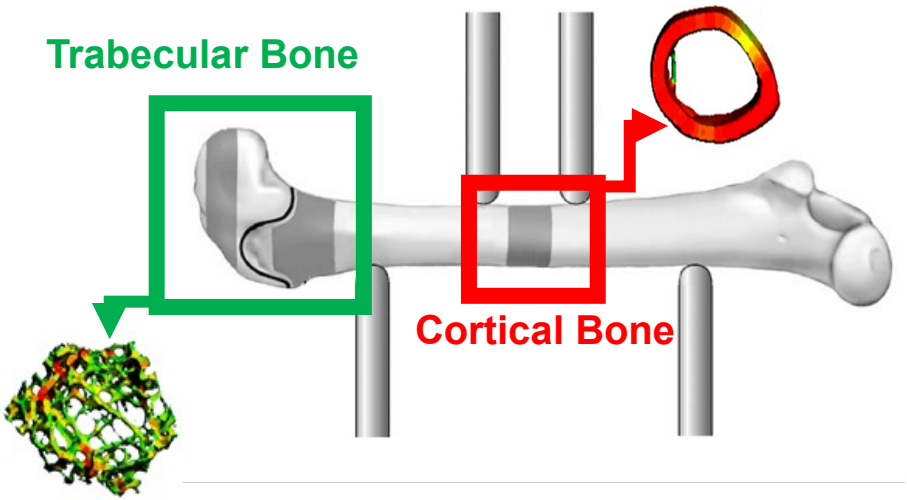
Müller, R. (2009) Hierarchical microimaging of bone structure and function *Nat. Rev. Rheumatol.* doi:10.1038/nrrheum.2009.107

Micro-CT imaging of Mouse Femur

- Mouse femur
- Compartments used to compute indices:
  - Gray: full bone
  - Red: cortical ring
  - Yellow: trabecular region

Müller, R. (2009) Hierarchical microimaging of bone structure and function *Nat. Rev. Rheumatol.* doi:10.1038/nrrheum.2009.107

Experimental Design



Appendicular Skeleton

- Humerus
- Femur
- Ulna
- Tibia

Blood Composition

- Hemolysis (LDH, Hb, hemopexin)
- Vascular injury (VCAM1, VE Cadherin)
- Antioxidants (HO-1, NO metabolites)
- Estradiol

MicroCT & Mechanical Testing

Histological Analysis

- Marrow Grading (Trichrome)
- General Staining (Toluidine Blue)

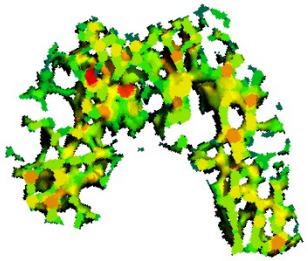
Glutamine Therapy

- Drinking water (ad libitum 1 g/kg/day)

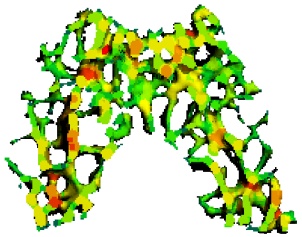
Femoral Epiphyseal Trabecular Bone

10 weeks

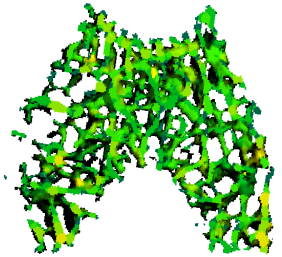
AA



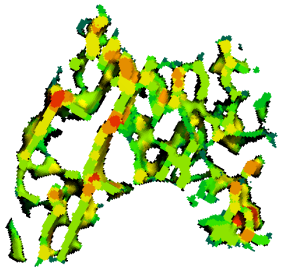
AS

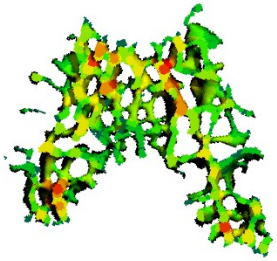


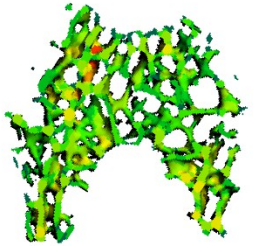
SS



21 weeks



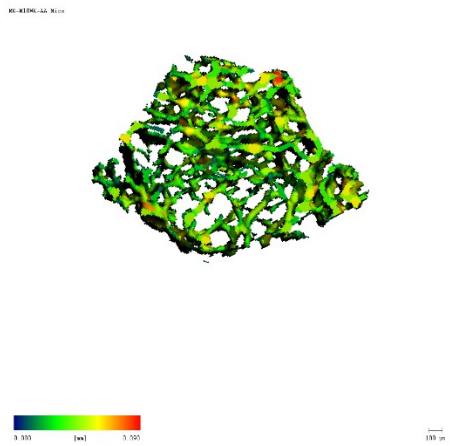




Femoral Metaphyseal Trabecular Bone

10 weeks

AA



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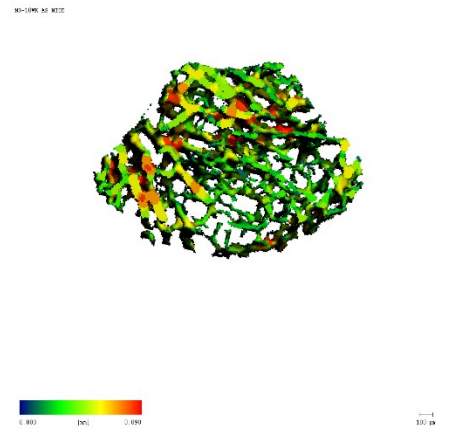

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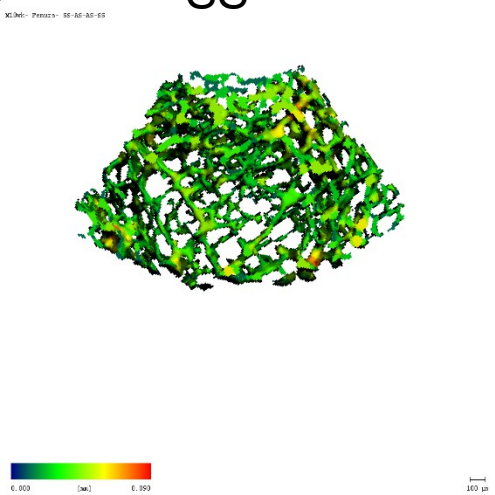
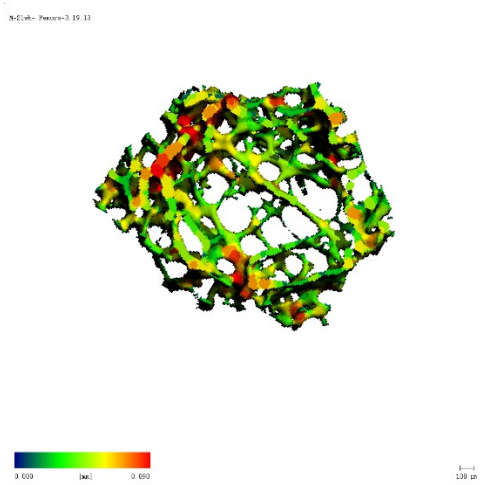
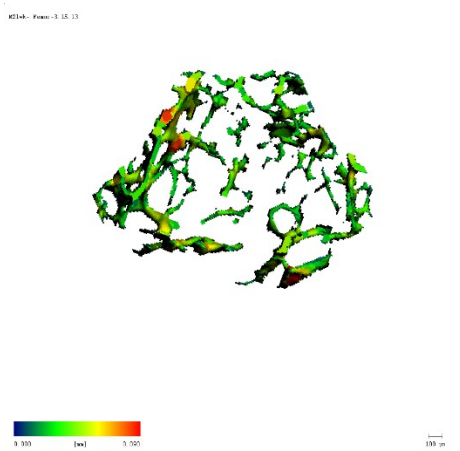
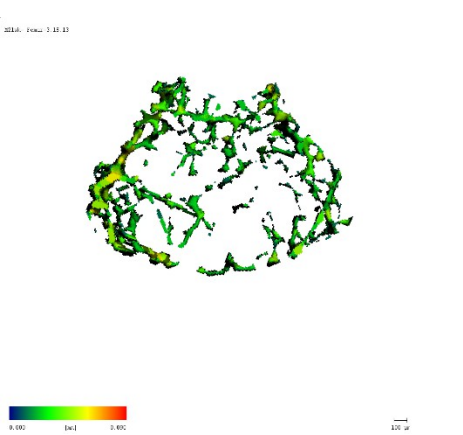


21 weeks

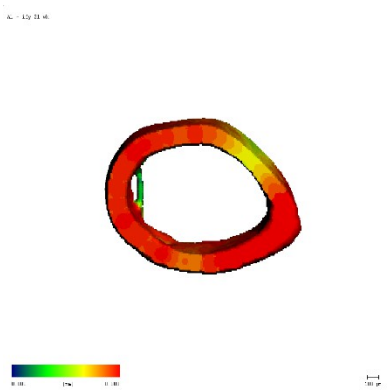






Femoral Mid-Diaphysis Cortical Bone

AA



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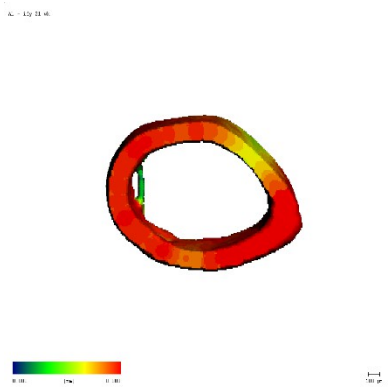
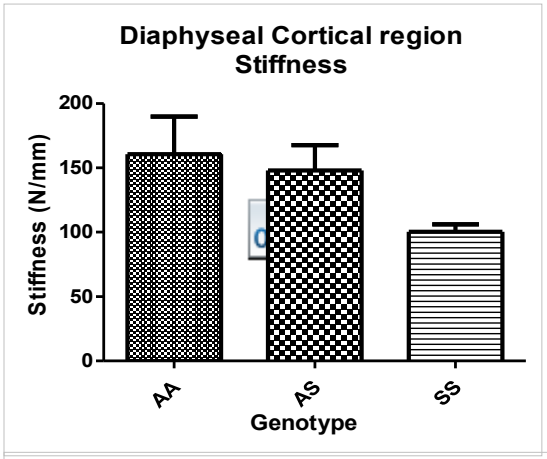
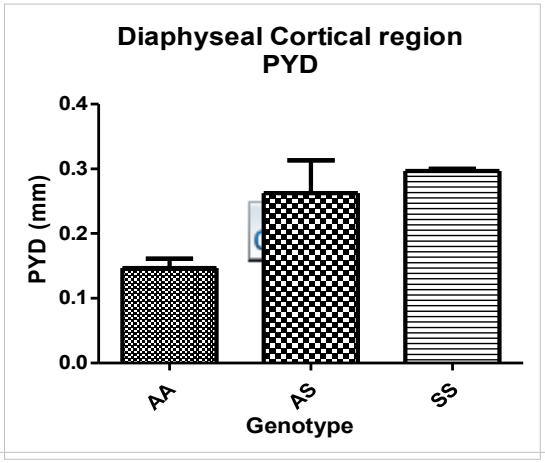


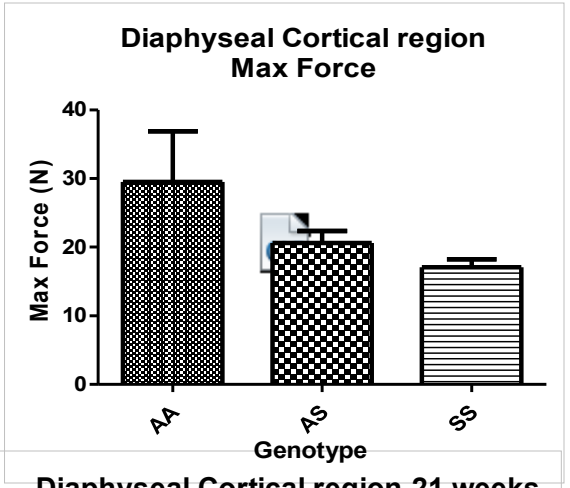
SS

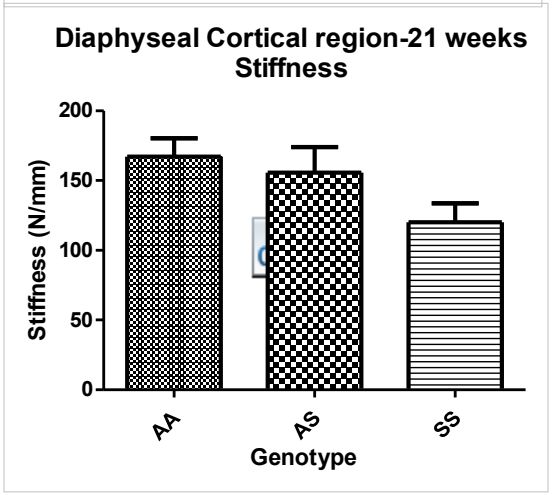
- Reduced bone volume fraction and cortical thickness correlates to mechanical testing data

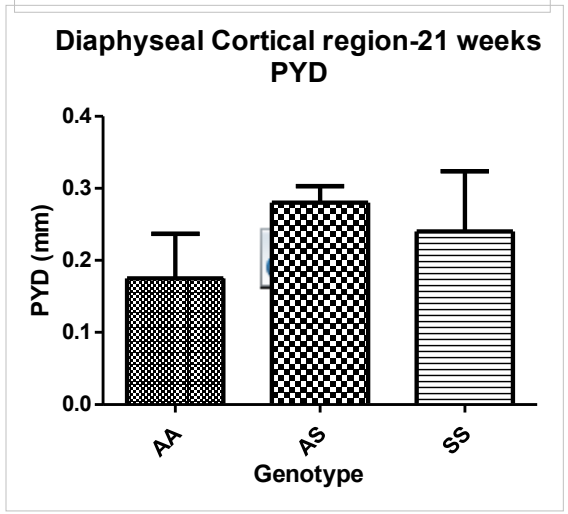
Bone mechanical properties in sickle mice at 10 and 21 weeks

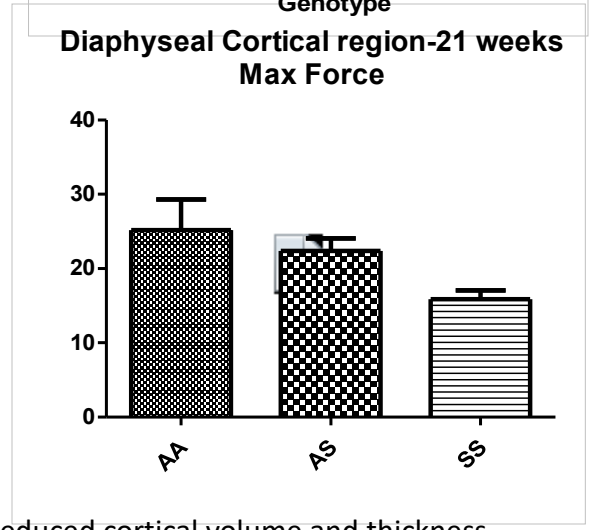












- Stiffness and maximum force to breakage is reduced in sickle mice, correlating with reduced cortical volume and thickness
- Post yield deflection (PYD), which is the opposite of stiffness is also increased in sickle mice

Sickle bone quality rapidly declines with age

SS cortical thickness 30% thinner

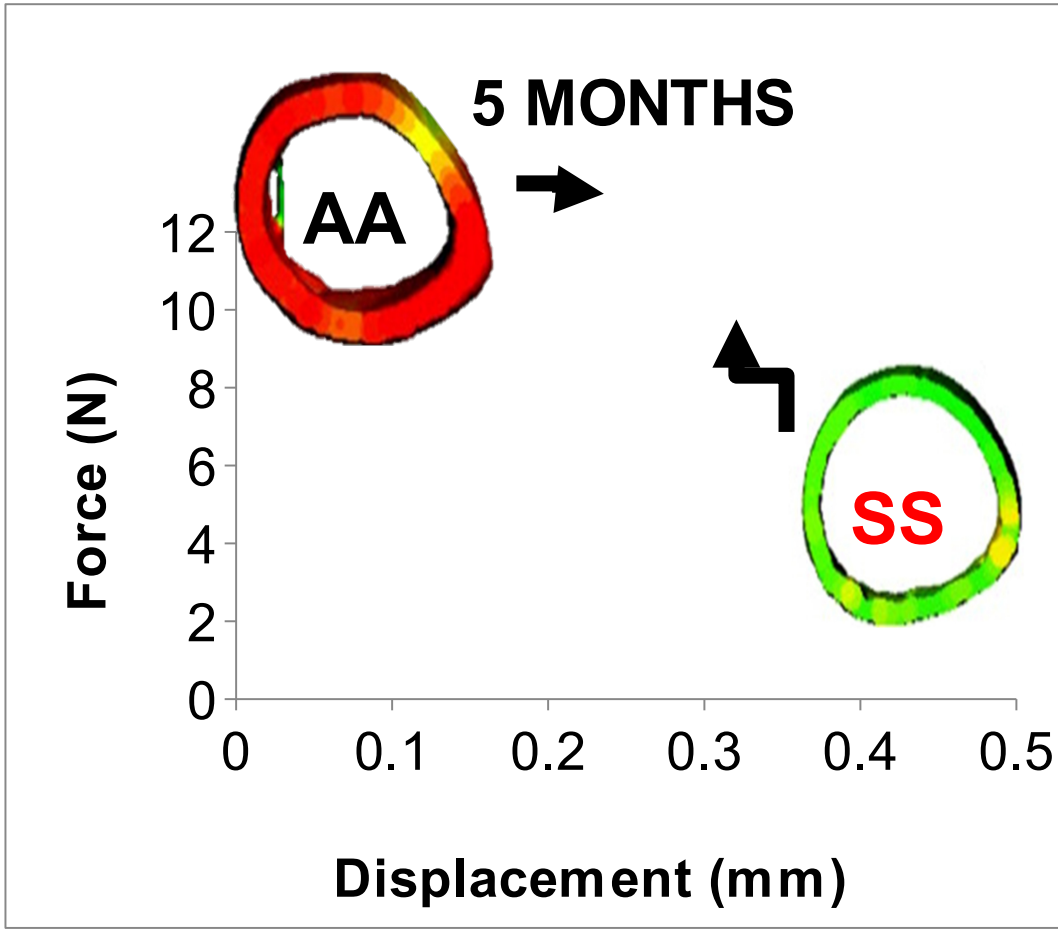
Reflected in sickle bone mechanics:

Strength

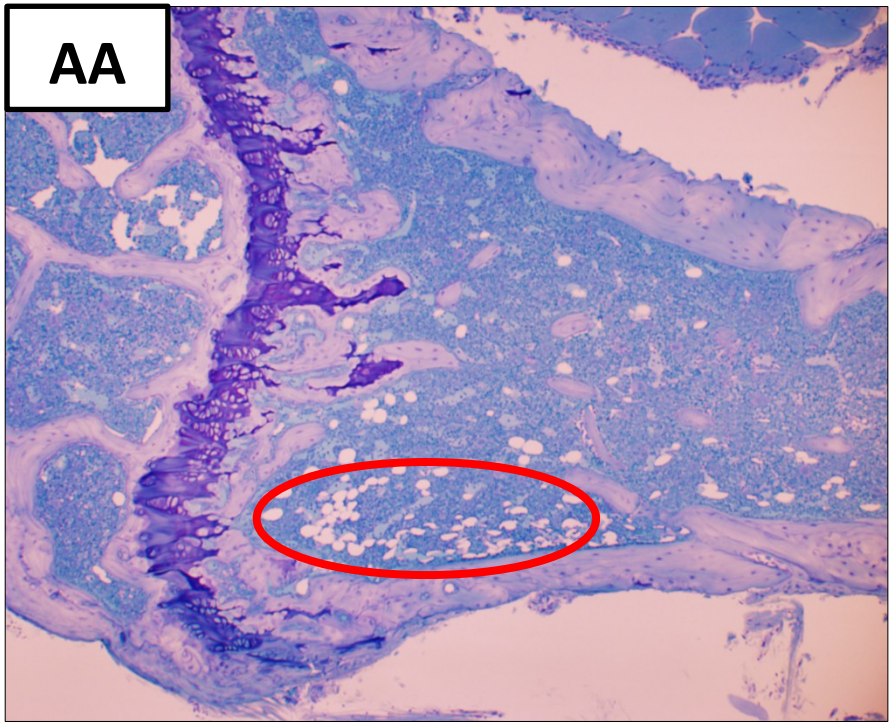
- 10% decrease Elastic Modulus
- 40% decrease in max and yielding force resistance
- 30% decrease stiffness

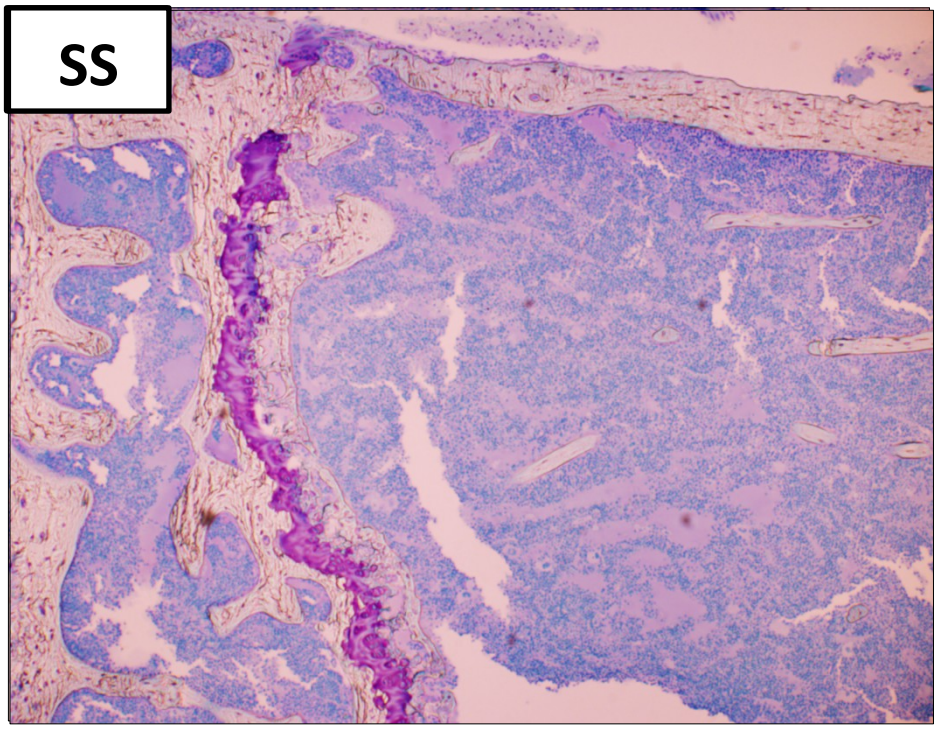
Toughness

- Post-yield deflection/deformation
- Energy needed to fracture (or work) were comparable to wild-type



Osteonecrotic features in sickle mouse tibia





Sickle bone quality rapidly declines with age

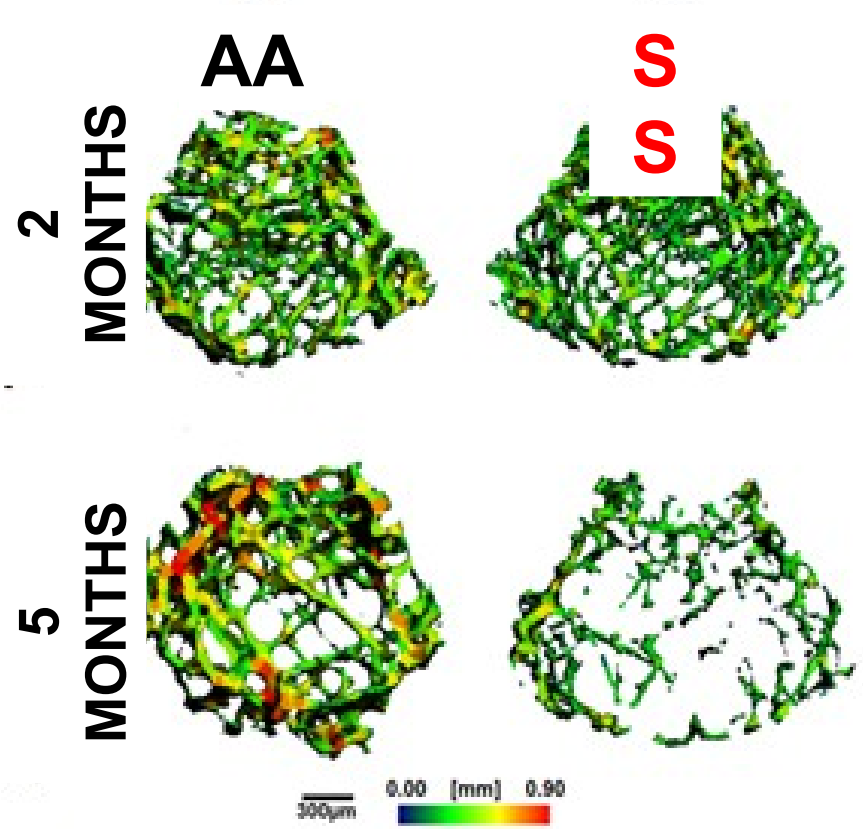
Femur length and tissue mineral density does not vary between genotypes.

Younger mice have comparable trabecular bone microarchitecture.

Older sickle bones reveal significant deterioration

- 70% Less Trabeculae
- 90% Connectivity
- 70% Thinner

Histological grading implies the progressive damaging of sickle bone

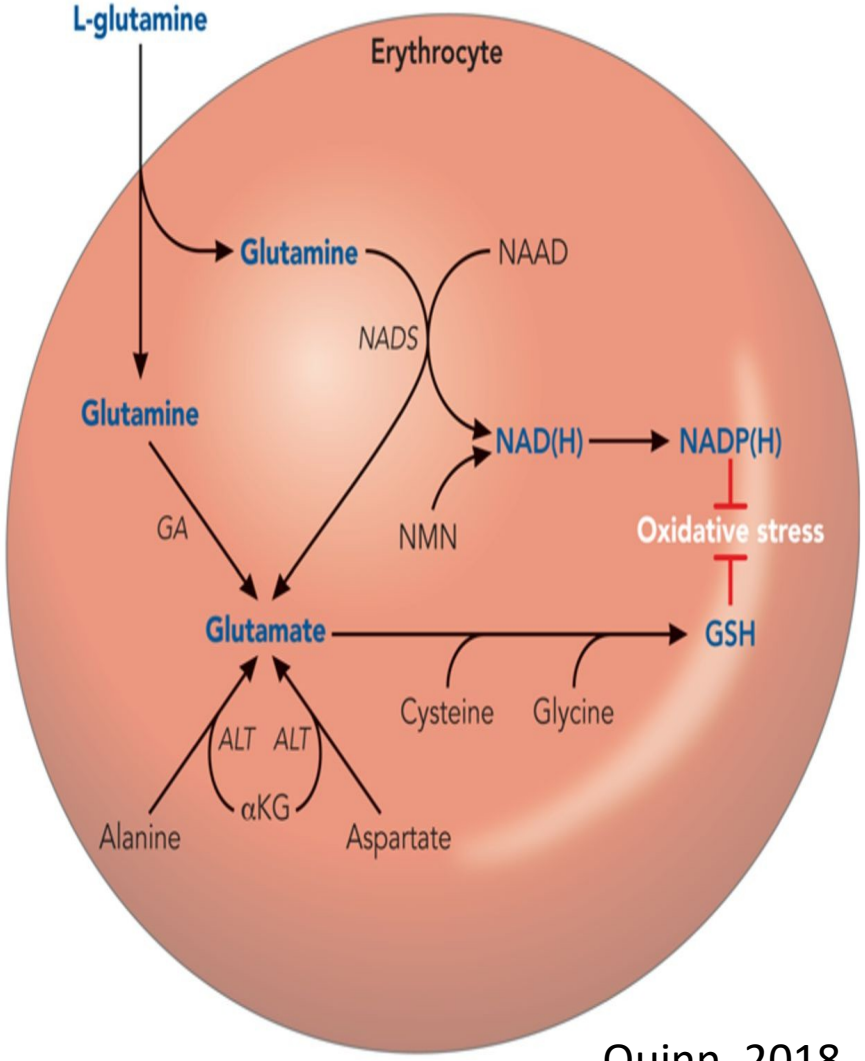


Hypothesis

Sickle RBC hemolysis derived oxidative stress disrupts osteocyte physiology and drives bone resorption

L-Glutamine (GLN) therapy for SCD: Evaluation through analysis of its impact on bone

- Glutamine is a conditionally essential amino acid required to synthesize NAD and NADP
- Glutamine is a precursor to glutamate which is used to synthesize glutathione, an antioxidant and apoptosis suppressor
- In oxidation stressed sickle RBC, uptake of glutamine is increased and the redox ratio NAD/NAD+NADH is decreased compared to normal
- Oral administration of glutamine is thought to increase the redox ratio
- Glutamine's therapeutic mechanism and long term safety are uncertain



Quinn, 2018.

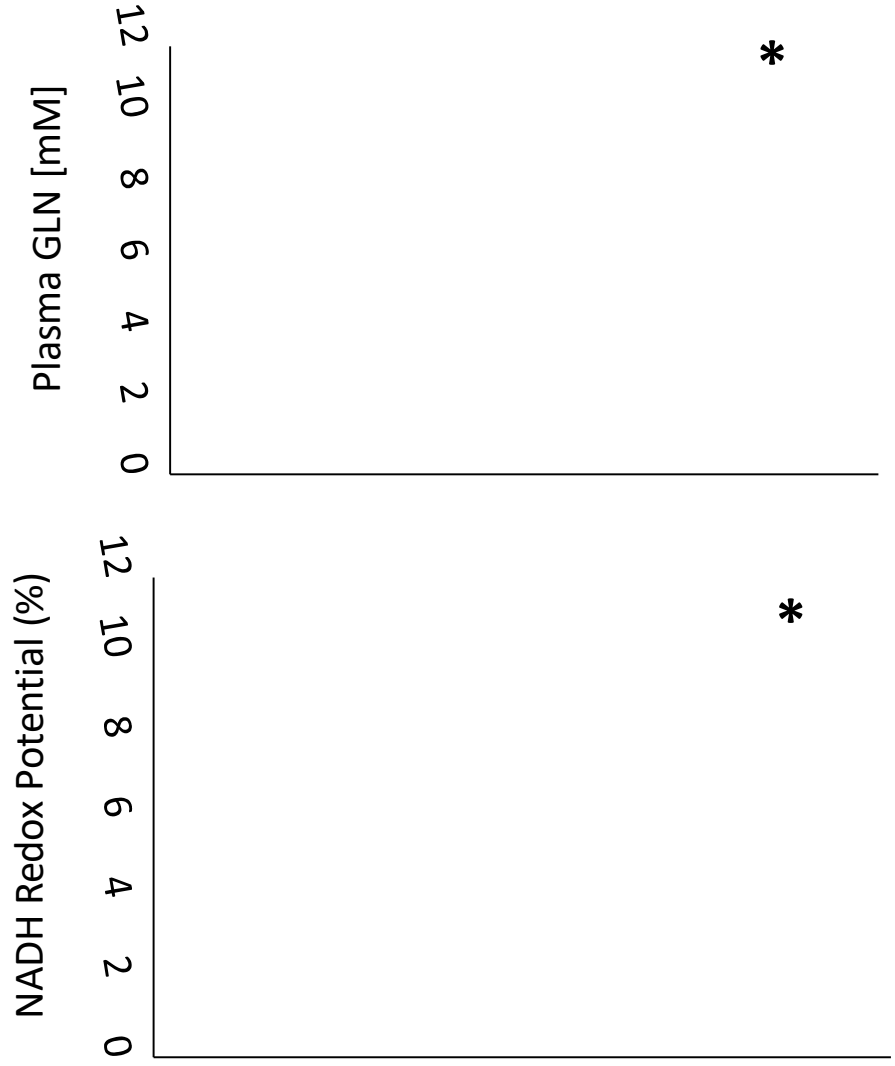
Experimental Model

8 Weeks of Age (Adolescence)

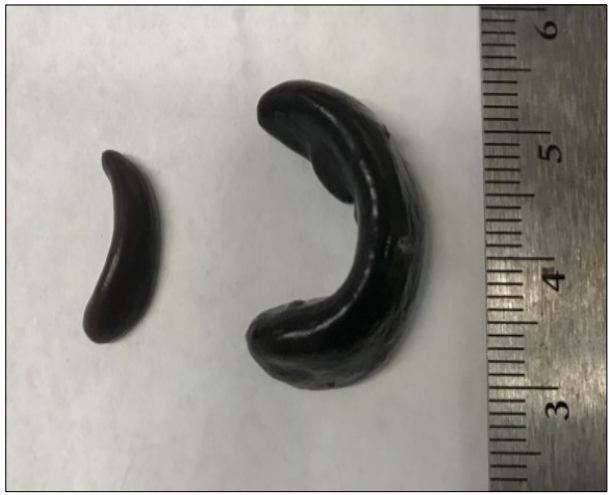
- Wild-type (AA)
- Sickle mice (SS)
- GLN treated sickle mice (SS + GLN)
  - Drinking water (1g/kg for 4 WKs)

- Expreses human hemoglobin
- Develops hemolytic anemia
- Exhibits severe organ pathology

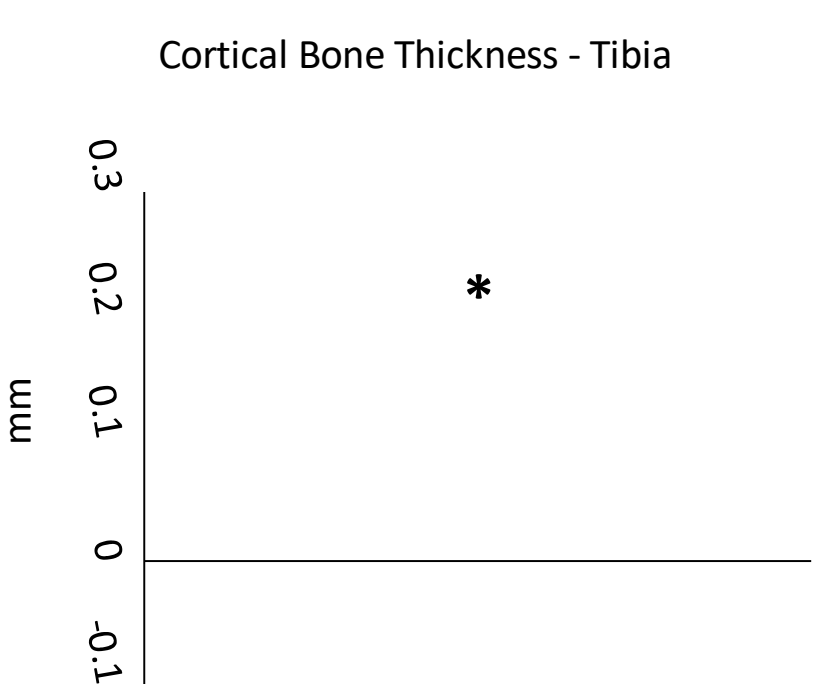
GLN reduces sickle whole blood redox potential

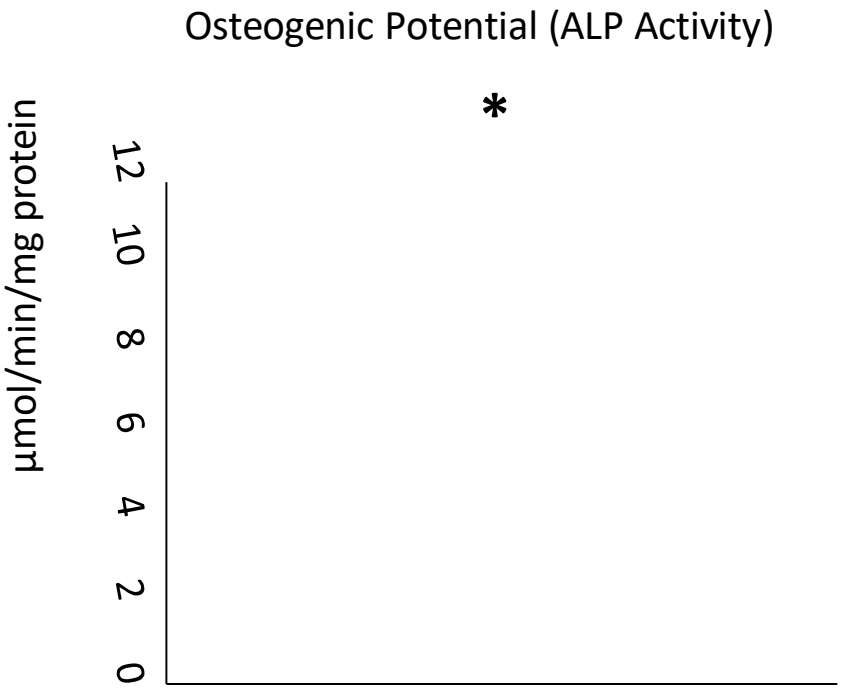


- Supplementation increases plasma GLN conc.
- GLN reduces redox potential by 25%
- GLN reduces sickle spleen mass by 89%



GLN maintains healthy bone tissue in SCD

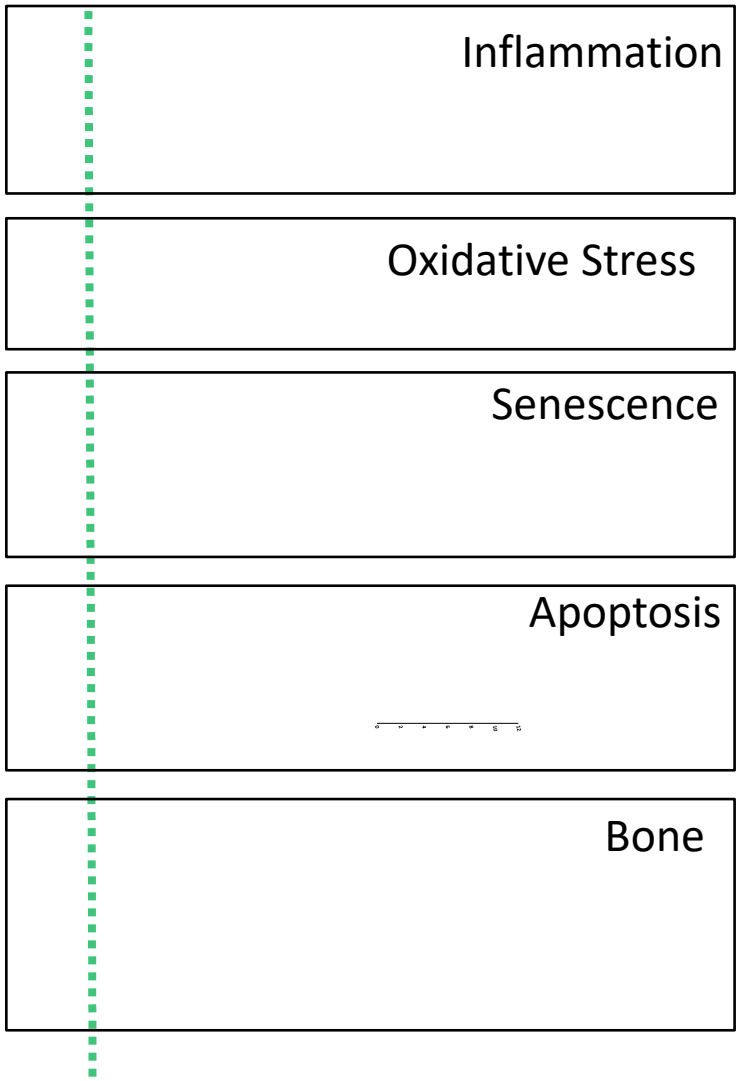




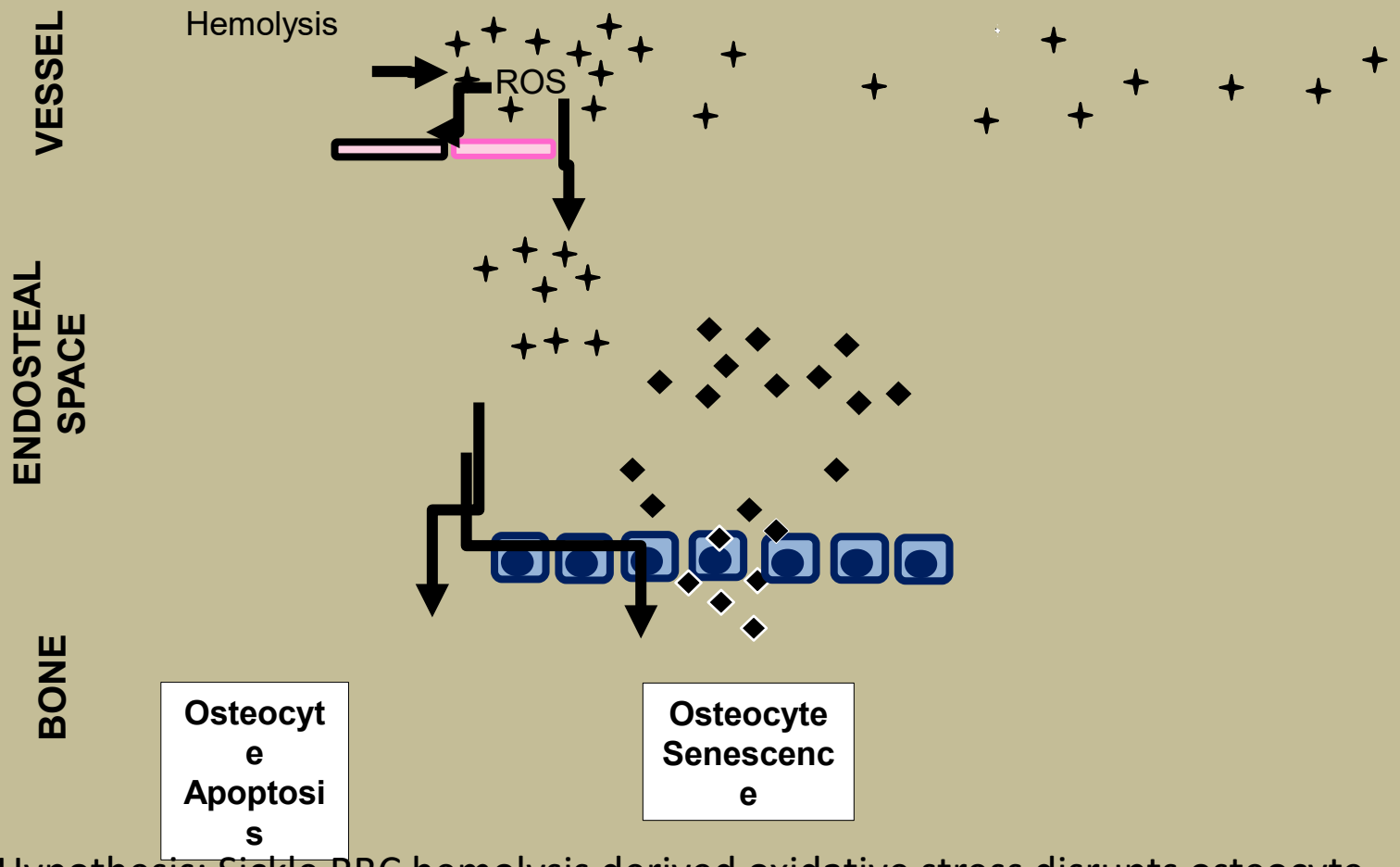
- GLN treated sickle bone has an average cortical thickness comparable to wild-type controls
- Untreated sickle marrow osteogenic potential is significantly higher than AA and SS +GLN

GLN maintains healthy bone tissue in SCD

- GLN supplementation significantly down regulates sickle osteocyte gene expression – recovery to that of wild-type (green) for genes associated with oxidative stress, senescence, and bone activity
- Considerations: inflammation and apoptosis are associated with a pro-bone resorption environment



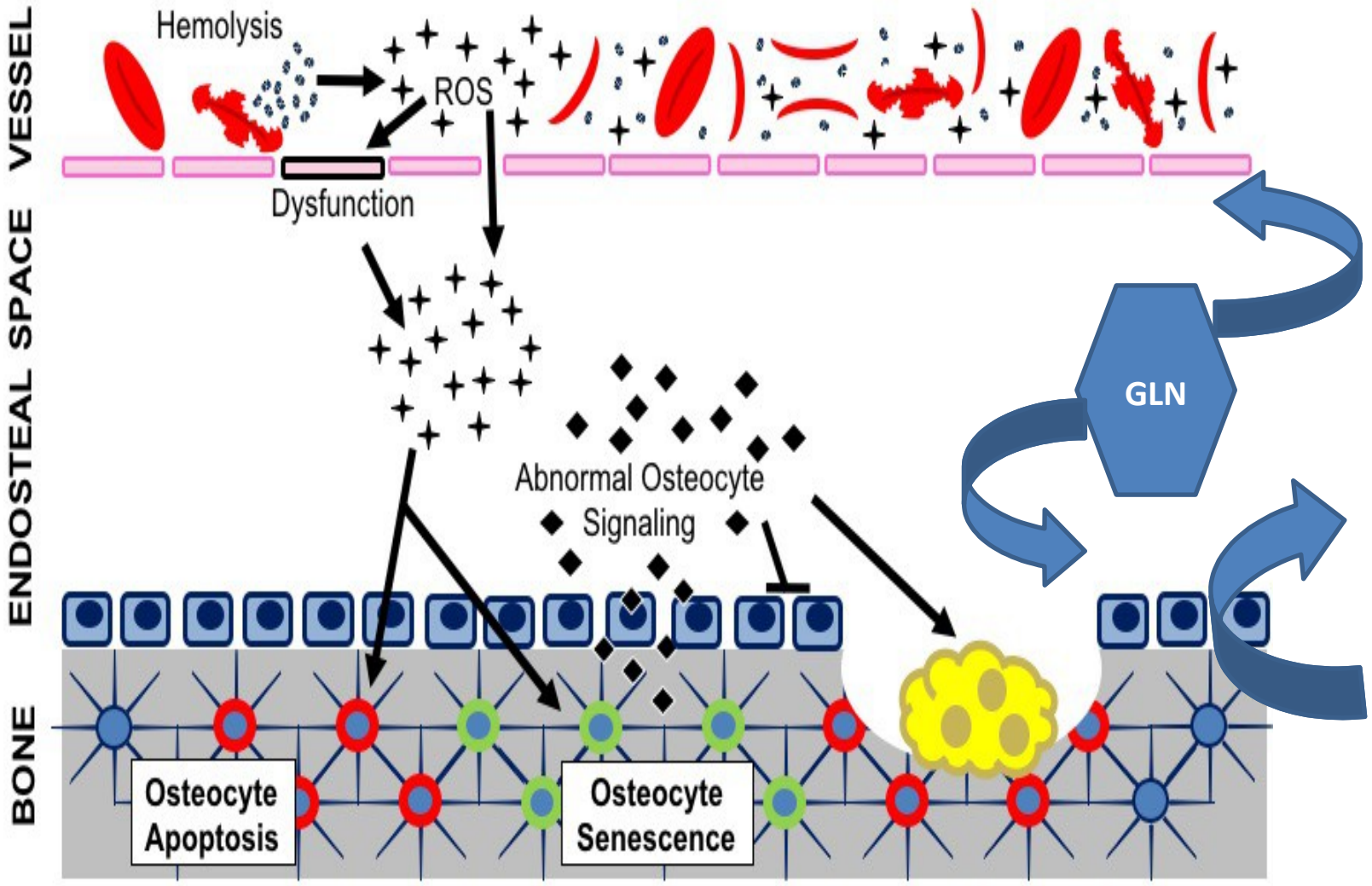
Conceptual Mechanism of Sickle Bone Pathology



Hypothesis: Sickle RBC hemolysis derived oxidative stress disrupts osteocyte physiology and drives bone resorption

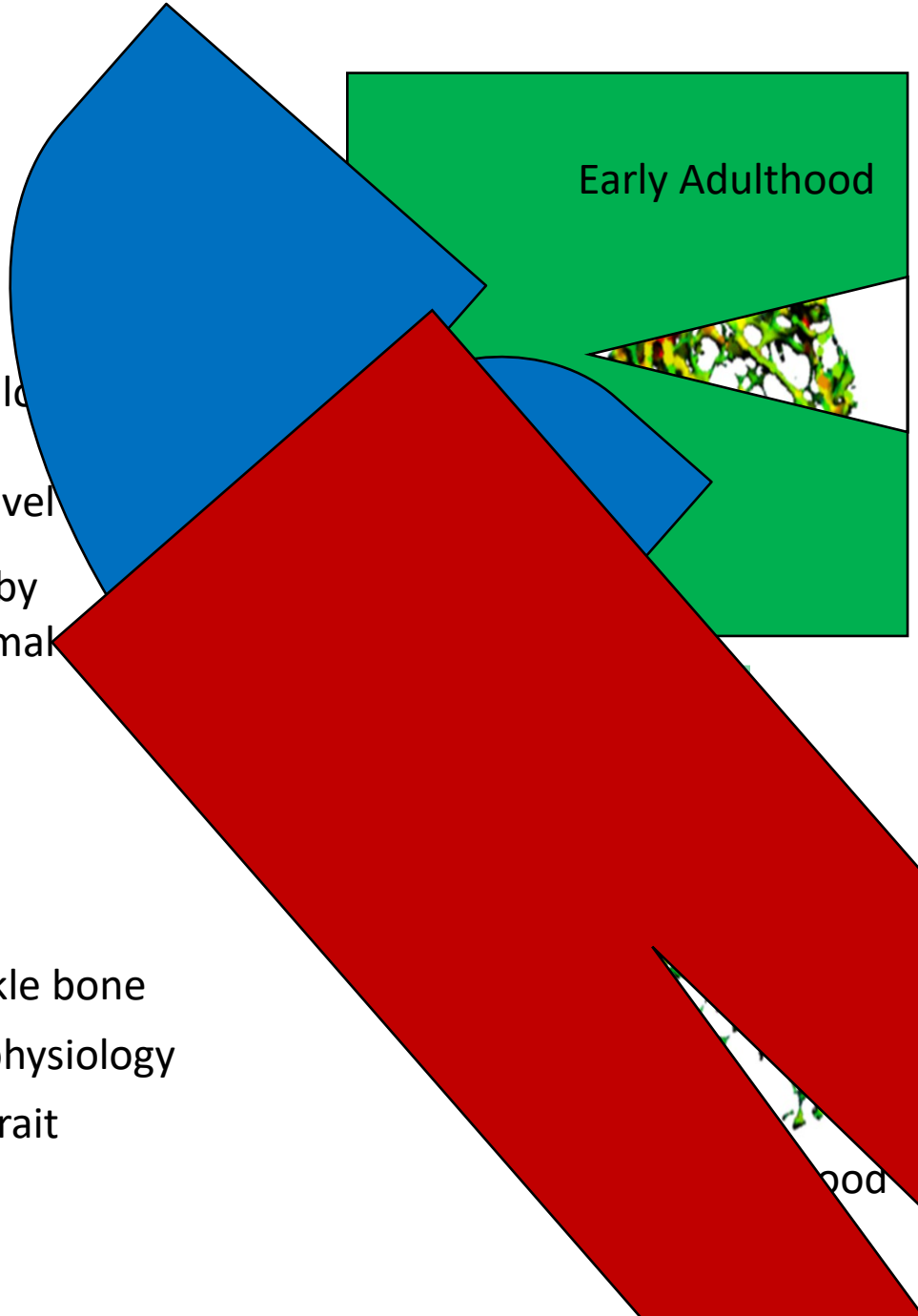
GLN supplementation may interrupt sickle pathology by targeting the vasculature or parenchymal tissue

Possible protection from oxidative stress



In Summary

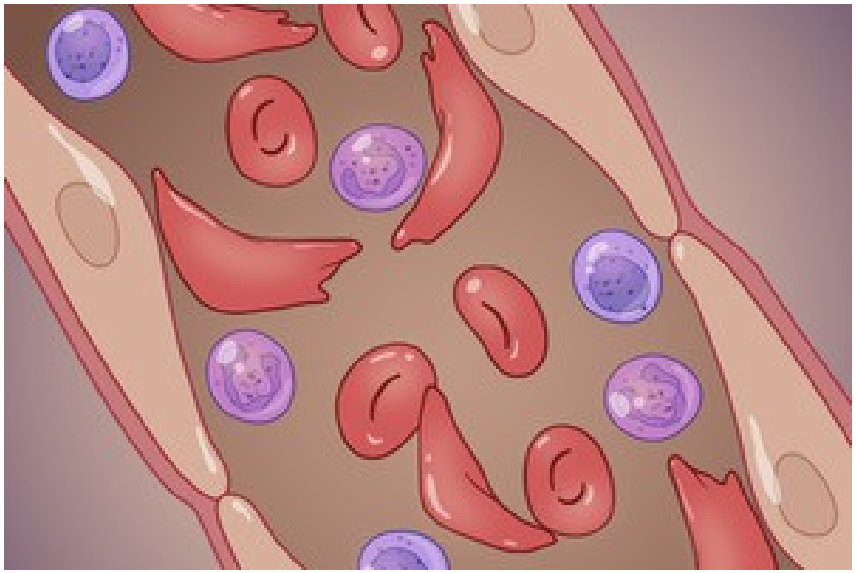
- Sickle RBC biomechanics damages the vasculature and surrounding tissue
- Sickle mouse bone is a unique model that allo for the in depth evaluation of sickle pathophysiology at the tissue and cellular level
- L-GLN therapy may maintain healthy tissue by protecting vasculature or altering parenchymal cell activity



Future Studies

- Assess the long term effects of GLN on sickle bone
- Determine whether SCD alters osteocyte physiology
- Identify differences between gender and trait

In Closing

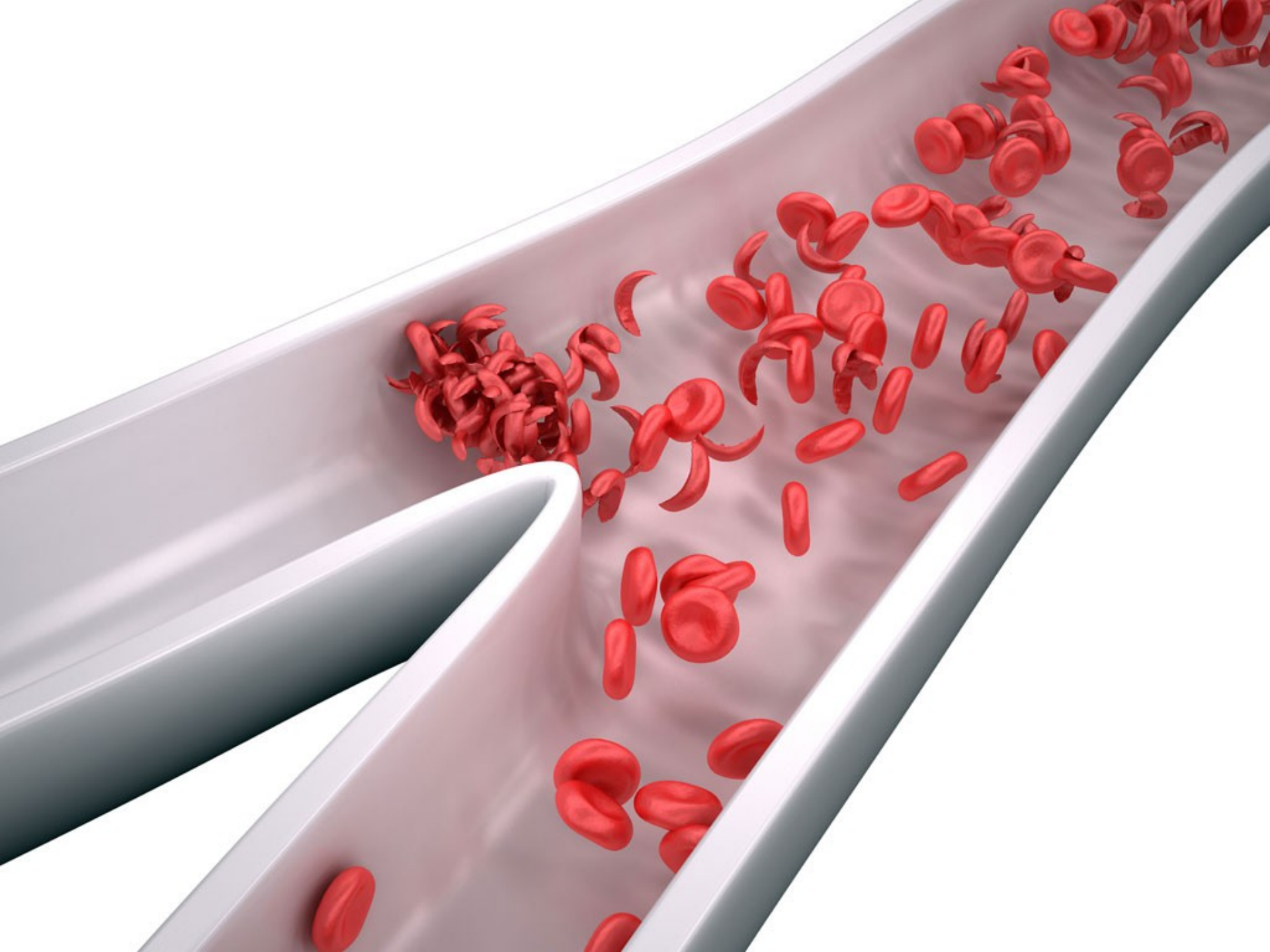


- Cell biomechanics (abnormalities in physical and structural characteristics) provide telltale signs of disease and can serve as indicators of the effectiveness of therapies

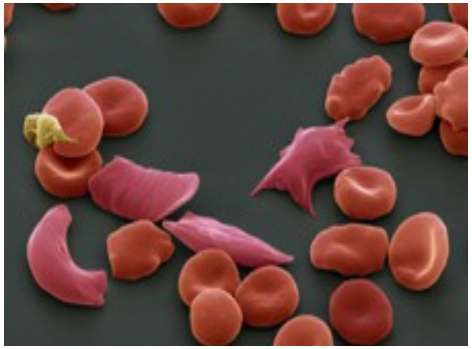
The City College of New York

BARABINO

THANK YOU



Sickle cell biomechanics, pathology and therapies



Patho-physiology

Hb polymerization and vaso-occulsion

Cell Mechanical Properties

RBC stiffness
RBC adhesiveness
Increased viscosity

Therapies

Transfusion
Hydroxyurea
Glutamine
Gene editing
Bone marrow transplant