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Sickle Cell Disease: An Arginine Deficiency Syndrome with Distinctive Nutritional Requirements

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Amino Acids (AA)

- Essential AA → diet dependent
- Non-essential AA → *de novo* synthesis
- *Conditionally essential* → Non-essential AA that become indispensable under stress/critical illness
  - *Capacity of endogenous synthesis surpassed*

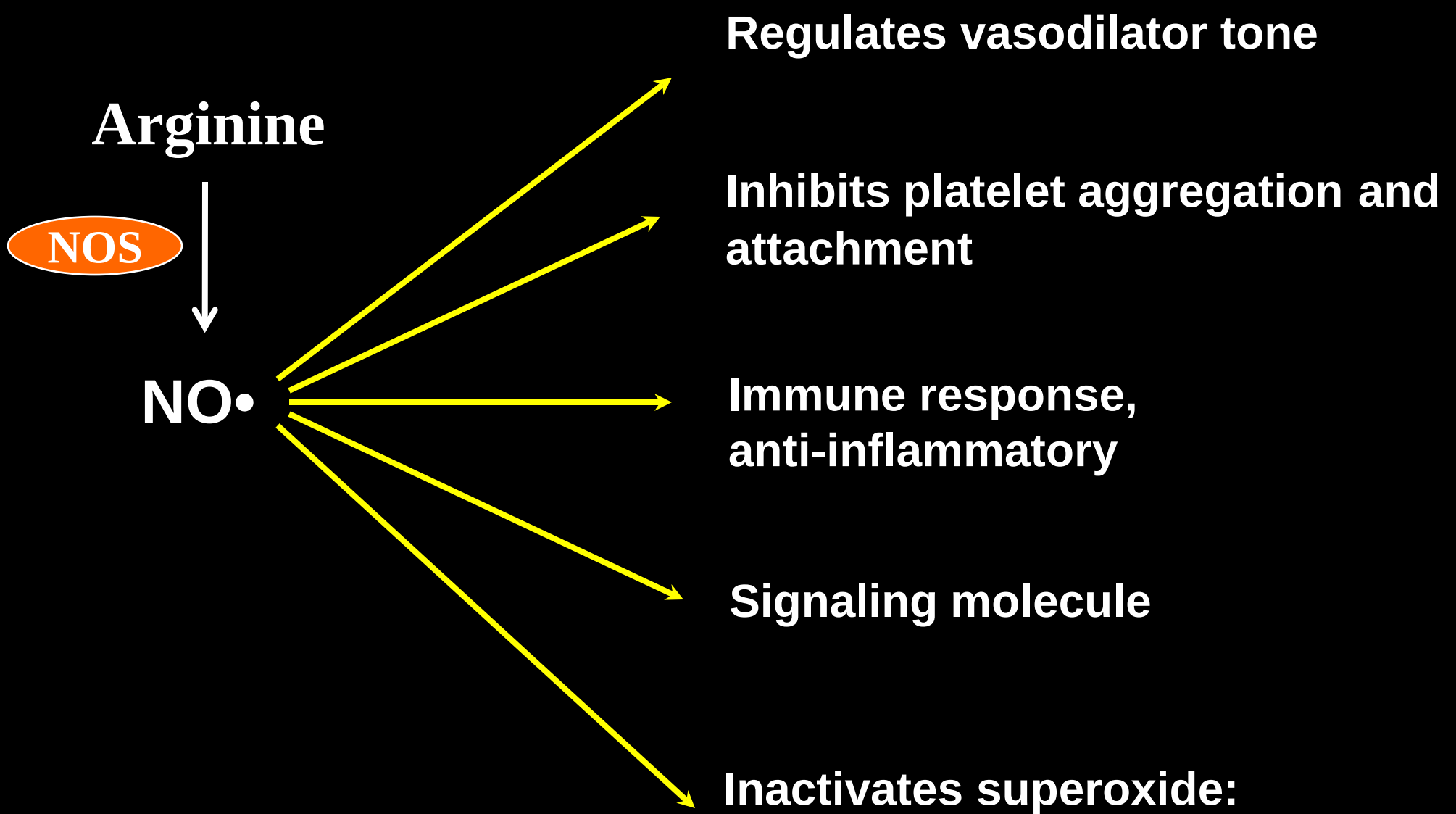
- Focus: *L-arginine in Sickle Cell Disease (SCD)*

Conditions Linked to Acquired Arginine (Arg) Deficiencies

- trauma
- critical illness
- burns
- surgery
- pregnancy
- sepsis
- pulmonary hypertension, asthma
- hemolysis: ***sickle cell disease***, thalassemia, malaria…

What is Arginine?

- Conditionally essential amino acid in dietary protein
- Synthesized through the intestinal-renal axis
- Becomes essential in stress, catabolic states & hemolysis: trauma, sepsis, burns, sickle cell disease, malaria, PH, ?? asthma
- Found naturally in diet: meat, dairy, seafood, nuts, seeds +
- Normal adult ingestion: 2-7 grams/day
- Nutritional supplement with low toxicity
- *Obligate substrate for NO production*

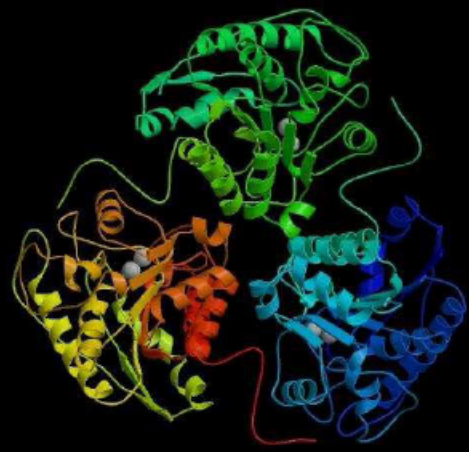

Arginine
NOS
NO•
Regulates vasodilator tone
Inhibits platelet aggregation and attachment
Immune response, anti-inflammatory
Signaling molecule
Inactivates superoxide:
Diffusion-limited antioxidant

What Is Arginase?

Mammals Express Two Arginase Isozymes

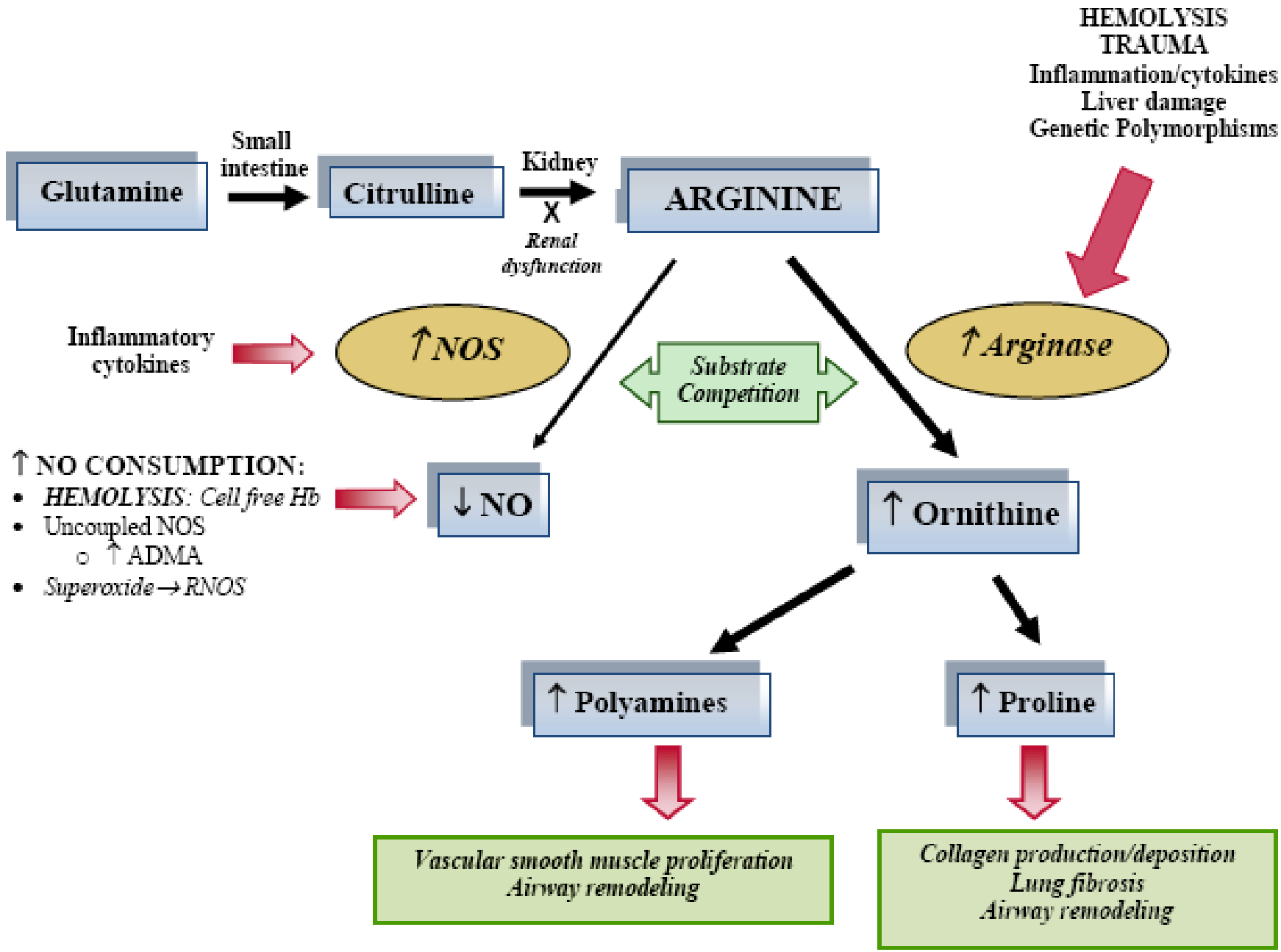
- Important enzyme in urea cycle
- 2 mammalian isoforms
  - *Arginase I (cytosolic)*
  - *Arginase II (mitochondrial)*
- Present in most cell types, including RBCs and MDSC
- Induced by cytokines
- Competes with NOS for common substrate → *Arginine*

- **Two isozymes: cytosolic (type I) & mitochondrial (type II)**
- **Homotrimer**
- **Require Mn^{2+} at active site**



Ornithine

- Arginine $\xrightarrow{\textit{arginase}}$ ***Ornithine*** + Urea
- Orn & Arg use same cationic amino acid transporter (CAT-1 and CAT-2)
- ↑ Orn competitively inhibits cellular uptake of Arg
  - *Limits Arg bioavailability*
  - *Translates to ↓ NO bioavailability*

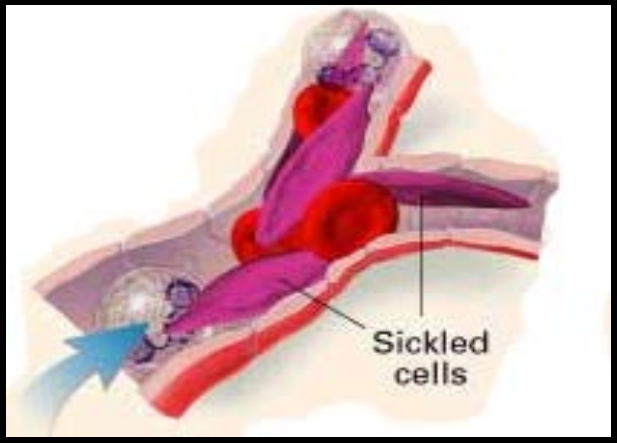

HEMOLYSIS
TRAUMA
Inflammation/cytokines
Liver damage
Genetic Polymorphisms
Glutamine
Small intestine
Citrulline
Kidney
X
Renal dysfunction
ARGININE
Inflammatory cytokines
↑NOS
Substrate Competition
↑Arginase
↑ NO CONSUMPTION:
• HEMOLYSIS: Cell free Hb
• Uncoupled NOS
○ ↑ ADMA
• Superoxide → RNOS
↓ NO
↑ Ornithine
↑ Polyamines
↑ Proline
Vascular smooth muscle proliferation
Airway remodeling
Collagen production/deposition
Lung fibrosis
Airway remodeling

Amino Acid Deficiencies

- Drop in any AA ≠ clinically significant deficiency
- For a nutritional deficiency to be present:
  - *Biological processes dependent on nutrient compromised*
  - *Compromise leads to abnormal physiologic response causative of poor outcomes*
  - *Poor outcomes reversed by amino acid replacement*

SCD is a model for distinctive nutritional requirements that arise from a chronic disease

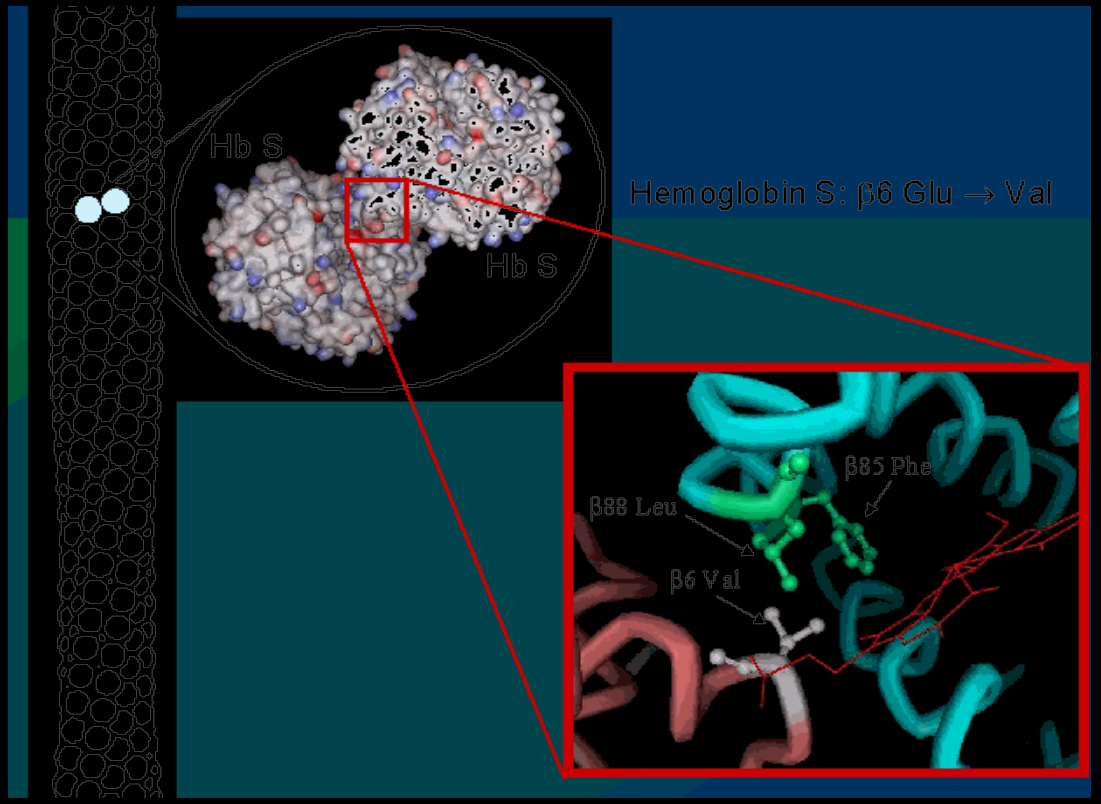
What Is Sickle Cell Disease?



- An inherited disease of red blood cells
- Affects hemoglobin
- Hb Polymerization leads to cascade of effects ↓↓ blood flow
- Tissue hypoxia causes acute and chronic damage

- 8% AA carriers of Hb-S (trait)
- 1/400 AA, > 100,000 with SCD in USA, millions worldwide
- > 75,000 admits; annual cost > $470 million

Why Do Cells Sickle?

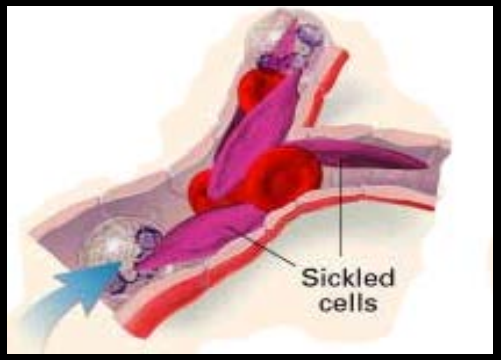


- Substitution of valine for glutamic acid in Hb molecule
- Polymerization of sickle Hb under the stress or deoxygenation

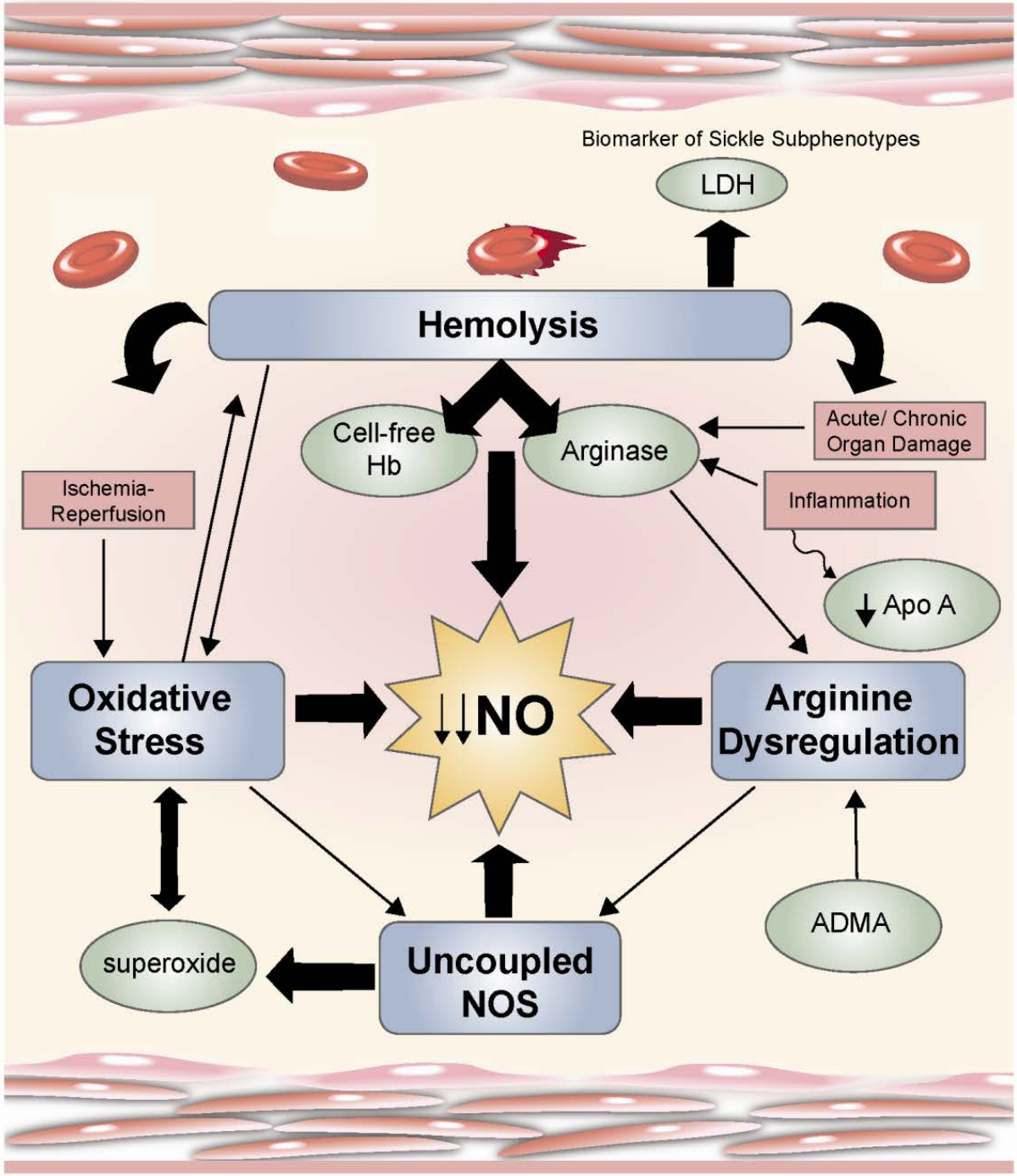
Cell Disease (SCD):
A Model for Vasculopathy & Endothelial Dysfunction

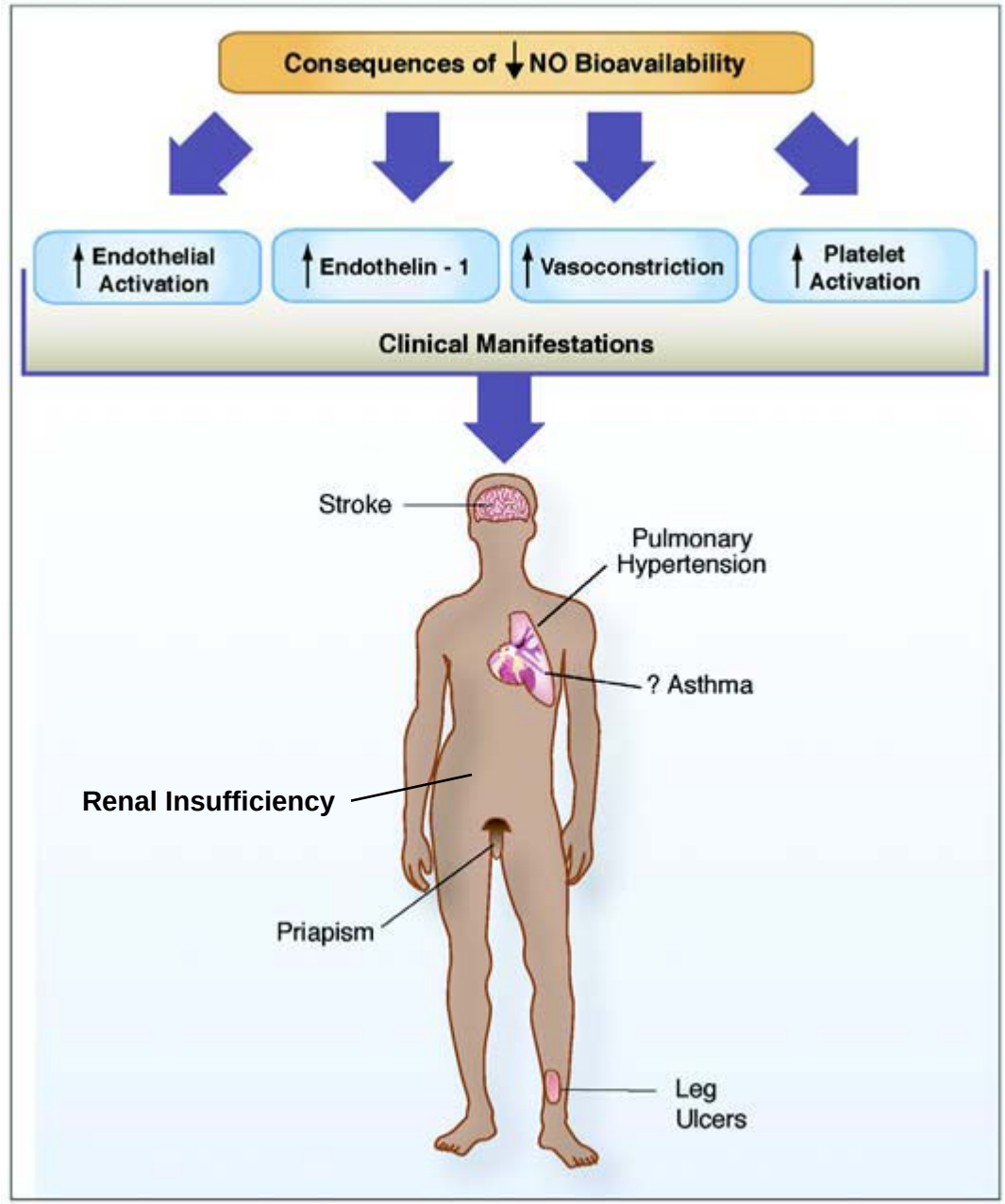
An inherited red blood cell disease: hemolytic anema

- Hemolysis
- Inflammation
- Nitric Oxide depletion
- Arginine depletion

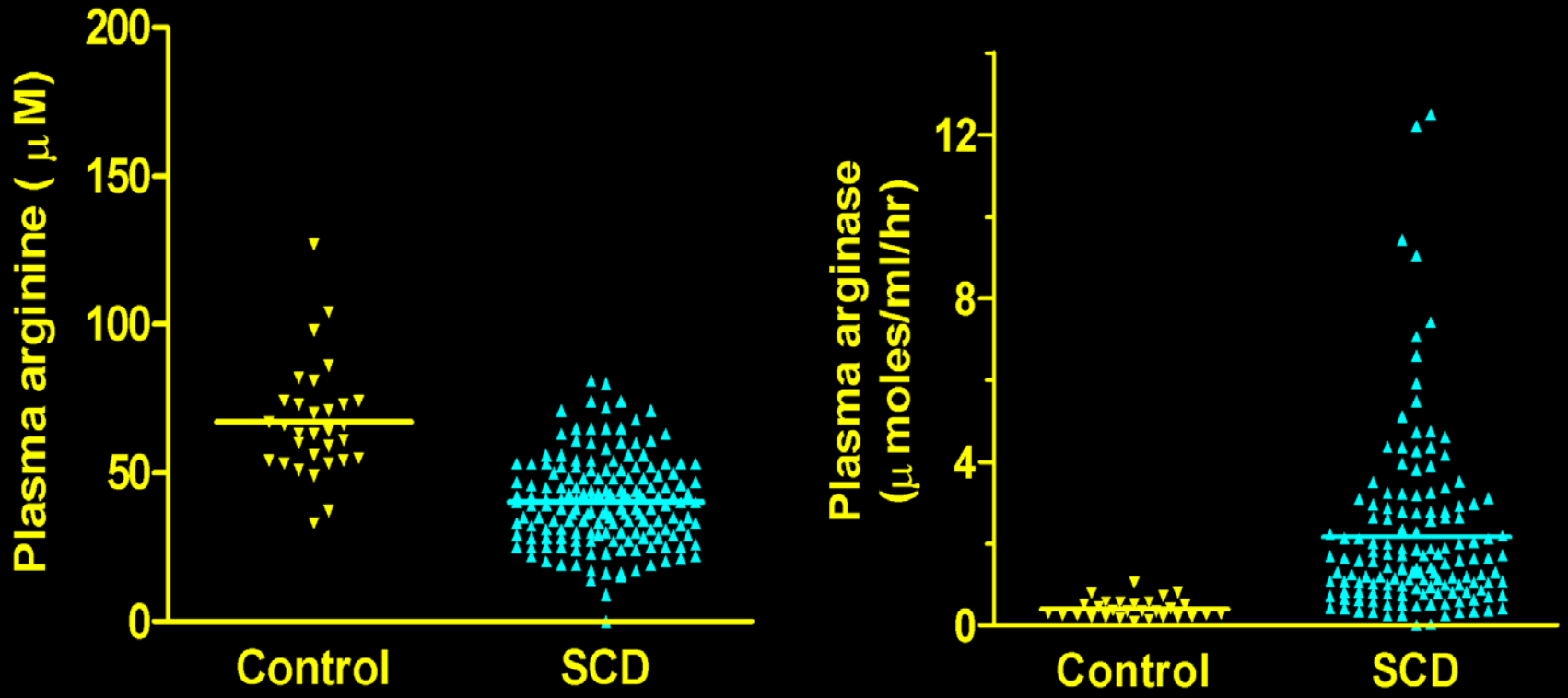


Mechanisms of Vasculopathy & Endothelial Dysfunction



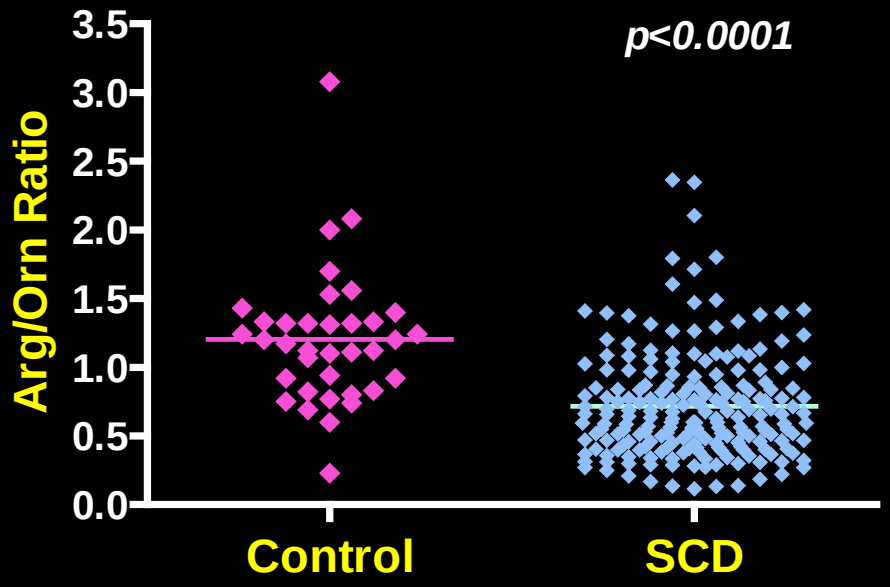

Consequences of ↓NO Bioavailability
↑Endothelial Activation
↑Endothelin - 1
↑Vasoconstriction
↑Platelet Activation
Clinical Manifestations
Stroke
Pulmonary Hypertension
? Asthma
Renal Insufficiency
Priapism
Leg Ulcers

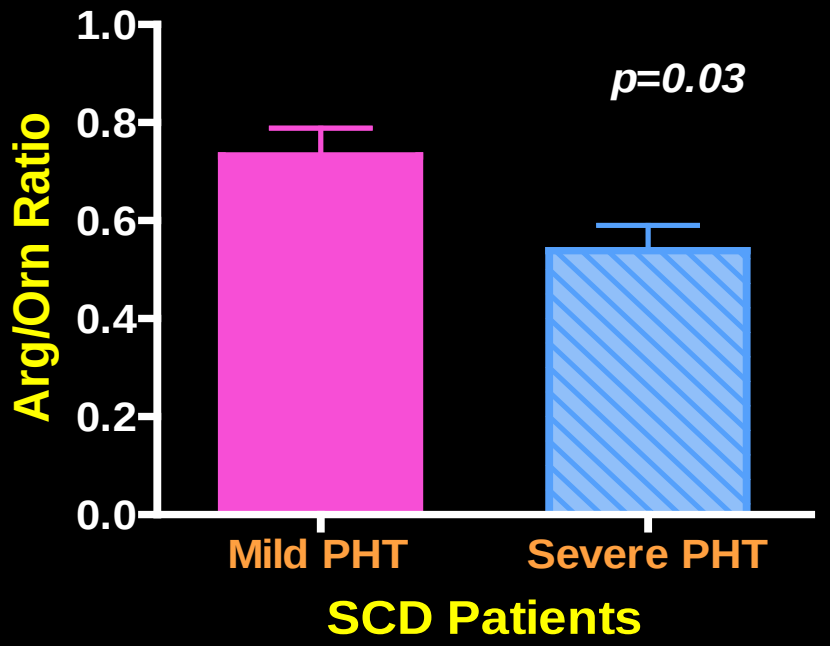
Arginine Insufficiency & Elevated Arginase in Sickle Cell Disease



(Morris et al, JAMA 2005)

Arginine-to-Ornithine Ratio





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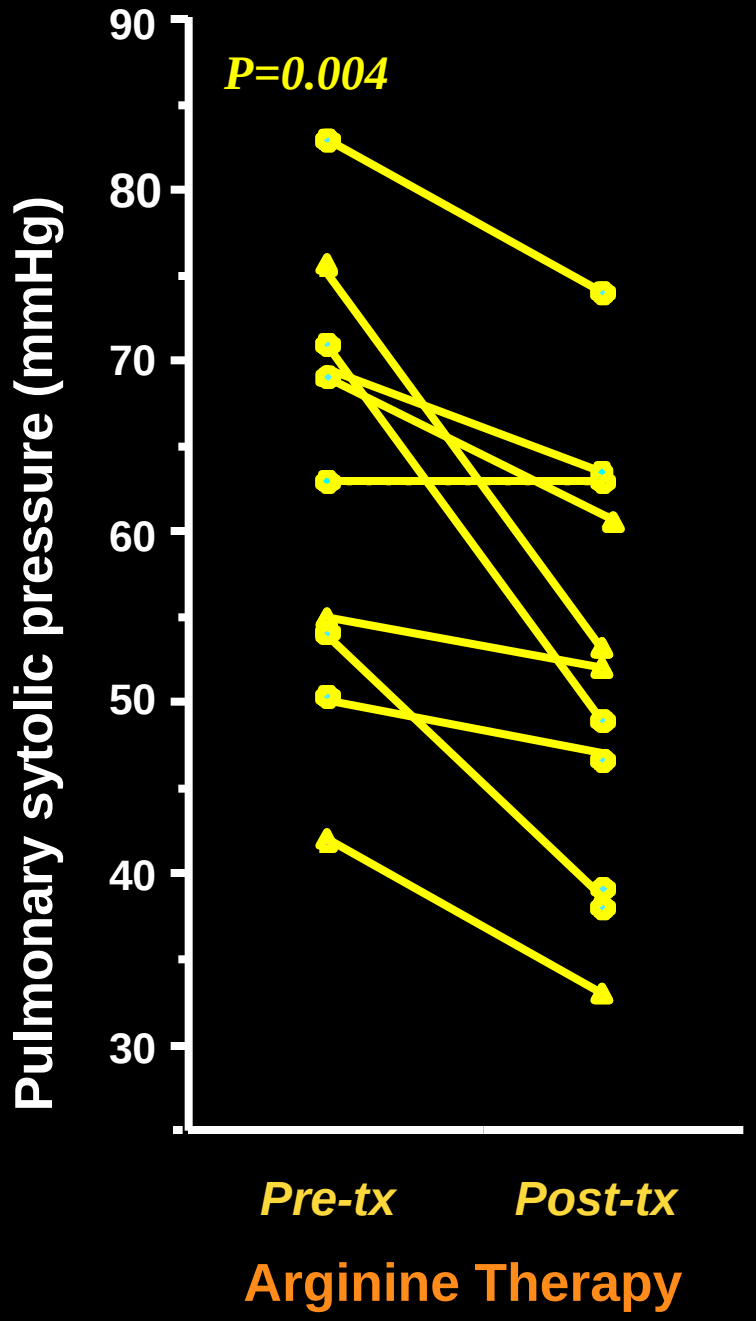
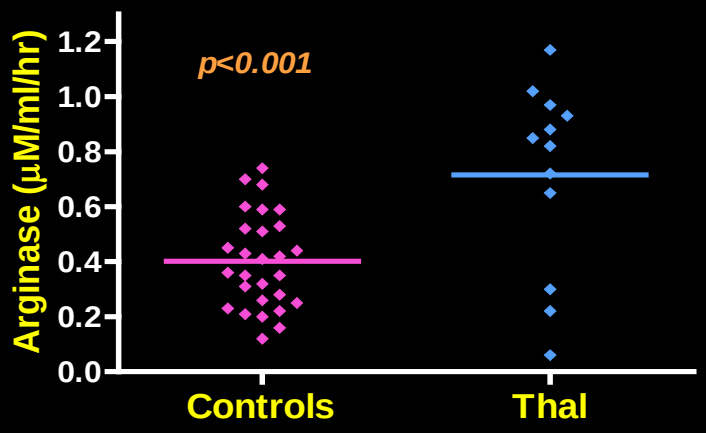


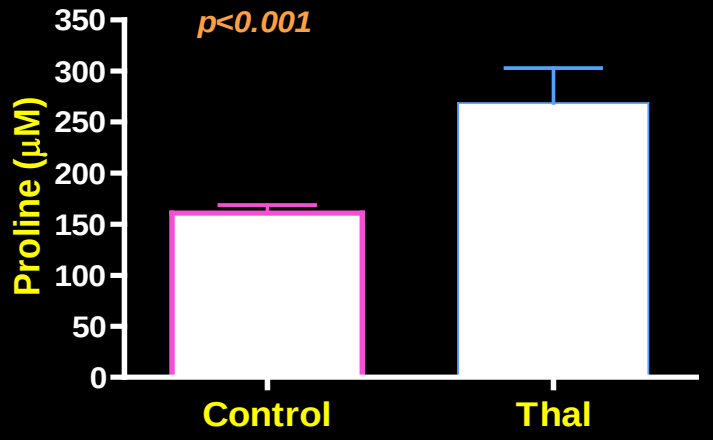
Changes in PASP before and after Arg

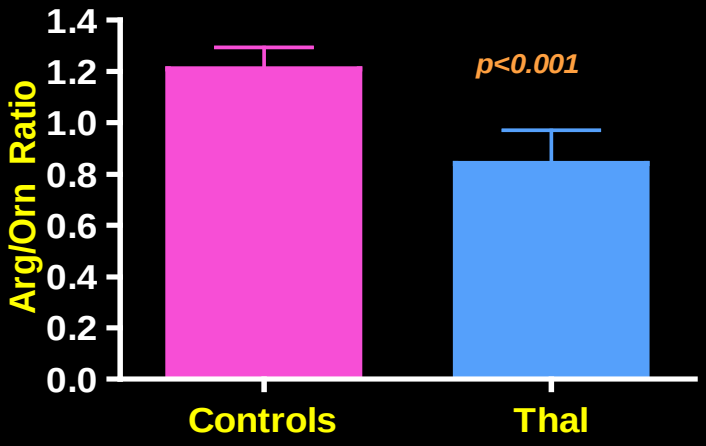
Results: ↓15.4 % in PASP

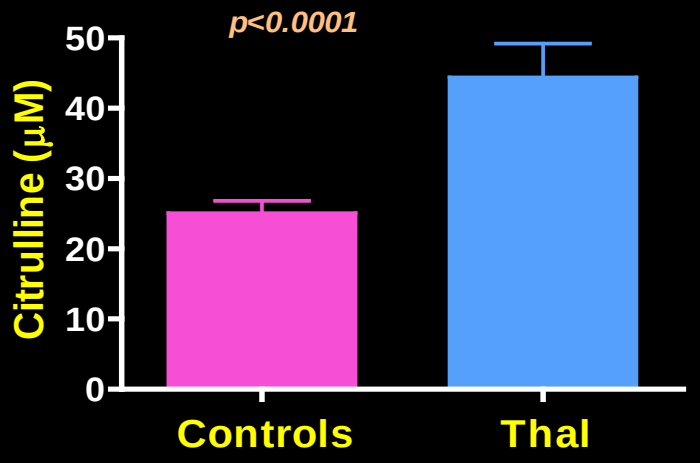
Morris et al. AJRCCM 2003

Dysregulated Arg Metabolism in Thalassemia









(Morris et al. Ann NY Acad Sci 2005)

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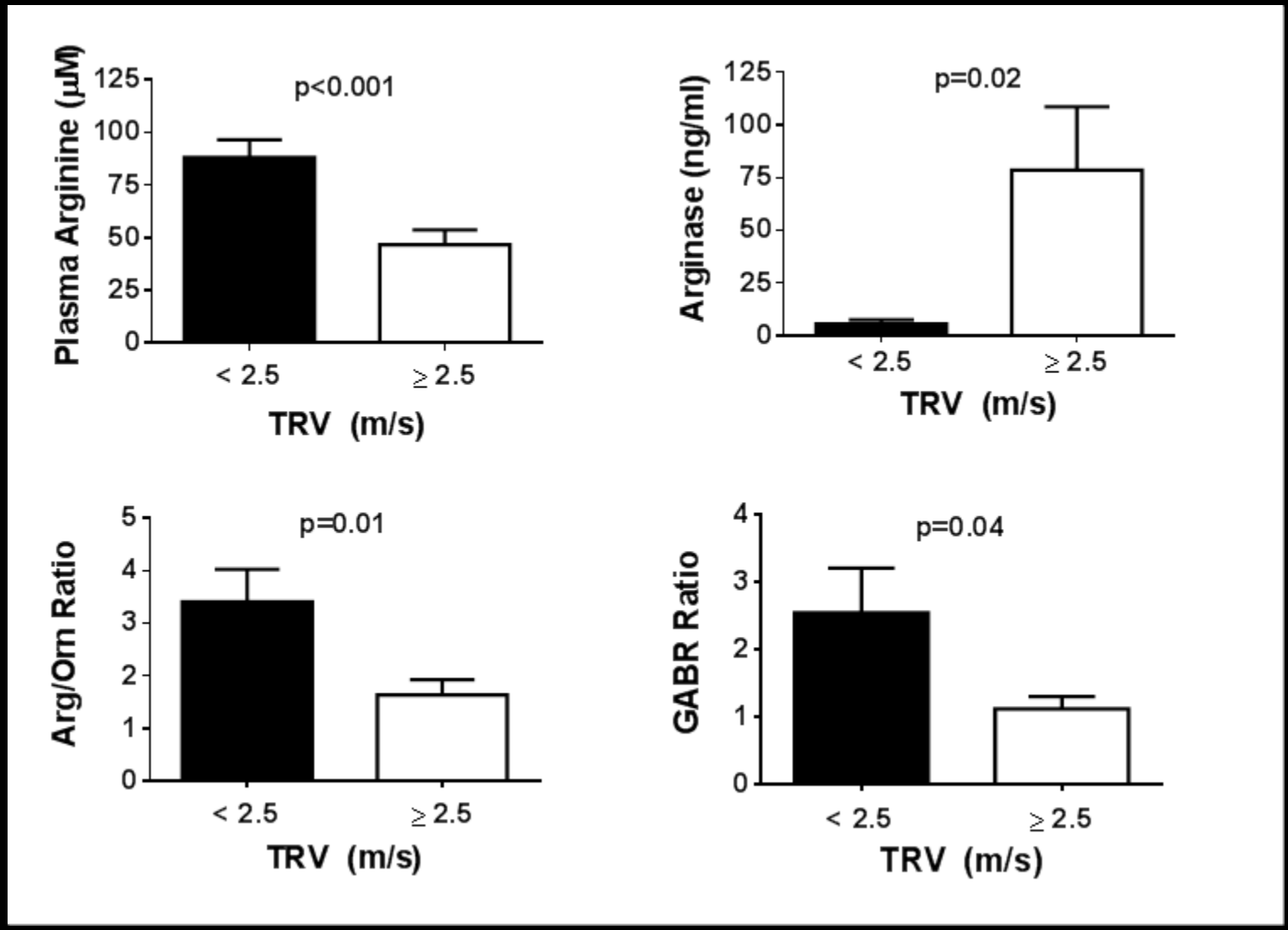
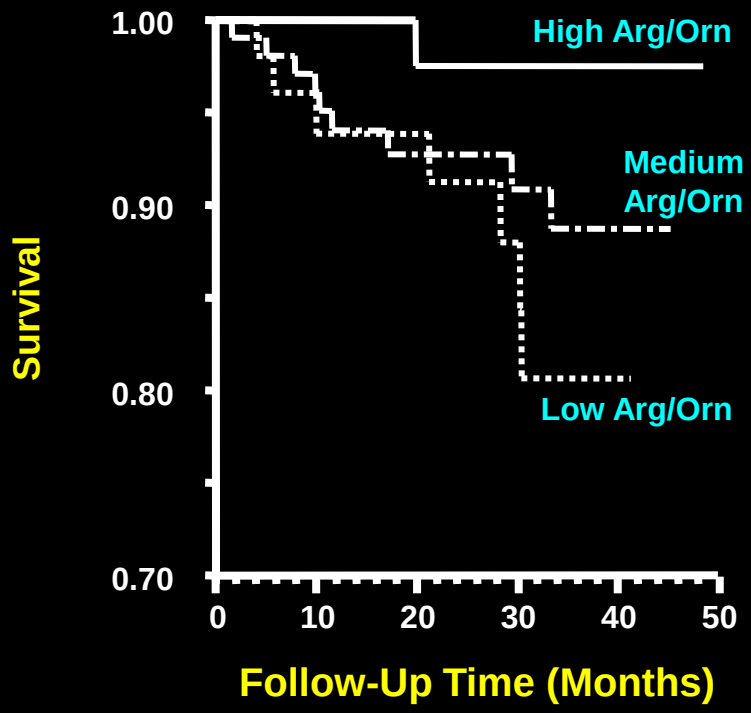


(Morris et al, Brit J Haematol, 2015)

Survival Proportions

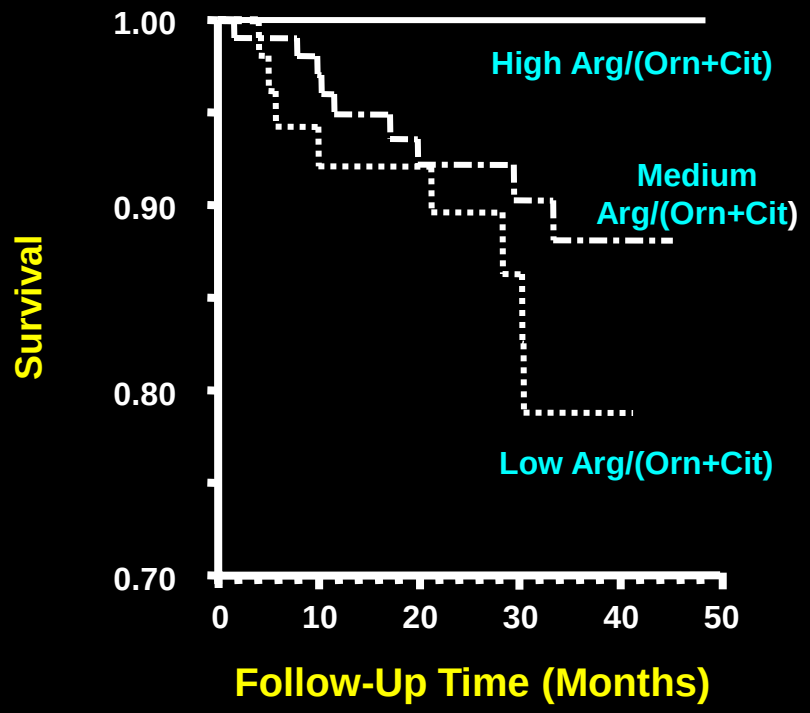
Low Global Arg Bioavailability ↑ Risk of Death

Arg/Orn



RR: 2.2 [1.0,4.9], *p=0.02*

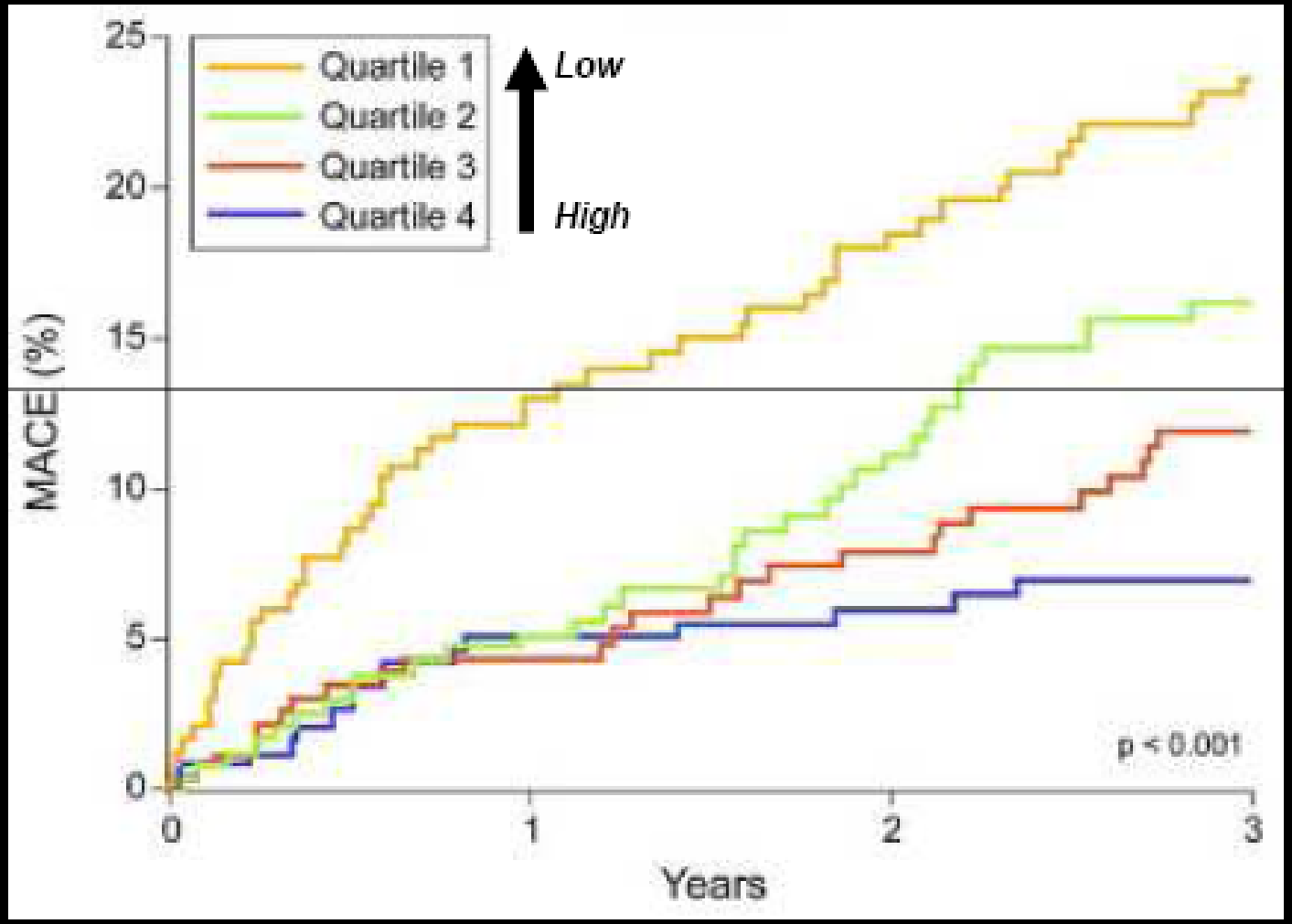
Arg/[Orn+Cit]



RR: 3.6 [1.5,8.3], *p<0.001*

(Morris et al, JAMA 2005)

Reduced GABR is Associated with Increased Incidence of MACE



MACE = Major Adverse Cardiovascular Events for ***n=991*** patients
(includes death, myocardial infarction, stroke)

JACC 53:2061-7 (2009)

Mechanisms of Arginine Dysregulation

- Hemolysis
- ↑ Arginase Activity
- Inflammation
- Myeloid-derived Suppressor Cells (MDSC)
- Intracellular Transport Abnormalities
- Renal Dysfunction
- Endogenous NOS inhibitors (ADMA)
- Uncoupled NOS

Arginine Deficiency Syndromes

Whether hepatic, immune or from hemolyzed RBC during hemolysis or transfusion, clinical consequences of excess extracellular arginase similar regardless of cellular origin

- Endothelial dysfunction – *sickle cell disease (SCD)*
- T-cell dysfunction – *trauma*

T-cell Dysfunction

- Linked to acute nutritional deficiencies in trauma patients
- Arginine essential for naïve T-cell activation
- T-cells sensitive to **arginine depletion**
  - *T-cell proliferation blunted*
  - *interferon-γ & IL-2 production inhibited*
  - *T-lymphocyte mediated cytotoxicity & memory responses nearly completely abolished*
- Provision of arginine to culture media restores T-lymphocyte function

Arginine Deficiency in Trauma

- > 15 million injuries/yr in USA
- ~10% trauma pts develop wound infections
- Infection risk ↑ 30% for pts in ICU > 48 hrs
- Infections: Leading cause late organ failure; 10% of trauma deaths

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- ↓↓ plasma Arg in trauma (in minutes to hours), low for ≥ 1 wk
- ↑ plasma arginase activity
- MDSC express arginase 1 after trauma
  - *depletes Arginine → T-cell dysfunction*
  - *↑↑susceptibility to infection after injury*

Strategies aimed at infection prevention after trauma should result in significant ↓ in morbidity, mortality and cost

What is the Therapeutic Potential of L-Arginine?

SCD

- Improves perfusion,↑ glutathione, ↓ inflammation, lung injury, microvascular vaso-occlusion and mortality in SCD mice
- Positive human phase 2 trials: leg ulcers; vaso-occlusive pain
- ↓ Pulmonary hypertension in SCD; improved priapism

Trauma

- Enhances would healing after trauma & hemorrhagic shock
- Immunonutrition improves immune responses/T-cell function
- High Arg formulas: ↓ infection complications in critically ill
- Benefits greatest in surgical patients
- *Evidence of harm in sepsis; following acute MI*

Methodologic weaknesses in most studies; Paucity of data in children

Immunonutrition in Critically ill Children

- 2009 Cochrane review found insufficient evidence for or against nutritional support in children during 1st week of critical illness, mainly because appropriate studies not performed
- RCT Arg/Gln fortified formula in 40 vented children with TBI
  - No difference in mortality vs. standard formula (*underpowered*)
  - Positive nitrogen balance by day 5 in 69% vs. 31% ($p<0.05$)
  - Critically ill pediatric pts (n=1245): decreased 60-day mortality with adequate protein intake: <20%, intake ≥ 60% of prescribed goal associated with OR of 0.14 (95% CI:0.04-0.52, p=0.003) for 60-day mortality (*enteral protein delivery, not overall calories*)
- Achieving delivery of >60% protein goals associated with ↓ mortality in mechanically ventilated children

Enteral protein delivery is a modifiable risk factor of mortality in dire need of a shift in current practice given the potential for improved outcomes

Therapeutic Strategies

- Arginine supplementation
- Arginine precursors: citrulline, glutamine
- Combination therapy
- Immunonutrition: targeted enteral formulas

Ideal formulas for trauma, critical illness, pediatrics do not yet exist. More research is needed.

Key Points

- Arg: conditionally essential AA→ essential under conditions of stress & catabolic states - capacity of endogenous AA synthesis is exceeded
  - *Includes critical illness, trauma, hemolysis*
- SCD & trauma represent arginine deficiency syndromes
  - *Distinct nutritional requirements; may benefit from arginine replacement therapy*
- At least 2 broad categories of arginine deficiency syndromes:
  - *T-cell dysfunction*
  - *Endothelial dysfunction*
- GABR: ?? novel biomarker of Arg deficiency; warrants further study
- Arg-fortified immunonutrition: Treatment for acquired Arg deficiencies
  - *Further study needed to identify sub-populations who will benefit while minimizing potential adverse events*

Nutrition is Medicine!

Questions?

Emory University School of Medicine