What Rare Diseases Can Teach Us About Common Ones: Lessons from the Urea Cycle and Other Biochemical Systems

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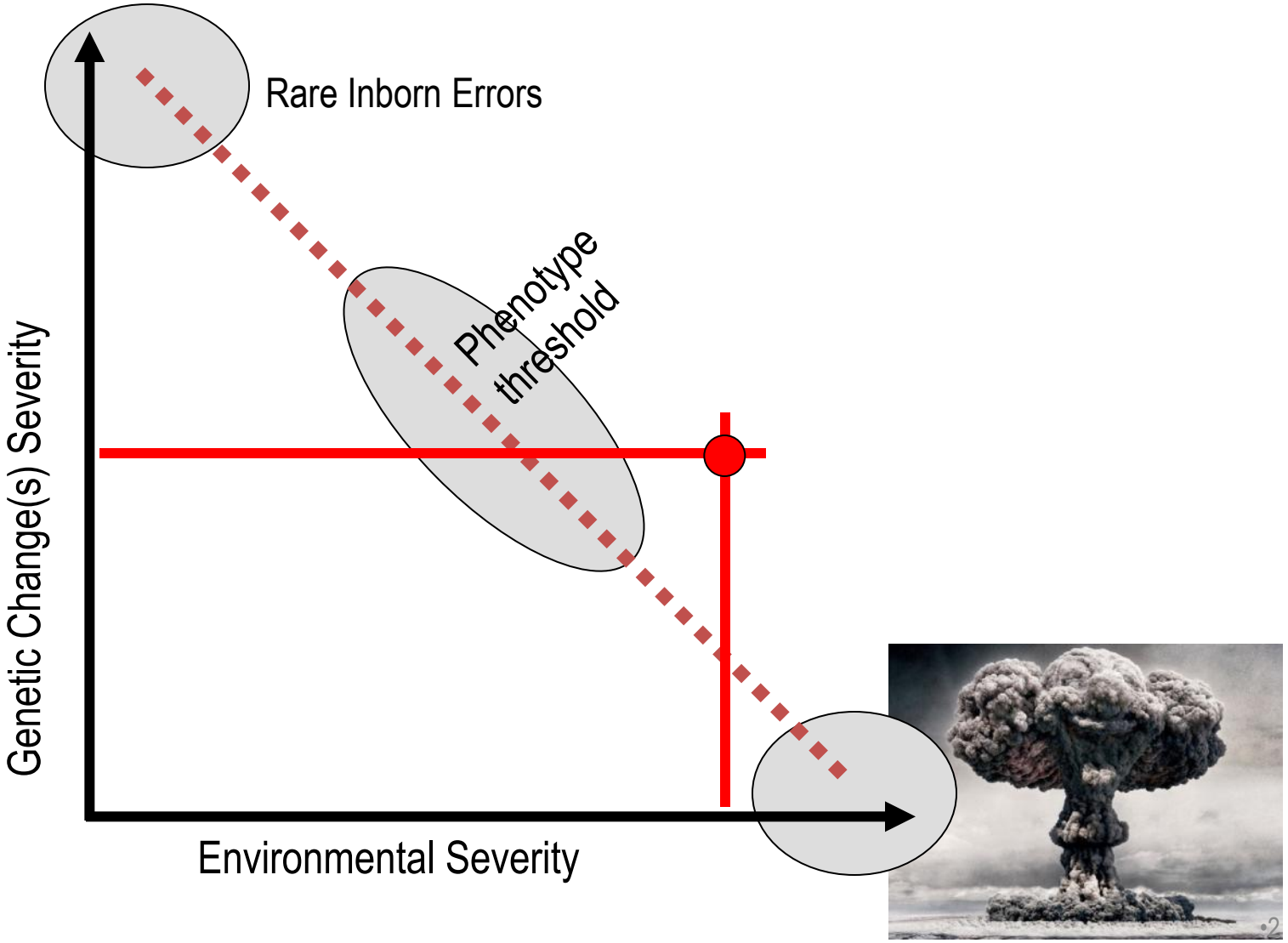
A Tale in Two Parts

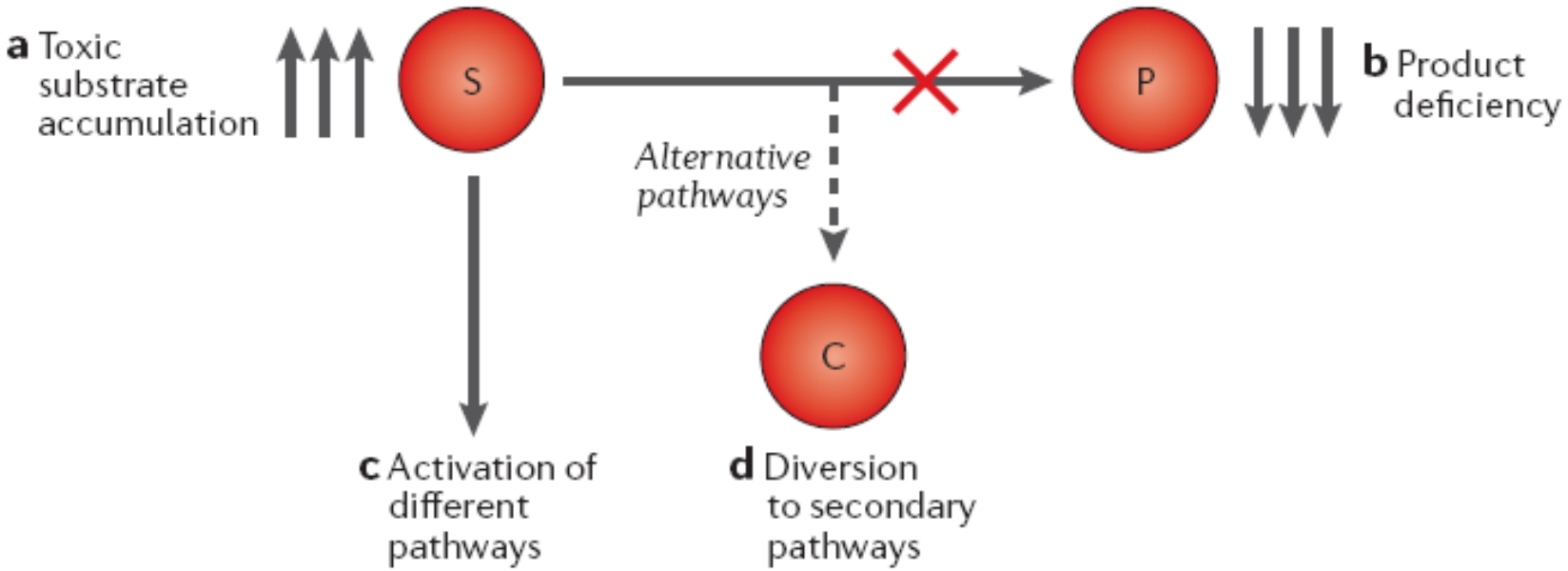
What? & So What?

Children's National Medical Center
WASHINGTON DC

Concept: Envirionmentally Determined Genetic Expression



THE CONCEPT SLIDE!

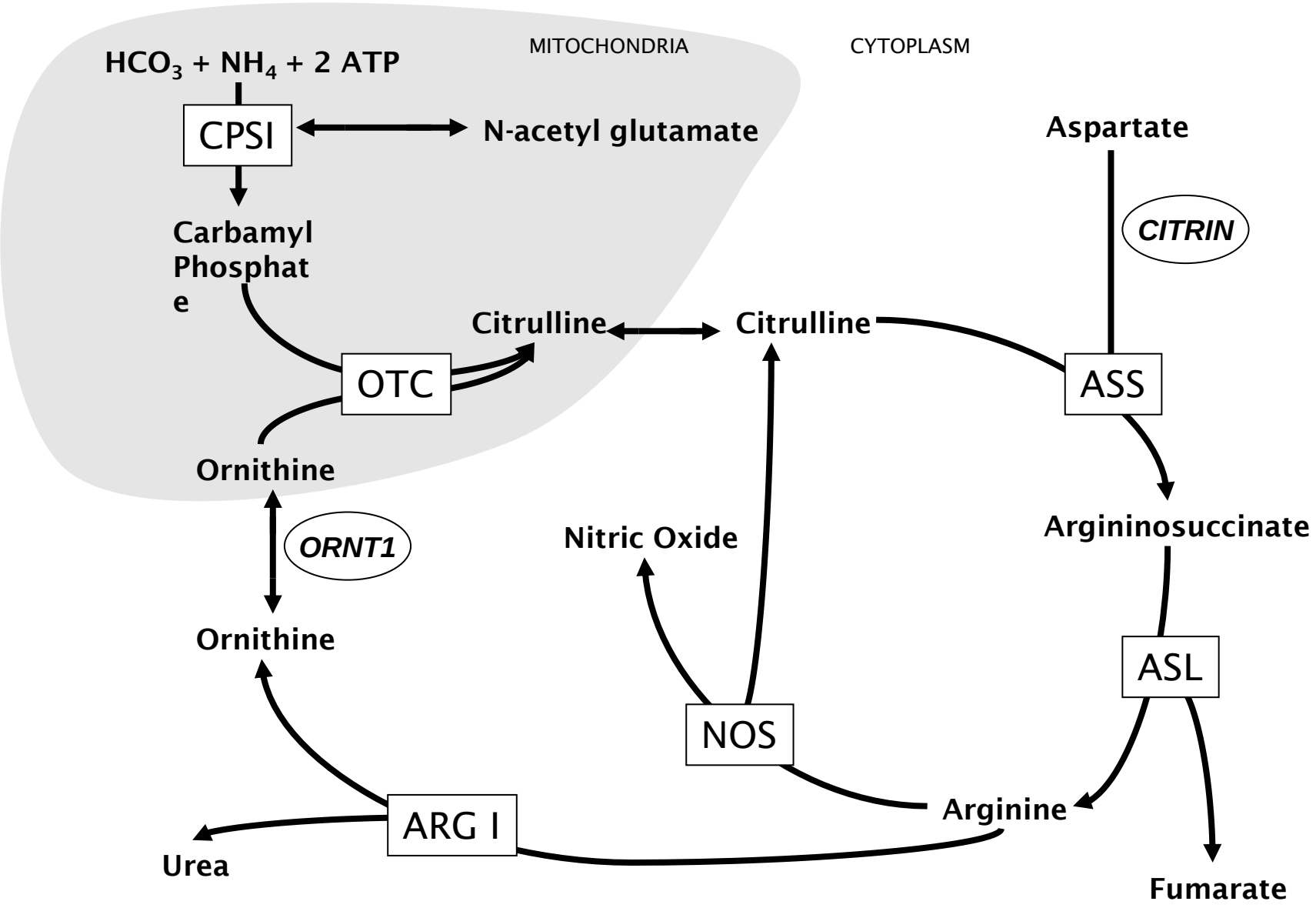


Presenting Symptoms in 260 Urea Cycle Disorder patients at first hyperammonemia

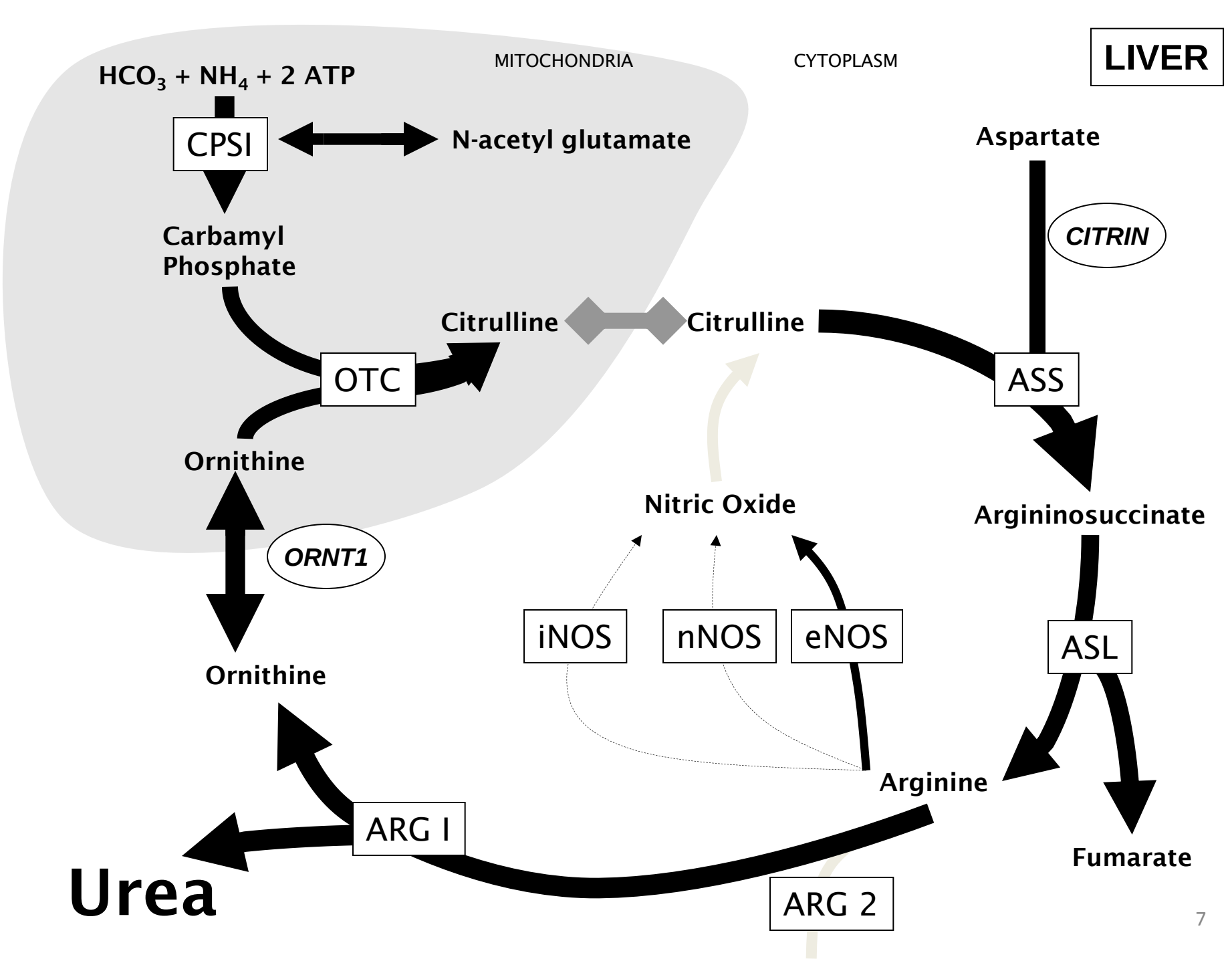
- **Neurologic symptoms (100%)**
  - Decreased level of consciousness (63%)
  - Abnormal motor function or tone (30%)
  - Seizures (10%)
- **Vomiting (19%)**
- **Infection (30%)**
- **Subjective: Decreased appetite, fussy**
- **Physiologic: Respiratory alkalosis (secondary to cerebral edema) followed by apnea**

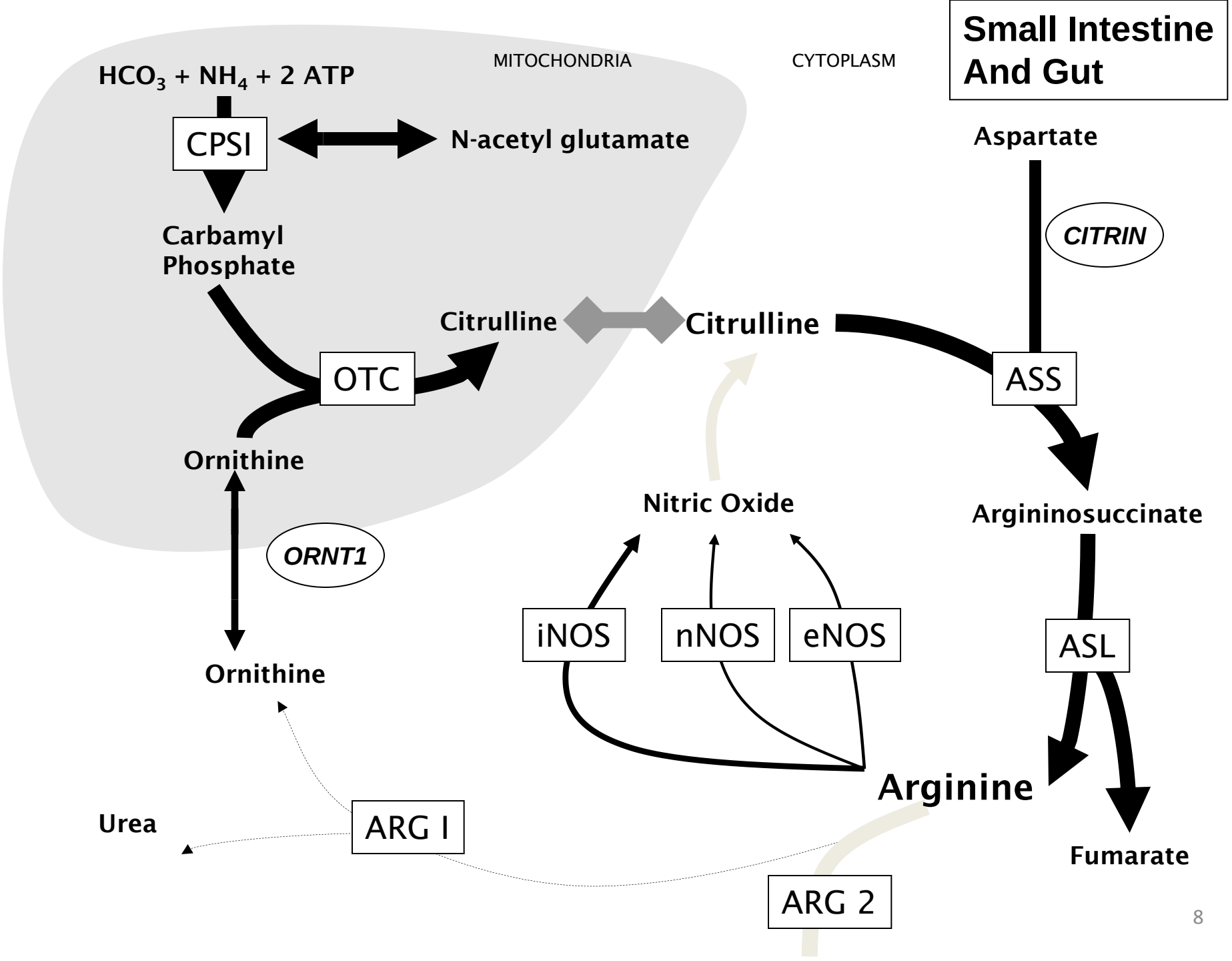
Common Themes to U.S. Treatment of UCDs

- Aggressive Treatment of Newborns, Protocol Driven
- Emphasize early recognition
- 3 arms of therapy
    - Use of Dialysis in encephalopathic treatment and rapidly rising ammonia levels
    - Aggressive reintroduction of essential amino acids (12-24 hours), high calories (100kcal/kg +), protein (up to 1.5+ gm/kg)
    - Full use of medications (phenylacetate, benzoate, arginine, citrulline, carbamyl glutamate.
- Common use of feeding tubes at discharge
- Aggressive treatment of newborns in anticipation of transplant at early opportunity.
- Use of full oral medication dose (phenylbutyrate) to allow liberalization of protein in diet.



•THIS FLAT DIAGRAM DOESN'T REFLECT REALITY


LIVER
MITOCHONDRIA
CYTOPLASM
$HCO_3 + NH_4 + 2$ ATP
CPSI
N-acetyl glutamate
Carbamyl Phosphate
OTC
Citrulline
Citrulline
Aspartate
CITRIN
ASS
Argininosuccinate
ASL
Fumarate
Ornithine
ORNT1
Ornithine
Nitric Oxide
iNOS
nNOS
eNOS
Arginine
ARG I
ARG 2
Urea


Small Intestine And Gut
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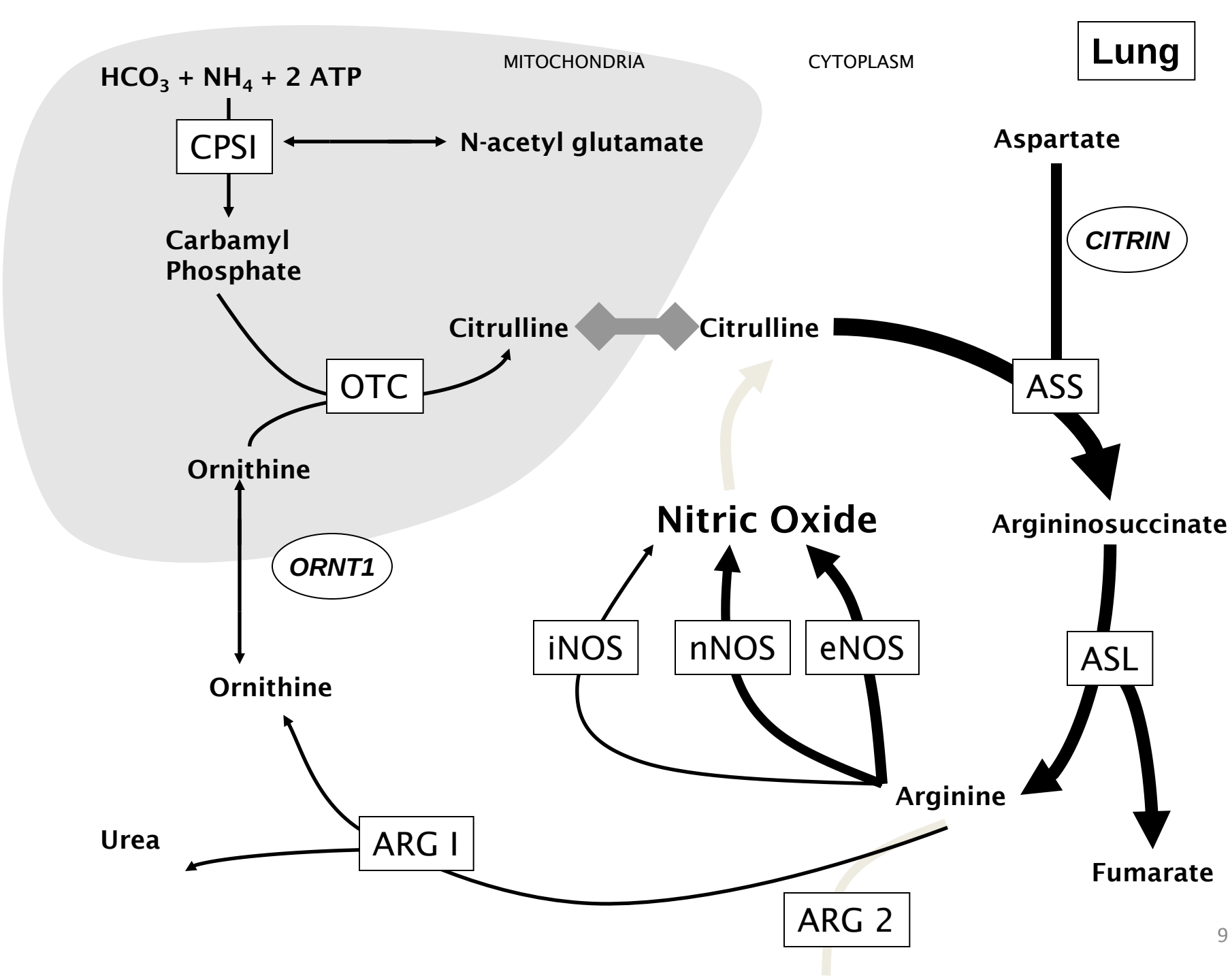
ARG 2


Lung
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ORNT1
iNOS
nNOS
eNOS
ASL
Ornithine
Arginine
Urea
ARG I
Fumarate
ARG 2

So…… If we break an enzyme like CPSI what happens?

-Ammonia builds up since it can't enter the pathway

-You can't make citrulline

-You can't make arginine

-You can't make new protein

-You can't make **nitric oxide**

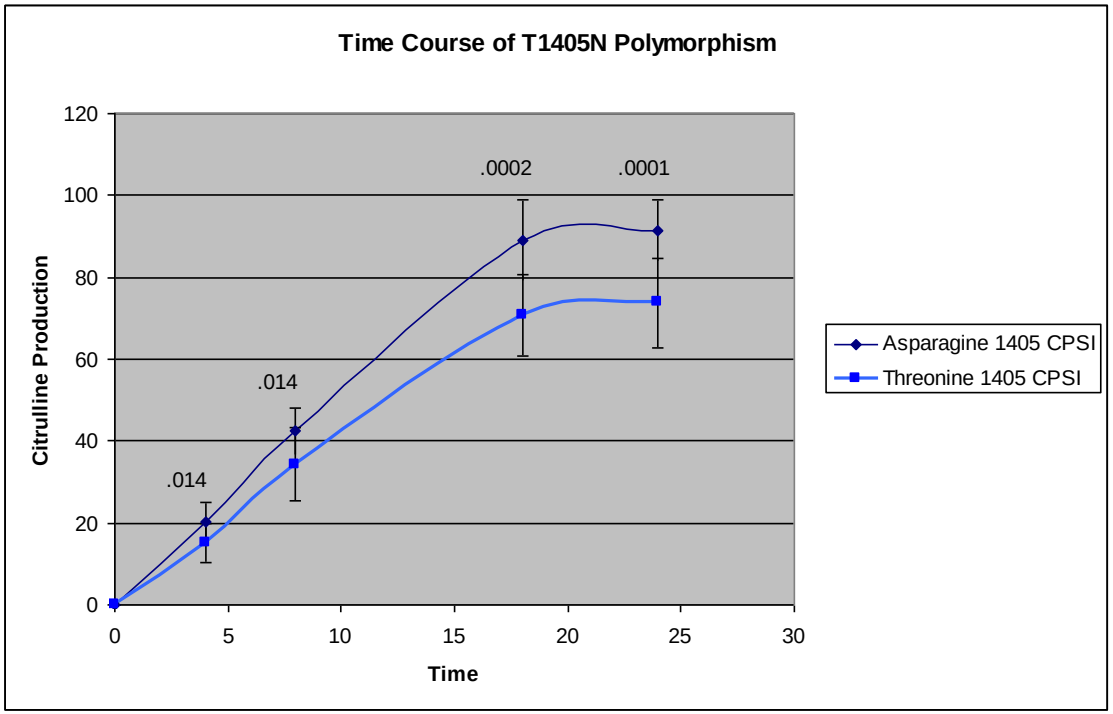
-You can't make polyamines

-You can't make urea

-You can't make ornithine

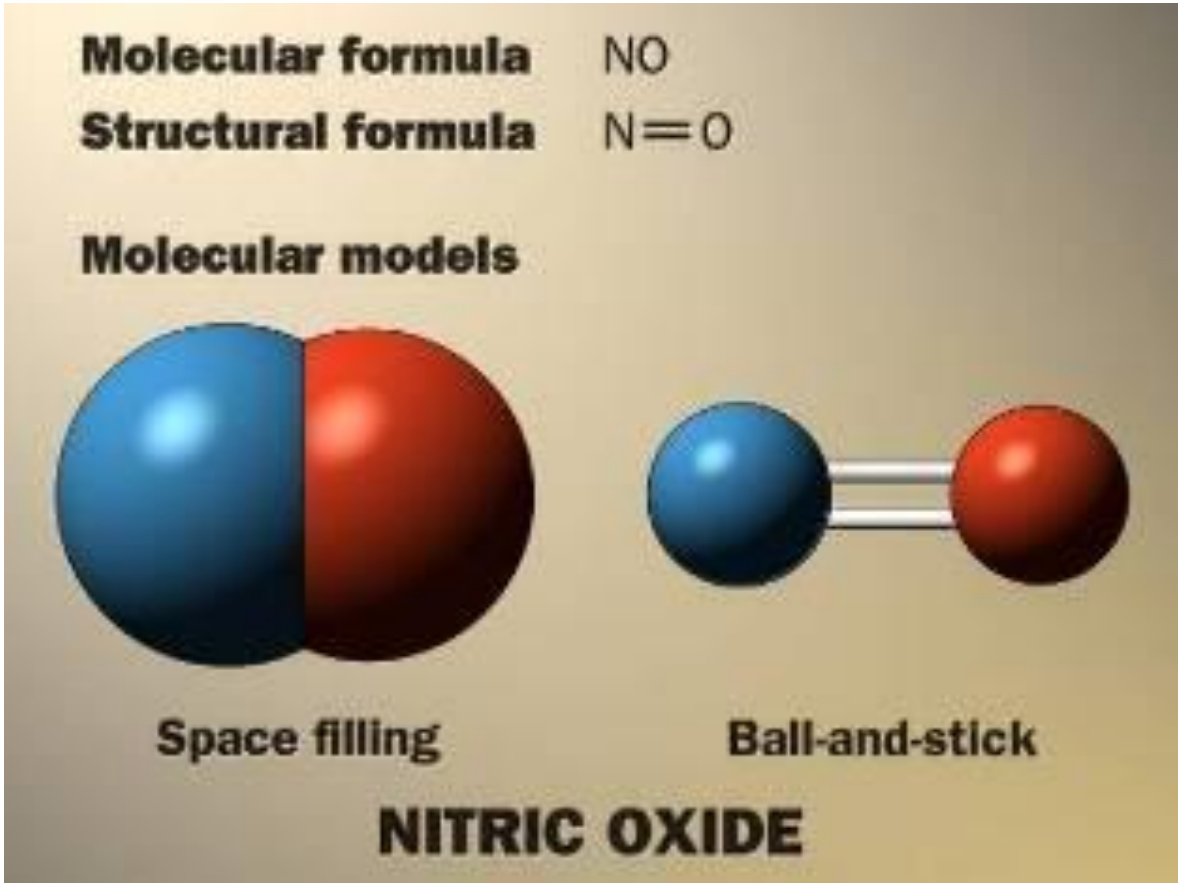
-TREATING these rare diseases means dealing with these consequences.

- Are there common genetic changes in the urea cycle enzymes that affect funtion?
  - Yes in almost every one
- Does the “choke point” enzyme CPSI have common genetic changes in it that affect its function.
  - Yes T1405N



•Things that Disrupt the Urea Cycle

- Rare genetic defects in a urea cycle enzyme
- Damage to the liver and gut
  - Viral
  - Chemical (ETOH or other)
  - Hypoxia, shock
  - Cardiopulmonary bypass
  - Metabolic (galactosemia, tyrosinemia, Wilson's dz, etc.)
- Vascular Bypass of the liver by cirrhosis or vascular damage
- Drug and Molecule effects
  - Valproic acid
  - Chemotherapy (cyclophosphamide primarily)
  - Organic acids (propionic, methylmalonic, isovaleric
- Mild Genetic Changes in the Cycle Combined with the above.

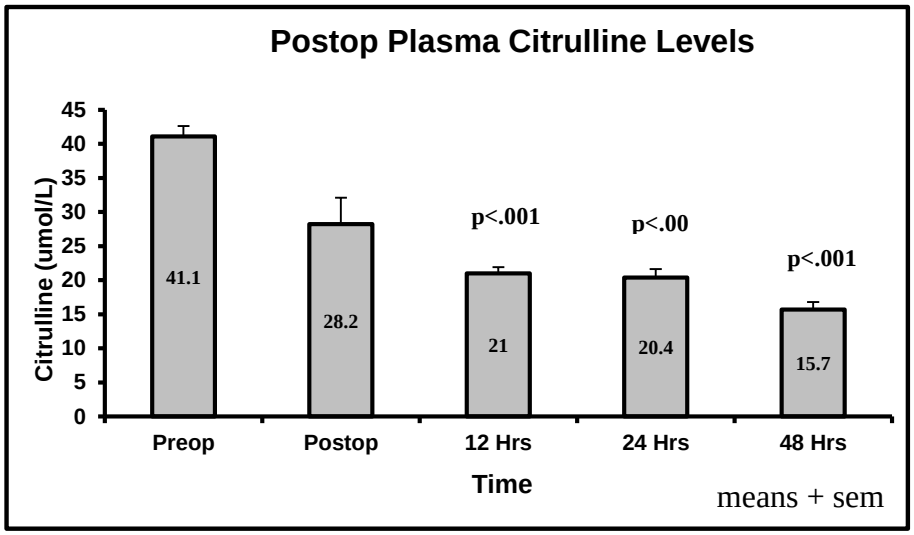


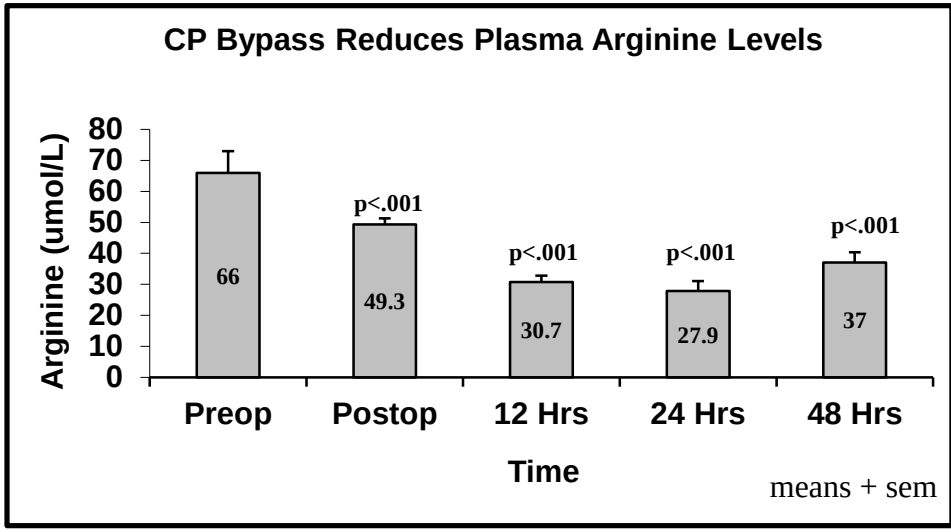
- As of 9:30 AM 124,285 articles on nitric oxide
- 5310 articles on urea cycle in PUBMED
- For our purposes think of it as a highly regulated vasodilator

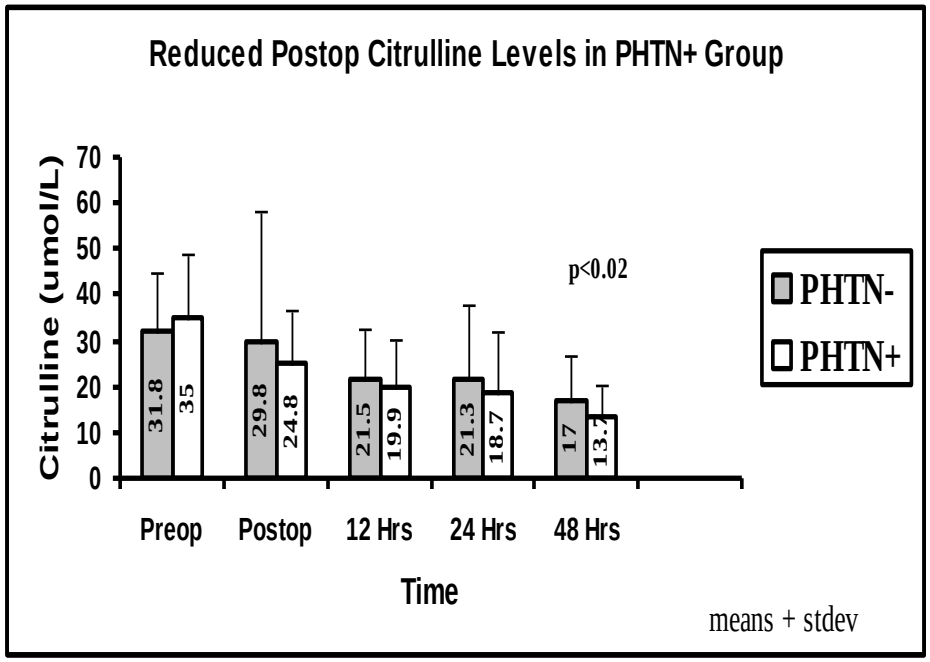
Cardiac Surgery in Children
In U.S. 25,000 per year

- Extremely stressful environment with hypoxia, vascular damage etc. Well documented damage to liver from bypass.
- Very controlled conditions with protocol treatment of patients
- 25-35% of patients develop the complication of post-cardiac surgery pulmonary hypertension

•What Happens to Urea Cycle Function after Surgery



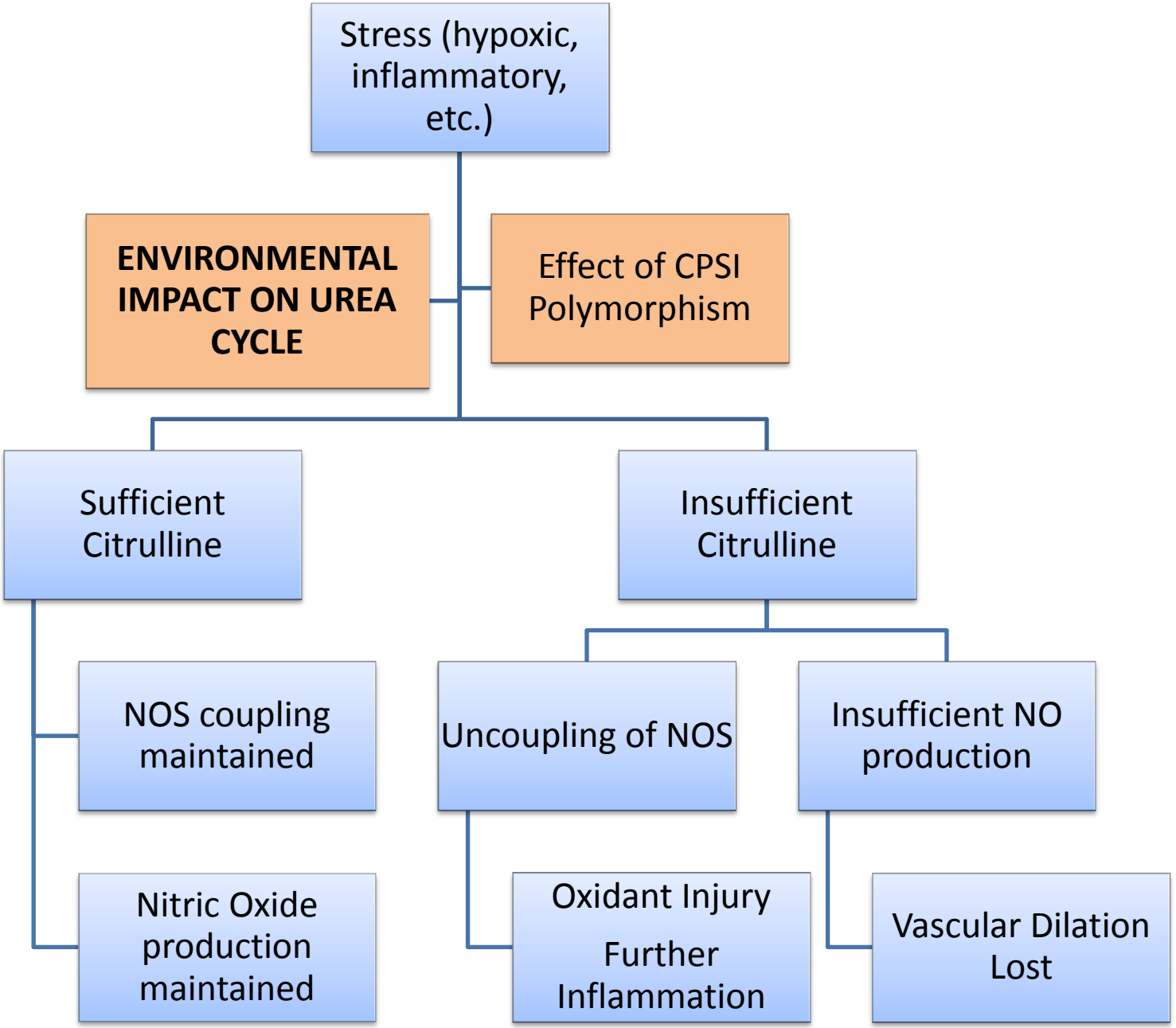




We utilized both clinical experience and MDR to select variables for modeling. In both methods, age and CPSI genotype created the best model.

Multivariate Modeling of Pulmonary Hypertension after Cardiac Surgery				
Variable	Odds Ratio	Std. Error	95% CI (lower)	95% CI (higher)
Age	0.92	0.03	0.87	0.98
Bypass Time	1.00	0.01	0.99	1.01
Down Synd.	5.25	4.15	1.11	24.7
AC vs. AA (CPS I T1405N)	4.08	2.85	1.04	16.04
CC vs. AA (CPS I T1405N)	5.97	4.12	1.54	23.15

This model was tested in a separate validation cohort and accurately (and significantly) predicted pulmonary HTN.


Stress (hypoxic, inflammatory, etc.)
ENVIRONMENTAL IMPACT ON UREA CYCLE
Effect of CPSI Polymorphism
Sufficient Citrulline
Insufficient Citrulline
NOS coupling maintained
Nitric Oxide production maintained
Uncoupling of NOS
Insufficient NO production
Oxidant Injury
Further Inflammation
Vascular Dilation Lost

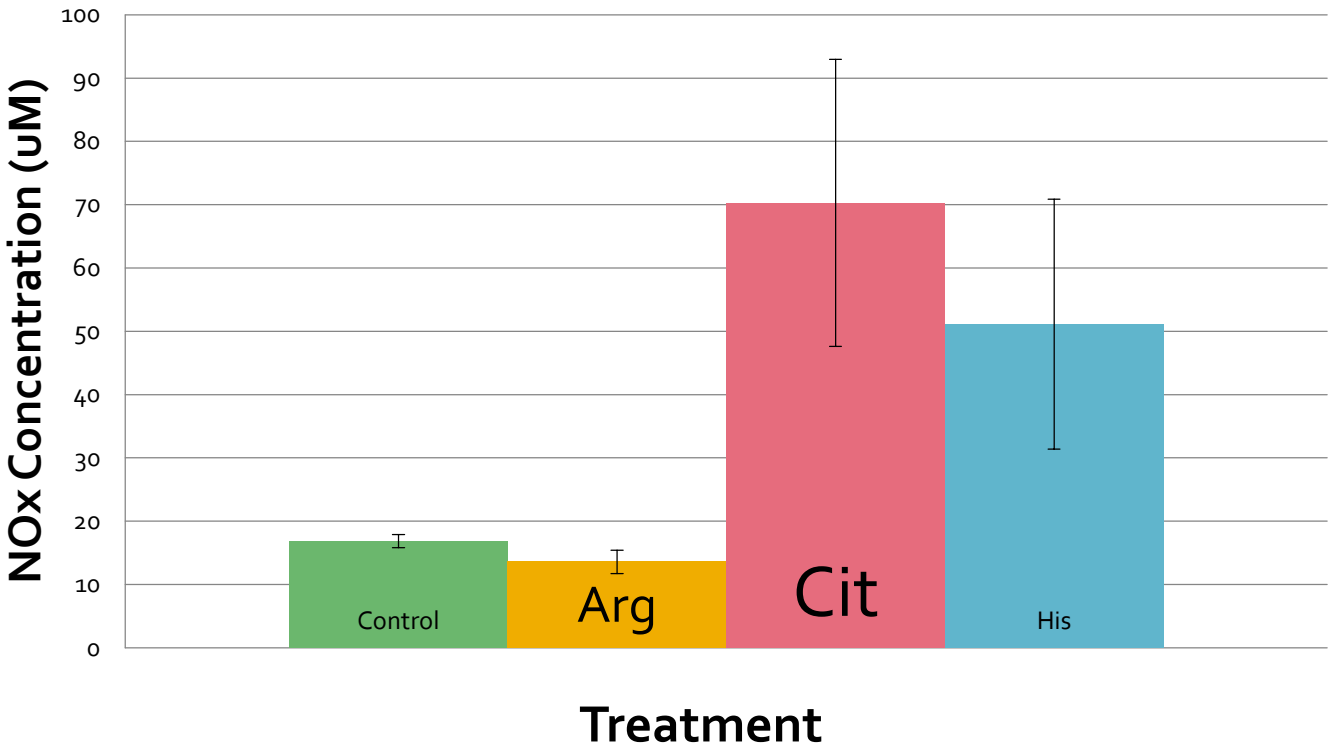

TRANSLATIONAL SCIENCE

Why Doesn't Arginine Fix These Problems

- **ARGININE**

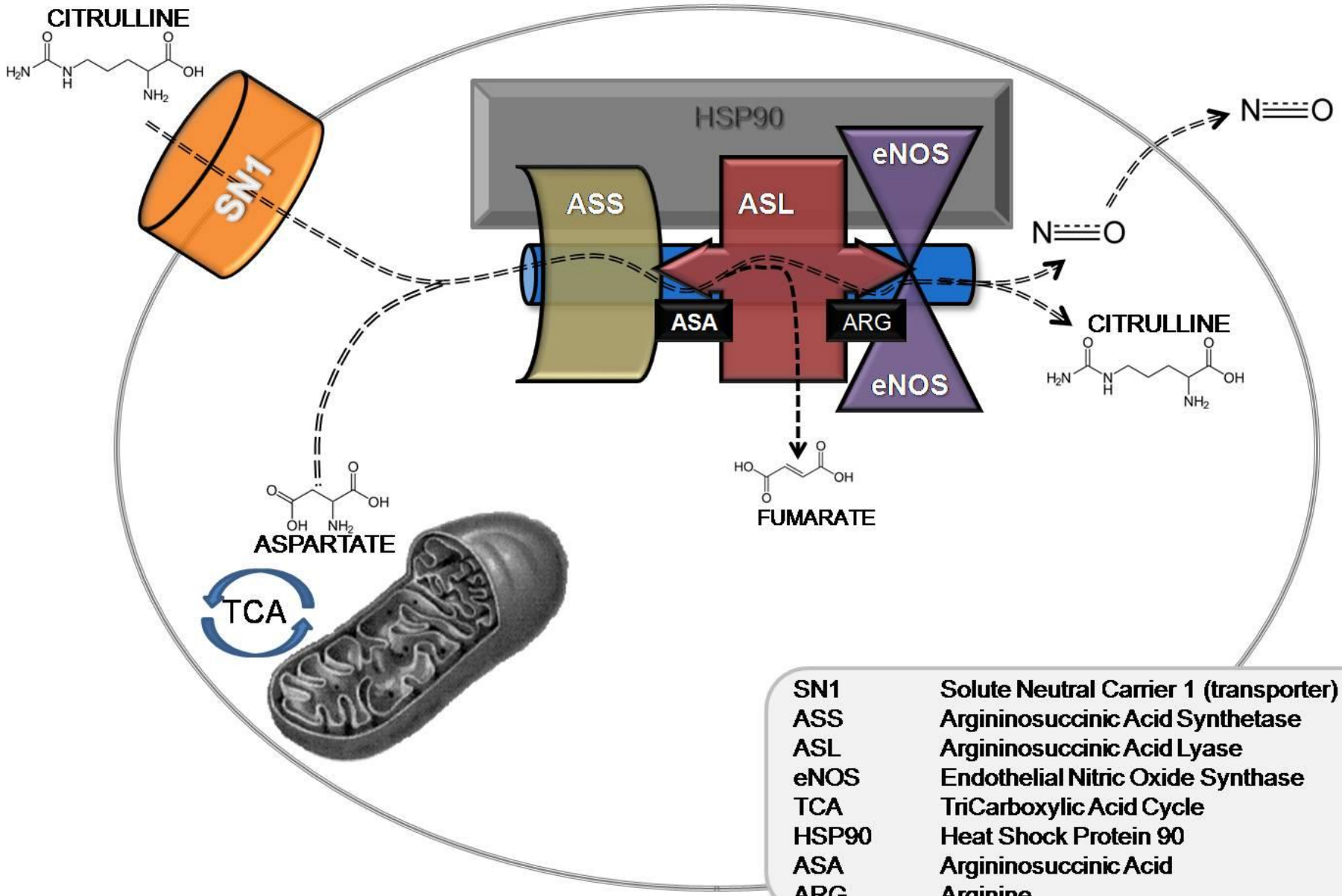
- **NO**

Human Vascular Endothelial Cells



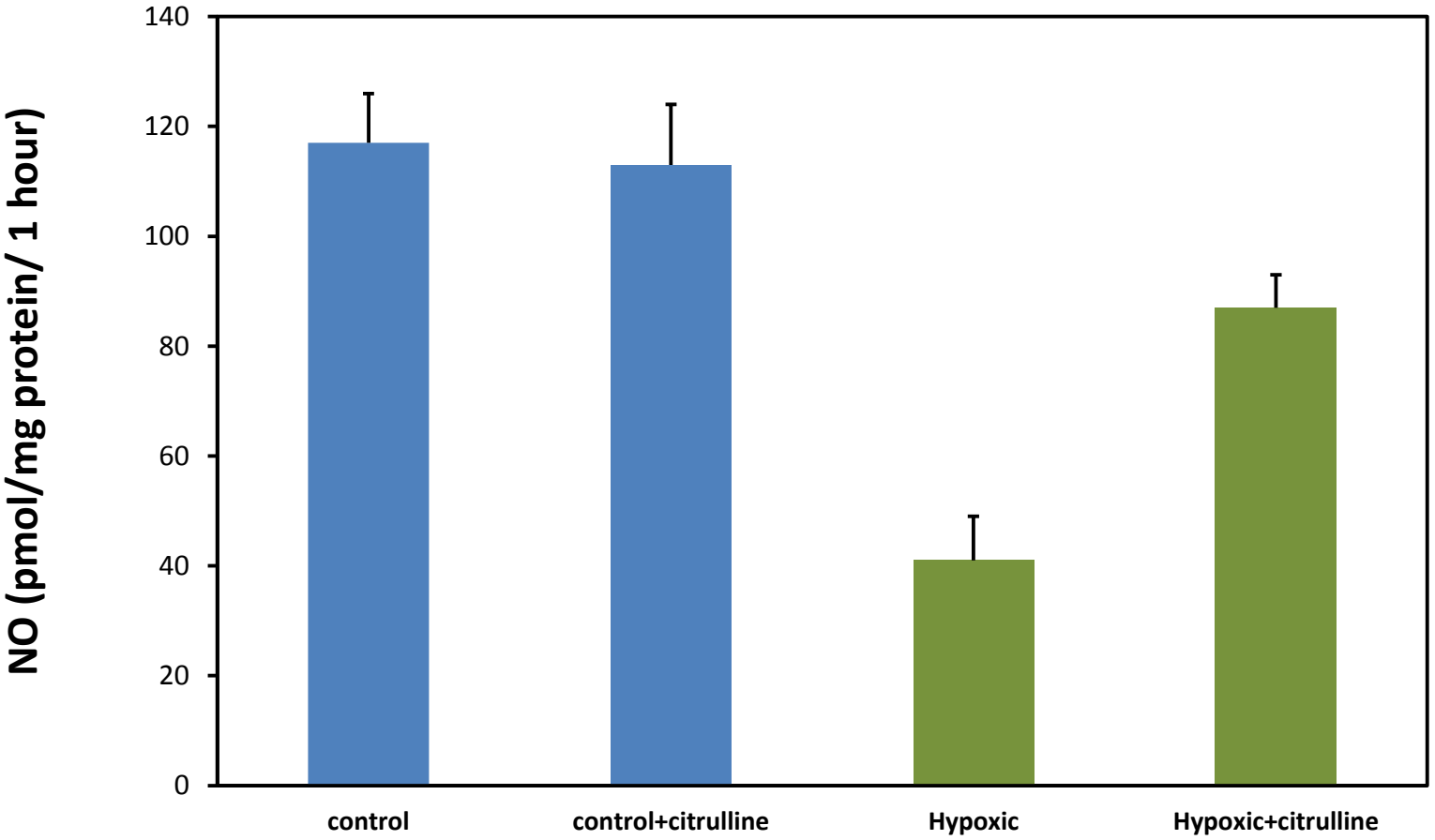
•**Intracellular Nitric Oxide Metabolites**

ENDOTHELIAL NO PRODUCTION

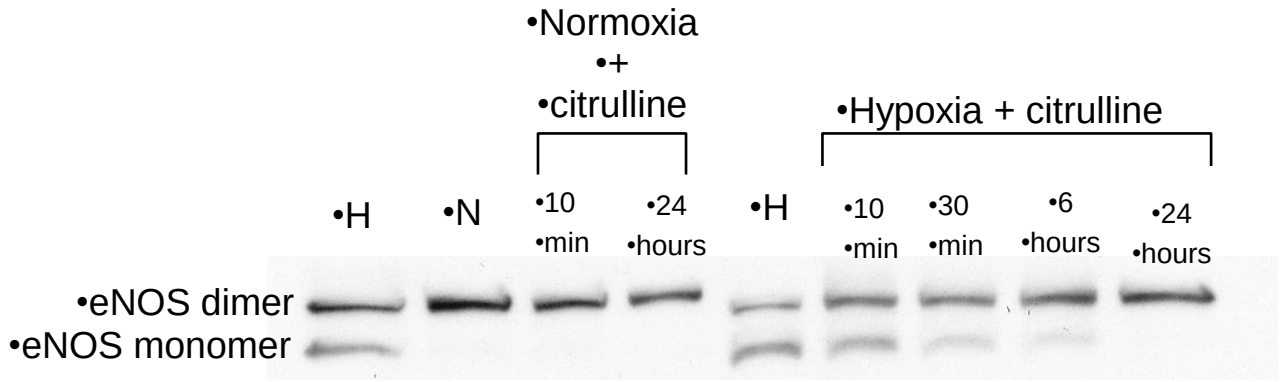


SN1	Solute Neutral Carrier 1 (transporter)
ASS	Argininosuccinic Acid Synthetase
ASL	Argininosuccinic Acid Lyase
eNOS	Endothelial Nitric Oxide Synthase
TCA	TriCarboxylic Acid Cycle
HSP90	Heat Shock Protein 90
ASA	Argininosuccinic Acid
ARG	Arginine

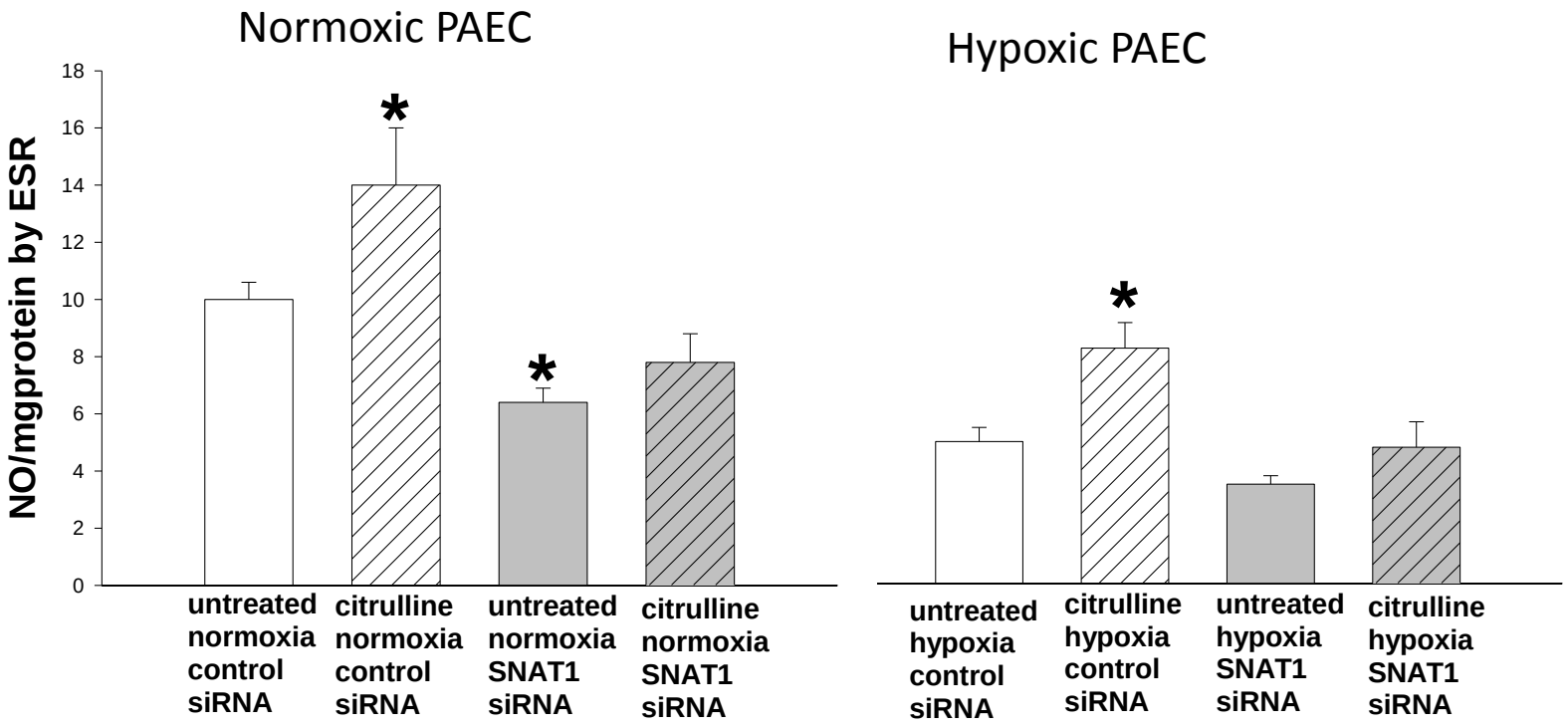
- **NO production in Stressed PAEC cells measured by ESR with Fe-DETC probe after 48 hours of hypoxia,**



•Citrulline recouples eNOS in hypoxic PAECs



Effect of citrulline treatment and SNAT1 knockdown on nitric oxide production in PAEC cultured under normoxic or hypoxic conditions for 48 hours

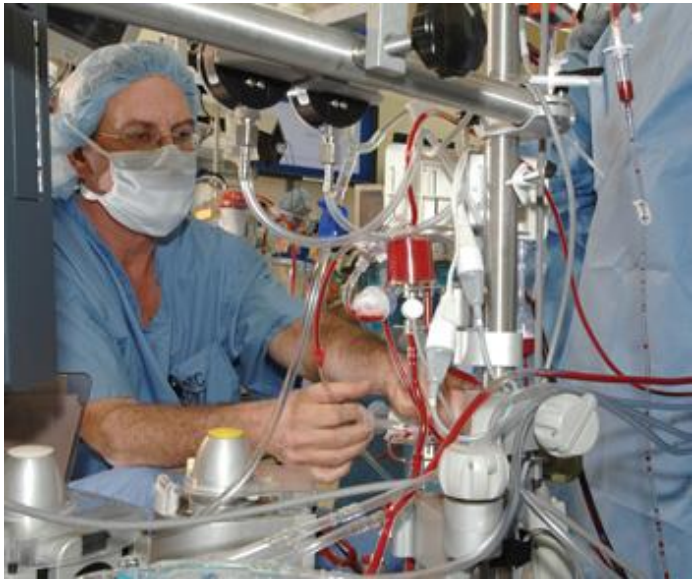


* Different from untreated normoxia control siRNA

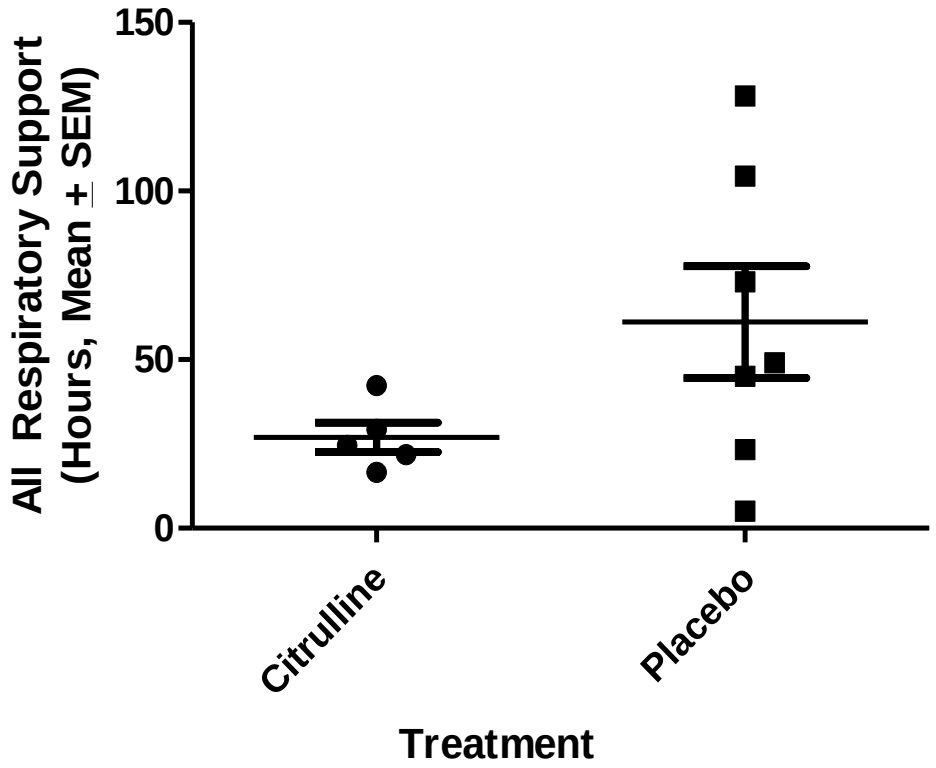
* Different from untreated hypoxia control siRNA

Advantages to Treating Post-Op Pulmonary HTN

- Current Treatment is Reactive (after disease happens)
- Prolonged hypoxia and post-cardiac stress
- Significant increase in measure of oxidant injury
- Significant increase in time on ventilator (3 fold, $P < 0.01$)
- Increase in length of hospital stay ($P = 0.06$)
- Increased mortality (1.4% to 6.2%, $P = 0.05$)
- Increased right ventricular strain
- ? Long term effects on Brain Development



Effect of Citrulline on Respiratory Outcome in Congenital Heart Repair Surgery



A Few of the Disease Models We Have Data On

System	Disease	Data Source	Treatment Data	U.S. Impact	Segment
Pulmonary	Post Cardiac Surgery PHTN	Biochemical Genetic	Animal Human	25,000/yr Orphan	Pediatric ? Adult
	Bronchopulmonary Dysplasia	Biochemical	Animal	60,000/yr Orphan	Pediatric
	Asthma, Emergency Room	Biochemical Genetic	Underway	> 1million/yr	Pediatric Adult
	ARDS	Biochemical Genetic	In Design	~100,000 Orphan	Adult Pediatric
CNS	Post-stroke, Subarachnoid Hemorrhage Vasospasm	Biochemical	Animal	>200,000	Adult
Liver	High Dose Chemotherapy HVOD	Biochemical Genetic	Animal Human	25-30,000/yr	Adult Pediatric
	Cirrhosis, Portal Hypertension	Biochemical	In Design	>200,000/yr	Adult

Cardiac Surgery: 23/1000 births, BPD 8.4% of 438K births, Asthma 3.9% of population

So What Have We Learned

- Lessons learned from rare genetic conditions are pertinent to common diseases
- Treatments can go both ways. Rare to common and back.
- Working on both helps both groups of patients
- NEXT
  - We are working with a number of new disease models.

This work represents the efforts of a great number of researchers, students, technicians, and others at Children's National and at Vanderbilt University

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