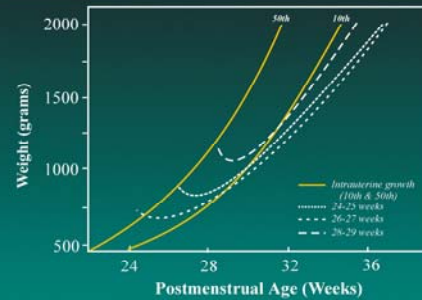


## Glutamine and Protein Metabolism in the Newborn

Satish C. Kalhan, M.D.  
Cleveland Clinic

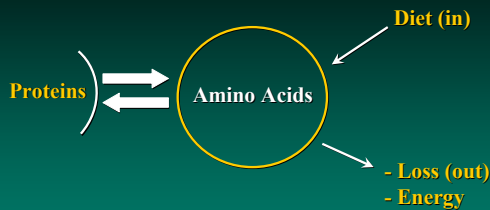


## Rate of Growth vs Postmenstrual Age of LBW Infants



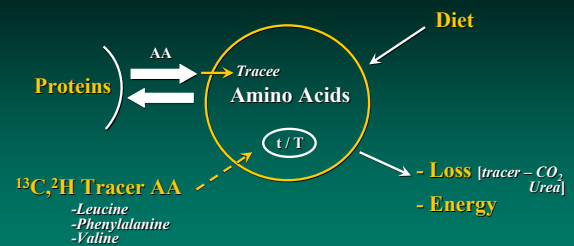
Pediatrics, 2000

## Protein Metabolism in Vivo



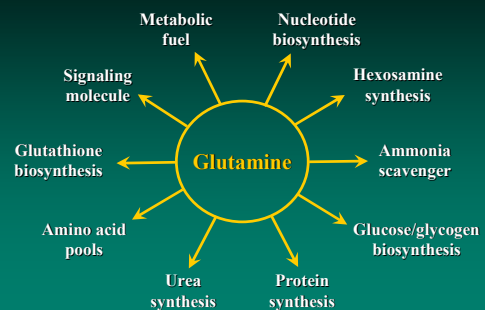
Equilibrium : Input = Output

## Quantification of Protein Metabolism in Vivo



- Glutamine
- Most abundant dispensable amino acid in blood and tissues.
- Virtually synthesized by every tissue
- Rapid decrease in tissue and plasma levels in acute stress

## Biological Actions of Glutamine



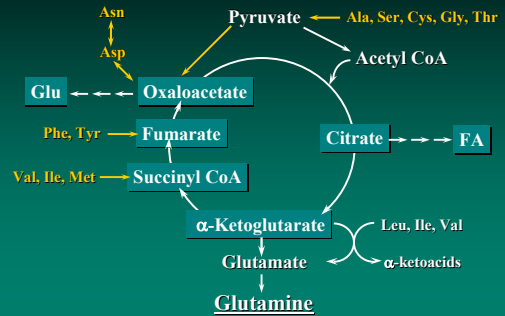
BRIEF REPORT

### Congenital Glutamine Deficiency with Glutamine Synthetase Mutations

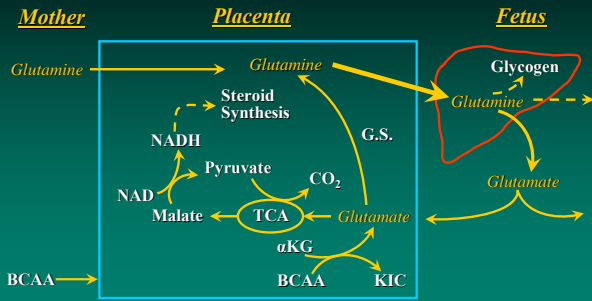
Johannes Häberle, M.D., Boris Görg, Ph.D., Frank Rutsch, M.D.,  
 Eva Schmidt, M.D., Annick Toutain, Ph.D., Jean-François Benoist, Ph.D.,  
 Antoinette Gelot, Ph.D., Annie-Laure Suc, M.D., Wolfgang Höhne, Ph.D.,  
 Freimut Schliess, Ph.D., Dieter Häussinger, M.D., and Hans G. Koch, M.D.

Nov 3, 2005

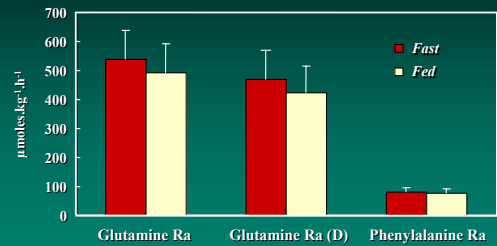
### Glutamine Synthesis from TCA Cycle Anaplerosis and Cataplerosis



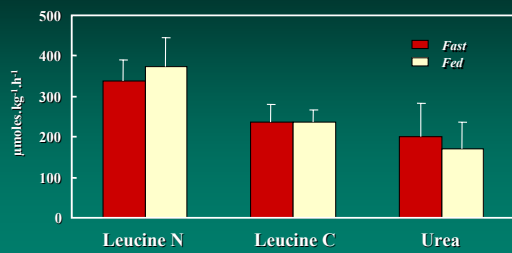
### Fetal-Placental Glutamine Exchange



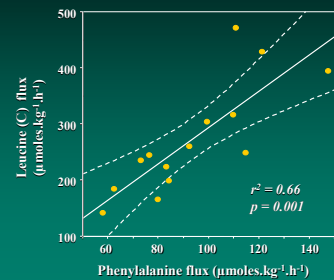
### Glutamine and Phenylalanine Kinetics in Term Infants



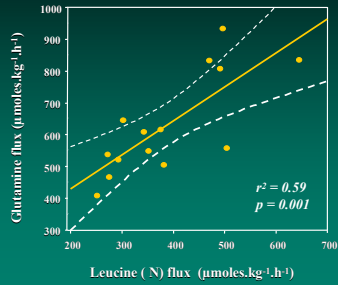
### Leucine and Urea Kinetics in Term Infants



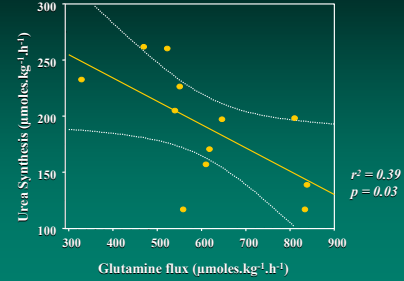
### Relationship Between Leucine (C) and Phenylalanine Flux in Neonates



## Correlation Between Rate of Appearance of Leucine (N) and Glutamine



## Correlation Between Ra Glutamine and Urea Synthesis in Newborn Infants



## Enteral Glutamine in LBW Infants

## OBJECTIVES

*Effect of 5 days enteral glutamine (0.6 g.kg<sup>-1</sup>.d<sup>-1</sup>) on*

- Whole body protein turnover (phenylalanine kinetics)
- Glutamine turnover and de novo synthesis
- Transamination of BCAA
- Urea synthesis

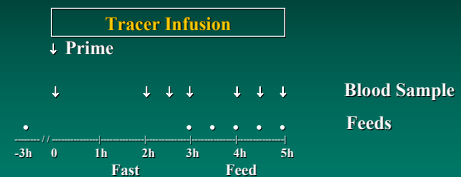
## Methods

**Preterm infants:** Gestational age <32 weeks  
Birth Weight <1500 g

Control (n=8)	Preterm infant formula (PF <sub>24</sub> ®, Ross Labs), 5d
Glutamine (n=9)	PF <sub>24</sub> ®+ glutamine 0.6g.kg <sup>-1</sup> .d <sup>-1</sup> , 5d

**Tracers:** [<sup>2</sup>H<sub>5</sub>]phenylalanine, [1-<sup>15</sup>N, <sup>13</sup>C]leucine, [5-<sup>15</sup>N]glutamine, [<sup>15</sup>N<sub>2</sub>]urea

## STUDY DESIGN



## Clinical Characteristics of Study Infants

	<u>Birth Weight</u> g	<u>Gestation</u> wk	<u>Age at Study</u> d	<u>Postconceptional Age at Study</u> wk	<u>Weight at Study</u> g
Control (n=8)	1161 ± 340	28 ± 3	39 ± 20	34 ± 1	1827 ± 330
Glutamine (n=9)	1216 ± 330	28 ± 2	41 ± 20	34 ± 1	1888 ± 307

## Glutamine Kinetics

	<u>Glutamine Ra</u>		<u>Glutamine De Novo Synthesis</u>	
	Fast	Fed	Fast	Fed
Control (n=8)	765 ± 165	654 ± 136	664 ± 157	539 ± 123
Glutamine (n=9)	690 ± 124	634 ± 95	588 ± 126	528 ± 97

$\mu\text{moles.kg}^{-1}.\text{h}^{-1}$

## Glutamine and Urea Kinetics

	<u>Glutamine from Proteolysis</u>		<u>Urea Ra</u>	
	Fast	Fed	Fast	Fed
Control (n=8)	101 ± 16	116 ± 24	133 ± 50	129 ± 47
Glutamine (n=9)	102 ± 12	110 ± 13	306 ± 111	292 ± 97
<i>P</i>			0.001	0.001

$\mu\text{moles.kg}^{-1}.\text{h}^{-1}$

## Phenylalanine and Leucine Kinetics

	<u>Phenylalanine Ra</u>		<u>Leucine (N) Ra</u>	
	Fast	Fed	Fast	Fed
Control (n=8)	96 ± 15	109 ± 22	453 ± 71	499 ± 94
Glutamine (n=9)	96 ± 11	103 ± 12	533 ± 86	587 ± 92
<i>P</i>	ns	ns	0.07	ns

$\mu\text{moles.kg}^{-1}.\text{h}^{-1}$

## Phenylalanine and Leucine Kinetics (cont)

	<u>Leucine (C) Ra</u>		<u>Leucine Reamination</u>	
	Fast	Fed	Fast	Fed
Control (n=8)	273 ± 44	271 ± 31	163 ± 85	230 ± 83
Glutamine (n=9)	298 ± 97	287 ± 65	250 ± 55	280 ± 71
<i>P</i>	ns	ns	0.05	ns

$\mu\text{moles.kg}^{-1}.\text{h}^{-1}$

## SUMMARY

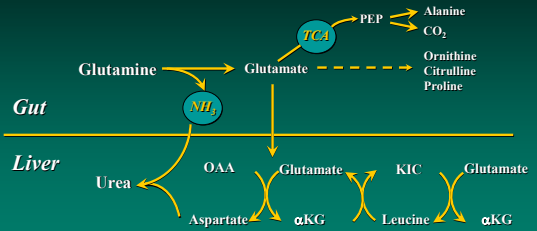
*Enteral administration of glutamine in growing LBW infants results in:*

- No measurable change in glutamine systemic Ra
- Increase in transamination of leucine
- An equimolar increase in urea Ra
- A *positive* linear correlation between reamination of BCAA and urea synthesis
- No detectable change in whole body phenylalanine or leucine C kinetics

## CONCLUSION

Enterally administered glutamine does not have a measurable impact on whole body protein kinetics. The equimolar increase in urea synthesis and unchanged systemic glutamine Ra suggests a first pass oxidation of glutamine in the gut.

## Splanchnic Metabolism of Glutamine



## Parenteral glutamine

## Glutamine Supplementation in LBW Infants

- Clinical advantages
  - Length of hospital stay
  - Ventilatory support
- Lower rate of sepsis
- Lower stimulation of immune system

(Neu J. et al; J Pediatr 1997)  
(Lacey et al; JPEN 1996)

## Parenteral Glutamine Supplement - Rationale

In isolated skeletal muscle:

- Positive correlation between (cellular) glutamine levels and protein synthesis
- Glutamine inhibited protein breakdown

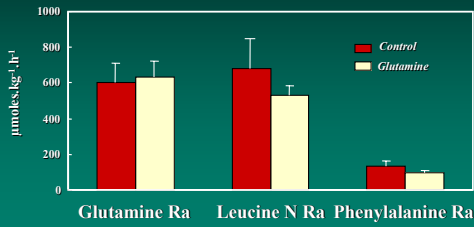
(MacLennan et al, 1987)

## Parenteral Glutamine Supplement - Rationale

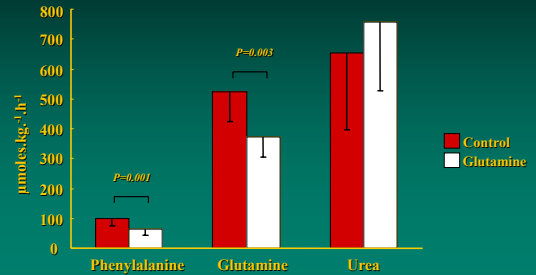
Experimental Depletion of Glutamine:

- No effect on Ra
  - Leucine*
  - Phenylalanine*
  - Glutamine*
- Decreased hepatic
  - (a) release of protein
  - (b) KIC oxidation

### Effect of parenteral Glutamine on Amino Acid Kinetics In Preterm Infants



### Endogenous Ra of Glutamine, Phenylalanine and Urea



### SUMMARY

*Our data show that in LBW infants, parenteral glutamine supplementation results in:*

- a decrease in the rate of appearance (Ra) of glutamine and phenylalanine,
- no change in the rate of urea synthesis, and
- a lower rate of turnover of leucine N.

### CONCLUSIONS

Since decrease in whole body proteolysis is associated with protein accretion, parenteral glutamine supplement may benefit LBW infants by enhancing protein synthesis and growth.

### Increased Parenteral Amino Acids and Protein Metabolism in LBW Infants

### Parental Amino Acids and Protein Kinetics

*In healthy adults, infusion of amino acids for 3-4 hours*

- decreased leucine Ra (proteolysis)
- increased NOD of leucine (synthesis)
- increased incorporation of leucine in skeletal muscle

Phenylalanine tracer – similar data

*(Pacey et al 1988; Bennet et al 1989, 1990)*

## Parental Amino Acids and Protein Kinetics

*Healthy full term and preterm infants respond to exogenous infusion of amino acids by suppressing protein breakdown (leucine, phenylalanine).*

*(Denne et al 1996; Clark et al 1997)*

## SPECIFIC AIM

Examine the effect of parenteral amino acids on whole body nitrogen kinetics.

## HYPOTHESIS

Administration of intravenous amino acids to LBW infants during the immediate neonatal period, when they are acutely stressed, will result in greater amino acid oxidation.

## Study Population

*Low birth weight infants, <32 weeks gestation*

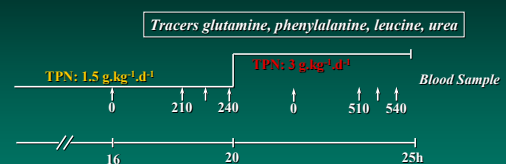
- (a) Age 3-5 days
- (b) Age <48 h

## Isotopic Tracers

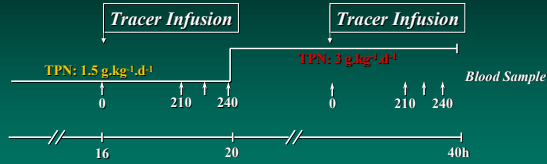
ring [ $^2\text{H}_5$ ]Phenylalanine  
[5- $^{15}\text{N}$ ]Glutamine  
[ $^{15}\text{N}_2$ ]Urea  
[1- $^{13}\text{C}$ , $^{15}\text{N}$ ]leucine

*Prime-constant rate infusion*

## Short Study



## Extended Study



## Clinical Characteristics

	Birth Weight g	Gestational Age wks	Age d	Weight at Study g
Group 1 (12)	1139 ± 276	29 ± 2	5 ± 1	1009 ± 270
Group 2 (5)	1189 ± 324	28 ± 2	5 ± 1	1093 ± 308

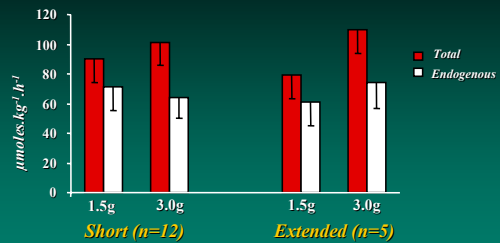
Group 1: 1.5g for 20h, 3g for 5h. Group 2: 1.5g for 20h, 3g for 20h.  
Data shown are mean ±SD

## Plasma Concentration of Amino Acid

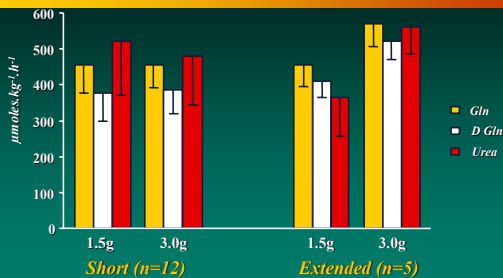
	Group 1		Group 2	
	1.5 g	3.0 g	1.5 g	3.0 g
Leucine	97 ± 24	174 ± 34	102 ± 17	198 ± 30
Phenylalanine	64 ± 19	85 ± 12	64 ± 12	93 ± 8
Glutamine	338 ± 156	437 ± 160	468 ± 195	707 ± 74
Alanine	90 ± 40	129 ± 50	84 ± 35	197 ± 93
Serine	114 ± 35	163 ± 47	144 ± 40	239 ± 70

mean ± SD

## Phenylalanine Ra During High and Low TPN



## Glutamine and Urea Kinetics

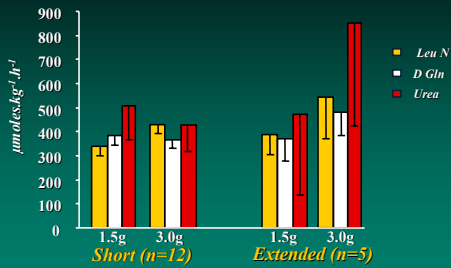


## Infants Less Than 48h Age

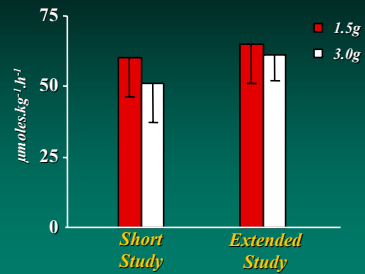
	Birth Weight (g)	Gestational Age (wks)	SNAP (median)
Short Study (7)	1234 ± 282	29.4 ± 2.4	8 (1-18)
Ext. Study (5)	1047 ± 229	28.6 ± 2.5	8 (1-18)

Data shown are mean ±SD

### Leucine, Glutamine and Urea Kinetics (<48 h age)



### Endogenous Phenylalanine Ra during TPN



### SUMMARY

Higher amino acid infusion causes a transient effect on whole body rate of proteolysis and protein oxidation (urea synthesis).

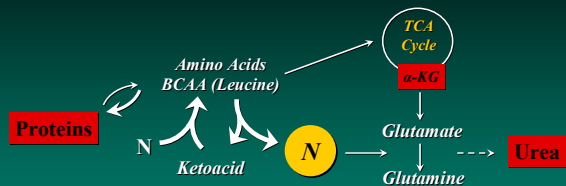
### SUMMARY (cont'd)

Acutely "stressed" LBW infants also respond to acute amino acid load by suppressing whole body proteolysis and protein oxidation.

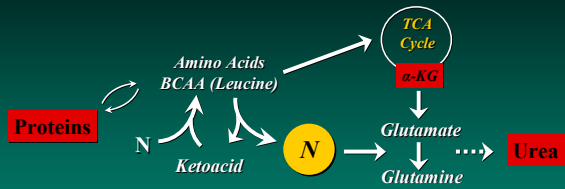
### SUMMARY (cont'd)

Sustained amino acid load, following the initial response, results in higher rate of glutamine and urea synthesis (cataplerosis and oxidation).

### Short Study



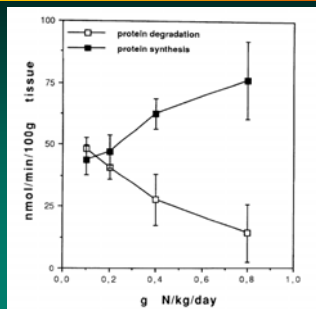
## Extended Study



## SPECULATION

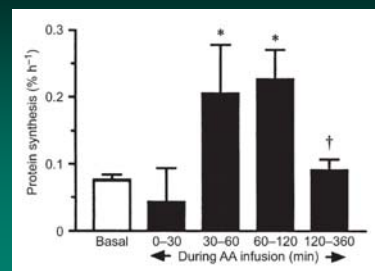
Adaptation to higher amino acid concentration may have resulted in down-regulation of amino acid transporter or other intracellular signaling systems.

## Effect of Increasing Dose of Amino Acids on Skeletal Muscle Protein Dynamics



Svanberg et al, AJP 1996

## Time Course of Synthesis of Mixed Muscle Protein During Amino Acid Infusion



Bohe et al, J Physiol 2001

## Amino Acids and Protein Dynamics

### Skeletal Muscle:

- Increase synthesis

### Splanchnic Compartment:

- Increase synthesis
- Inhibit breakdown

### Proteolytic Pathways

- Autophagic - lysosomal
- Ubiquitin - proteasome

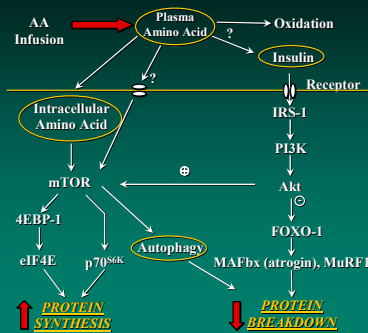
## Autophagic – Lysosomal Pathway

- Controlled by plasma amino acids
- Activated by:
  - Starvation
  - Amino acid deficiency
  - Hypoxia
  - High temperature

## Autophagic – Lysosomal Pathway

- “Partial autodigestion” to provide nutrients to maintain cell viability.
- Responsible genes: atg 1.....12
  - Regulated by P13 kinase
  - IgF<sub>1</sub> and others activate P13 and inhibit autophagy

## Synthesis and Breakdown of Proteins by Amino Acids



## Clinical Trials

### Parental Glutamine Supplement in LBW Infants

Lang <i>et al</i> (1996)	<i>Shorter duration of ventilation and TPN; Early full feeds</i>
Thompson (2003)	<i>Early full feeds</i>
Poindexter (2004) (NICHD)	<i>No effect</i>

### Parental Glutamine Supplement in LBW Infants

#### Why variable response?

- Heterogeneity of population
- Difficulties in delivery targeted amino acids and glutamine
- Complex outcome parameters  
Mortality, sepsis, etc.

## Enteral Glutamine Supplement in LBW Infants

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- Lower rate of sepsis
  - Less feeding intolerance
    - *Neu, J Pediatr 1997*
    - *van den Berg, AJCN 2005*
    - *Vaughn P, J Pediatr 2003*
- 

### My colleagues

Prabhu Parimi	Ed Burkett
Chantal Cripe-Mamie	Carole Bennett
Mark Kadrofske	Clarita Duenas
Colleen Nye	Jose Mariappuram
Joyce Nolan	Lourdes Gruca

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*Thank You*

## Acknowledgment

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My colleagues:

Prabhu Parimi, M.D.  
Mark Kadrofske, M.D.  
Lourdes Gruca, M.S.

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