Interorgan exchange of Amino Acids: What is the driving force?

NEP Deutz
Center for Translational Research in Aging & Longevity. Donald W. Reynolds Institute on Aging. UAMS, Little Rock
Interorgan exchange of Amino Acids

- Introduction
- Methods to measure interorgan exchange of Amino Acids
- Interorgan exchange Amino Acids during feeding
- Impact Diet on interorgan exchange
- Impact disease on interorgan exchange
- Conclusion
Interorgan exchange of Amino Acids

- Is highly active and regulated process
- Provides and delivers Amino Acids to all organs and tissues for
  - protein synthesis
  - Specific metabolic functions
- Plays a role in the maintenance of plasma Amino Acid homeostasis
- Major role of (patho)physiological state and nutrition
Interorgan exchange of Amino Acids

- After Feeding and initial phase of fasting
  - Dominant flux from the Gut to other tissues
- During prolonged fasting
  - Dominant flux from muscle to liver and kidney
Interorgan Amino Acid transport: Transition from feeding and early fasting period to prolonged fasting and starvation
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Peroperative Interorgan measurement

Direct puncture of portal vein (either blind or direct)
Catheter placement in the pig (full model)

- To measure plasma flow, PAH is infused through the splenic vein (S) and abdominal aorta (A1)
- For turnover measurements, tracer is infused through the caval vein (V2) for turnover measurements
- Blood is sampled from the abdominal aorta (A2), caval vein (V1), hepatic vein (H) and portal vein (P)
The Interorgan Pig Model

Implantation of the splenic and portal vein catheter
The Interorgan Pig Model

Implantation and checking of the position of the hepatic vein catheter
The Interorgan Pig Model

Implantation of the gastric catheter and closing
The Interorgan Pig Model: Nutrition and Sampling

Lab Anim 30(4): 347-58
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Interorgan exchange of Amino Acids: Flow from the Gut

Delivery of amino acids from luminal and arterial site
Use of tracers to study gut metabolism
Phenylalanine kinetics across the gut

- **Net Balance**
  - Control
  - Caseine
  - Soy

- **Release**
  - Control
  - Caseine
  - Soy

- **Protein Synthesis**
  - Control
  - Caseine
  - Soy
Interorgan exchange of Amino Acids: Muscle - % uptake of intake

% net Muscle Uptake

PIG Interorgan GLN and GLU transport

Clin Sci (Lond) 2003;104:127-41
Changes during fasted-fed state transition

Glutamate

- Gut
- Liver
- Muscle

Gut Liver Muscle
-2500 0 2500
5000
7500
10000
nmol/kg BW/min

Glutamine

- Gut
- Liver
- Muscle

Gut Liver Muscle
-7500 -5000 -2500 0 2500 5000
nmol/kg BW/min

Alanine

- Gut
- Liver
- Muscle

nmol/kg BW/min

-7500 -5000 -2500 0 2500 5000

Clin Sci (Lond) 104(2): 127-41
Changes in BCAA during fasted-fed state transition

Clin Sci (Lond) 104(2): 127-41
PIG Interorgan GLU, GLN, ALA and BCAA transport
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Amount of protein given

- With a balanced meal ±90% of the dietary amino acids are absorbed and about 30-50% will be used by the intestine itself and the rest released into the portal vein.

- Excessive amount of proteins potentially could lead to limitation of gut absorption. However, it is more likely that the maximum that gut cells can use for own metabolism is reached faster and that consequently, higher protein intake will reduce the % amino acids extracted.

- During a low protein intake, the % amino acids extracted by the gut will be higher, although the intestine can adapt to reduced protein intake by reducing its amino acid oxidation.
Quality of the protein

- It is postulated that a high quality dietary protein stimulates the amino acid utilization in the gut and is therefore of benefit for the gut and for the rest of the body.

- Metabolic utilization of amino acids in the gut also depends on the composition of the meal with respect to the presence or absence of (in)dispensable essential amino acids. Lack of an amino acid in a protein meal makes the protein of low quality.

- For example, ingestion of an isoleucine-poor blood protein in pigs resulted in high amino acid release, while concomitant intravenous isoleucine infusion promoted amino acid retention in the gut.
Making a low quality protein a better protein by infusion the limiting Amino Acid

Less amino acids come out of the Gut: More Gut protein synthesis!

Gastroenterology 1991;101:1613-20
The gut as a metabolically-active organ during feeding

- Not all amino acids that pass the gut enter the circulation, since a part of the amino acids is used for local metabolism (e.g. oxidation, protein synthesis)
- There is also substantial use from arterial supply
- The gut has one of the highest protein synthesis rates
The gut as a metabolically-active organ

- About 50% of the dietary amino acid intake is used by the Portal Drained Viscera (PDV), but this percentage varies between different amino acids and other factors.
- During feeding, amino acids that come from either the lumen or circulation contribute to protein synthesis, but at a different level.
- Malnutrition or starvation, protein depletion or deficiencies of specific nutrients all inhibit the growth and turnover of the intestinal mucosa and therefore will affect absorption kinetics.
The gut as a metabolically-active organ

- The gut has a high protein synthesis rate that is affected during many conditions.
- Special role of the Amino Acids
  - Glutamine: Main energy source in small intestine
  - Citrulline: Important source of Arginine in and out
  - Arginine: Role in NO production
  - Threonine: As precursor of mucus protein
  - Cysteine: As precursor of GSH
The gut as a labile pool of protein

- After a meal there is a net accumulation of protein in the gut whereas in the post-absorptive state a net loss of protein takes place.

- When a protein is rapidly digested, absorbed and the amino acids are directly released to the portal system, this large flux of highly concentrated amino acids in the portal vein would give rise to a high rate of urea production, gluconeogenesis and amino acid oxidation.

- A more gradual release of amino acids from the gut would ensure a more prolonged supply of amino acids in the portal vein, resulting in lower plasma concentrations in the portal vein, lower urea production and potentially more muscle anabolism.
Addition of Macronutrient: CHO

In the multi-catheterized pig model, pigs were given a bolus meal consisting of Whey protein with and without carbohydrates (CHO).
In the multi-catheterized pig model, pigs were given a bolus meal consisting of Whey protein with and without carbohydrates (CHO).
Addition of Macronutrient: CHO

Glutamine

Alanine
Addition of CHO to a protein meal

- Results in increased intestinal amino acid retention, increased Gut GLN uptake and ALA release, indicating stimulated gut metabolism
- Results in lower plasma concentrations, thus lower muscle delivery
- But, it improves the anabolic quality of a protein meal for the gut and probably on a day-to-day basis also whole body anabolism
PDV flux

AA flux (µmol/kg/min)

Time (h)

High Qual Prot
High Qual Prot + CHO
Slow vs fast protein

- A more prolonged positive net protein balance was observed with casein protein or with repeated meals of whey protein to mimic slow digestion rate.

- When comparing protein sources, their digestibility and/or absorption rates can potentially have an effect on gut retention and consequently absorption kinetics.
- Small peptides can be actively absorbed via a separate transporter in the enterocyte.
- In the enterocyte, the peptides are converted to single amino acids, released to the portal system or are utilized in the gut itself.
- It is expected that only marginal amounts of peptides “escape” the mucosa cell to be released into the portal vein.

"Small peptides can be actively absorbed via a separate transporter in the enterocyte. In the enterocyte, the peptides are converted to single amino acids, released to the portal system or are utilized in the gut itself. It is expected that only marginal amounts of peptides “escape” the mucosa cell to be released into the portal vein."
Absorption kinetics tripeptide

Fraction appearing tripeptide in plasma: 0.05-0.1%

Plasma concentration-time curve for tripeptide in pig after intragastric infusion of 4.0 mg tripeptide/kg BW

Unpublished
Does the form matter?

Whey protein as intact protein (WPI), partially hydrolyses (hWPI) and as mixture of free amino acids (aWPI) was given to pigs.

Uptake of peptides and amino acid mixtures

- We did not observe a difference, because
  - whey protein was a fast protein with no digestion limitation
  - No limitation of peptidase activity in the mucosa (pigs have very high capacity?)
  - the amount of protein given was below daily intake
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Septic pigs Model validation

Hyperdynamic Sepsis model
• LPS
  • E.Coli 055:B5
  • 24h  i.v. at 3 µg.kg bw\(^{-1}\).h\(^{-1}\)
• High Fluid Support
  • 150 mM NaCl i.v.
  • 30 ml.kg bw\(^{-1}\).h\(^{-1}\) first 8h after start LPS
  • 20 ml.kg bw\(^{-1}\).h\(^{-1}\) 8-30h after start LPS
• NO MORTALITY
• This pig model is good animal model for human sepsis

Body Temperature

MAP
Changes during sepsis in fasted pigs

Glutamate

Glutamine

Alanine

Clin Sci (Lond) 104(2): 127-41
Interorgan BCAA in fasted pigs during sepsis

Control

LPS

Gut

Liver

Muscle

nmol/kg BW/min

Clin Sci (Lond) 104(2): 127-41
PIG Interorgan GLU, GLN, ALA and BCAA transport

Glutamate

Alanine

Glutamine

BCAA

SEPSIS

FASTED

Clin Sci (Lond) 104(2): 127-41
Changes post-sepsis in fed pigs

Glutamate

Glutamine

Alanine

Interorgan BCAA Changes post-sepsis in fed pigs

![Bar chart showing changes in nmol/kg BW/min for Gut, Liver, and Muscle tissues under control, 24h after LPS, and 96h after LPS conditions.](chart.png)

PIG Interorgan GLU, GLN, ALA and BCAA transport

Glutamate

Glutamate

Glutamine

Alanine

BCAA

Alanine

Alanine

Glutamine

BCAA

Post SEPSIS

Interorgan GLU to GLN transport in humans
Severe sepsis in humans

![Graph showing GLN and GLU levels in Healthy Control, ICU Controls, and ICU severe sepsis groups.](chart)

Unpublished
Interorgan GLU to GLN transport in humans

SEPSIS

Plasma GLU Pool

Plasma GLN Pool

Gut dysfunction

Glutamine

Glutamate

Unpublished
GLU as marker for survival during and after sepsis

<table>
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<tr>
<th></th>
<th>Survivors</th>
<th>Non-survivors</th>
<th>p-value</th>
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<tr>
<td>Number</td>
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<td>10</td>
<td></td>
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<tr>
<td>Age (years)</td>
<td>51.2 (3.7)</td>
<td>66.6 (5.3)</td>
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<tr>
<td>Gender (ratio M: F)</td>
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<td>1.0</td>
<td>0.4</td>
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<tr>
<td>BMI</td>
<td>25.4 (1.1)</td>
<td>23.4 (1.0)</td>
<td>0.2</td>
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<tr>
<td>Diagnostic group</td>
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<td>Abdominal</td>
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<td>7</td>
<td>0.1</td>
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<tr>
<td>Pneumonia</td>
<td>8</td>
<td>3</td>
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<tr>
<td>Liver OF score at 24 hrs</td>
<td>1.2 (0.8)</td>
<td>1.2 (0.9)</td>
<td>0.7</td>
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<tr>
<td>MOF-score after 24 hours</td>
<td>8.1 (0.5)</td>
<td>8.7 (0.6)</td>
<td>0.5</td>
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<tr>
<td>APACHE-II</td>
<td>26.7 (2.6)</td>
<td>33.9 (2.4)</td>
<td>0.06</td>
</tr>
</tbody>
</table>

All values expressed as mean (SEM). BMI, body mass index; (M)OF, (multiple) organ failure; APACHE, acute physiological and chronic health evaluation. Patients were grouped according to their 60-day survival.

Unpublished
Arginine metabolism

- Arginine
  - 15% from Ornithine
  - 85% from Food and Body Protein

- Ornithine
  - 15% from Proline Glutamate

- Citrulline
  - 15% from Arginine
Interorgan CIT and ARG transport

Plasma ARG Pool

Arginine

Arginine

Citrulline

Glutamine

AJCN 2004; 79: 185-97
Interorgan GLU to GLN to Cit to ARG transport in humans

Plasma ARG Pool

Arginine

Glutamine

Glutamate

Citrulline
Interorgan ARG during sepsis in the pig


More ARG necessary
Interorgan CIT and ARG transport in the pig

Plasma ARG Pool

Sepsis

Arginine

Arginine

Citrulline

Glutamine

Gut dysfunction

Severe sepsis in humans

- **Comparative Analysis**
  - **WbRaCit**
    - Healthy Control
    - ICU Controls
    - ICU Severe Sepsis
  - **WbdeNovoArg**
    - Healthy Control
    - ICU Controls
    - ICU Severe Sepsis
Interorgan GLU to GLN to Cit to ARG transport in humans

Plasma ARG Pool

SEPSIS

Arginine

Citrulline

Glutamine

Glutamate

Gut dysfunction

Unpublished
Lower plasma ARG during sepsis is related to reduced Nitric Oxide production

NO (Nitric Oxide) regulates vascular tone and microcirculation and is essential in the immune response
Arginine supplementation after sepsis in the pig

Protein Breakdown

Sepsis
Sepsis+ARG

nmmol/kg BW/min

Gut
Muscle

AJCN 2002;75:1031-44
Arginine metabolism in septic patients: hypothesis

In cell
- Protein Synthesis
- Protein Breakdown

L-Arginine

+ Inflammation

Urea

L-Ornithine

NO

Plasma [Arg]

Arg supplementation

Crit.CareMed 2004;32:2135-45
Placebo-controlled Arginine infusion in septic patients: HAEMODYAMICS

Daily average values of 2h-interval measurements

- Significant increase in mean arterial blood pressure in both groups
- No effect of Arginine infusion

Crit.CareMed2007; In press
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Interorgan exchange of Amino Acids: What is the driving force?

- **Driving forces**
  - The fasting-feeding transition
  - Presence of disease

- **Quantity of the driving forces**
  - Role of the Gut as regulator!
  - Role of the quality and form/quantity of the dietary proteins

- **Quality of the driving forces**
  - The GLU → GLN → CIT → ARG route is important in relation to disease as it drives the ARG production and this also the Nitric Oxide production