Amino acids: fed or fasted?

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Amino acids: fed or fasted?

- Brief introduction
- Overview of amino acid metabolism
- The importance of muscle
- Starvation/isocaloric protein deprivation
- Amino acid analysis
- Summary
- What next?
Introduction

- Maintenance of protein content of certain tissues essential – skin, heart, brain & liver
- Postabsorptive state - rely on steady supply of amino acids (aa) via blood
- Muscle – principal reservoir of aa supply in absence of nutrient intake
- Fasting state – continued availability of aa for protein synthesis & gluconeogenesis
- Protein mass & plasma glucose maintained provided adequate muscle mass
Overview of AA metabolism

Dietary protein → Blood amino acids → Intracellular protein & amino acid pool → Intracellular amino acid pool → Carbon skeletons → Energy, Glucose, Ketone bodies

Nutritionally Non-essential Amino acids → Non-protein derivatives → Urea

Non-essential Amino acids → Carbon skeletons → Urea

NH₃
Non-essential amino acids

- Most mammals can synthesise 10 amino acids listed here
  - Alanine (Ala)
  - Asparagine (Asn)
  - Aspartate (Asp)
  - Cystine (Cys)
  - Glutamate (Glu)
  - Glutamine (Gln)
  - Glycine (Gly)
  - Proline (Pro)
  - Serine (Ser)
  - Tyrosine (Tyr)
Man cannot synthesise carbon skeletons of the following amino acids:

- Iso-leucine (Ile)
- Leucine (Leu)
- Lysine (Lys)
- Methionine (Met)
- Phenylalanine (Phe)
- Threonine (Thr)
- Tryptophan (Trp)
- Valine (Val)
- Arginine* (Arg)
- Histidine* (His)

*Impt in prems and growth in children
Overview of aa metabolism

- Dietary protein
  - Blood amino acids

- Intracellular protein & amino acid pool
  - Nutritionally Non-essential Amino acids
    - Non-protein derivatives
  - NH₃

- Intracellular amino acid pool
  - Carbon skeletons
    - Urea
    - Ketone bodies
    - Glucose
    - Energy
Nitrogen containing compounds

- Purines & Pyrimidines
- Choline
- Creatine
- Niacin
- Bile salts
- Melanin
- Nitric oxide
- Putrescine
- Cadaverine
Overview of aa metabolism

Dietary protein

Blood amino acids

Intracellular protein & amino acid pool

Nutritionally Non-essential Amino acids

Non-protein derivatives

NH₃

Urea

Carbon skeletons

Energy

Glucose

Ketone bodies
Overview of amino acid metabolism

Dietary protein → Blood amino acids → Intracellular protein & amino acid pool

Nutritionally Non-essential Amino acids → Non-protein derivatives

Intracellular amino acid pool → NH₃ → Carbon skeletons

Energy → Glucose → Ketone bodies

Urea
7 common metabolic intermediates

- aa - degraded by 20 different pathways
- Converge to 7 metabolites:
  - Pyruvate
  - $\alpha$-ketoglutarate
  - Succinyl-CoA
  - Fumarate
  - Oxaloacetate
  - Acetyl CoA
  - Acetoacetate
Tricarboxylic acid (TCA) cycle

Entry points for amino acid carbons in the TCA cycle.
Importance of muscle

- Mammals lack an “energy storage protein”
- Most proteins – specified roles but also donors of aa when required
- Free aa ~ 100-200g in 70kg male
- Protein turnover ~ 300g/day
- Protein intake Western world 70-100+ g/day
- Half life regulatory enzymes: mins – hrs
- Structural proteins: days – months
- Free aa exchanged between muscle, kidney & splanchnic tissues (liver & gut)
Importance of muscle: continued

- Muscle – remarkable capacity to maintain aa levels; ~ 60 days fasting!
- Severe muscle mass depletion - incompatible with life
- Warsaw ghetto studies – death from starvation when muscle unable to supply sufficient aa
- Stressed state eg sepsis, burns greater demand on muscle than fasting
- Individuals with low muscle mass respond poorly to stress
Fasted state

Muscle

Kidney

Liver

Glutamine

Alanine

Glucose

Urea

NH₃

Gut
Glucose/Alanine cycle

Glucose → (2)Pyruvate → (2)Alanine → Urea

6 ATP

Glucose → (2)Pyruvate → (2)Lactate

2 ATP

(2)Alanine

Urea

[2]-NH₂

4 ATP

LIVER

MUSCLE

ALT

α-amino acid (glutamate)

α-keto acid (α-ketoglutarate)

BLOOD

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Glutamine cycle

- Gln: many biosynthetic uses – supplies most nitrogen for purine & pyrimidines
- Gln readily synthesised from glutamate
- Can be degraded back to glutamate
- Gut – gln converted to & released as ala – fuel for cells lining gut and available to liver
- Kidney: Gln derived NH₃⁺ control urinary pH
- Gln nitrogen waste excretory molecule for muscle
Starvation (total calorie deprivation)

- Late 60’s Felig et al starved a group obese subjects 5 – 6 wks
- Analysed
  - plasma amino acids
  - Splanchnic aa uptake in
    - Postabsorptive state
    - Briefly fasted (36 – 48 hrs)
aa: transient early increase

Figure 1: Plasma concentration of amino acids demonstrating a transient early increase in starvation. Seven obese subjects were studied during prolonged fasting at the intervals indicated.
Figure 2  Plasma concentration of those amino acids demonstrating a delayed increase in starvation. The early fall in threonine was statistically significant.
aa: ultimately decreased

Figure 3  Plasma concentration of those amino acids which fell below baseline levels (day 0) during the course of prolonged fasting. Valine, isoleucine, and leucine which ultimately decreased are presented in Fig. 1. Tyrosine and phenylalanine which did not change significantly until day 40 are not shown here. Note the separate scale for alanine.
Isocaloric protein deprivation

- Western world: sufficient calorie intake but potentially insufficient protein
- Treated metabolic patients
- BCAA decrease
  - Presumably muscle uptake for release of ala & gln
  - Muscle proteolysis control by Leu & insulin
- Val disproportionately lower than Leu or Ile
- gly & ala increase
  - Gly - muscle proteolysis turns protein synthesis off so gly accumulates
  - Ala - sufficient calories so slow hepatic uptake for gluconeogenesis
- Gln utilised by gut & kidneys as in starvation
- Data based on overnight fast
Alanine levels & fasting

Postabsorptive plasma levels before (d0) during (d1-6) & after (d7-8) dietary deprivation (Values are mean +/- SEM (n=6)
Amino acid analysis

- Number of factors affect analysis
  - Timing of sample
  - Sample quality
  - Age
  - Protein intake and calorie content
  - Infection
  - Liver failure
  - Renal failure
Amino acid analysis

- Sample timing
- Accurate knowledge of control data
  - Reference intervals wider for random sampling than in fasting population
  - Overnight fast of ~8 – 12 hrs
  - Pre-feed samples taken on children on 4 hourly feeds
Amino acid analysis

- Most aa’s peak ~ 2½ hrs post meal
- BCAA’s peak ~ 5hrs post meal
- Samples taken <8hrs post meal may give inconsistent results
- Consider cit supplementation to OTC
  - Sample at point of stable whole body metabolism or
  - At a time potential toxic metabolite levels
- Answer is probably both
Amino acid analysis

- Interpretation is never going to be easy
- Improve diagnostic sensitivity by
  - Standardised diets
  - Fasting
  - Ratios
  - Stress tests
  - Pictorial or graphical representation results
Amino acid analysis

- Ratios need to be used with caution
  - Hormonal effects on phe/tyr ratios
  - Give no indication of magnitude
- Ratios can be used as means of reducing metabolic noise
Amino acid analysis

- **BCAA** - largely independent of liver metabolism are frequently used.

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**Figure 2.** ‘Ratiogram’ of \( \text{ala} / (\text{val} + \text{leu} + \text{ile}) \) as indicator of caloric intake plotted against gly\((\text{val} + \text{leu} + \text{ile})\) as indicator of protein intake. Derived from published and author’s own data (see Ref. 4). Reproduced with permission from Amino Acids. Chemistry, Biology and Medicine. In: Proceedings of 1st International Conference on Amino Acids, Vienna, 1989. Vienna: Escom Science Publishers, 1990.)
Amino acid analysis

- Can we evaluate muscle breakdown & nutritional status?
- Can we assess protein requirement?
- Can manipulating dietary protein control metabolic disease?
- Muscle proteolysis overload liver in UCD?
  - Despite restriction dietary protein
  - Low BCAA’s in phenylacetate treated UCD pts
  - Gln deficit due to excretion phenylacetylglutamine
- BCAA may be indicators protein deprivation in pts with defects in propionate metabolism
Summary

- Whole body physiological mechanisms
- Sample timing
- Availability control data
- Use of ratios
- Fed or fasted?
  - General population fasting samples probably better but not readily available
  - Monitoring pts with IEM may be better taken at a consistent time post feed
What next?

- Not considered effect individual genetic differences on diet & nutrition.
- Need more control data to get added value from aa results.
- Metbionet aa working group
While everyone knows that the Matthew was the ship in which John Cabot sailed on his famous 1497 voyage of discovery to North America, almost nothing is known about the vessel that took him there.
aa: ketosis
oa: ketosis
Protein Turnover and Nitrogen Balance

Most intra-cellular proteins are undergoing continual breakdown and synthesis. The rate of turnover of these proteins is variable and usually will vary depending on the nature of the protein and the metabolic state of the individual. Two major pathways are involved in protein turnover; one is carried out by proteases in lysosomes and a second major pathway involves a ubiquitin dependent pathway working in conjunction with a macromolecular protease complex called a proteosome. The amino acids released in this process can then enter into the same pathways as the amino acids derived from the diet.
## Nitrogen Balance

Nitrogen intake = Nitrogen excretion

<table>
<thead>
<tr>
<th>Positive Nitrogen balance</th>
<th>Negative Nitrogen balance</th>
</tr>
</thead>
<tbody>
<tr>
<td>intake &gt; excretion</td>
<td>excretion &gt; intake</td>
</tr>
<tr>
<td>- growth of children</td>
<td>- starvation</td>
</tr>
<tr>
<td>- pregnancy</td>
<td>- malnutrition</td>
</tr>
<tr>
<td>- wound healing</td>
<td>- disease (burns, trauma, surgery)</td>
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<tr>
<td>- convalescing adult</td>
<td></td>
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