Amino acids: fed or fasted?

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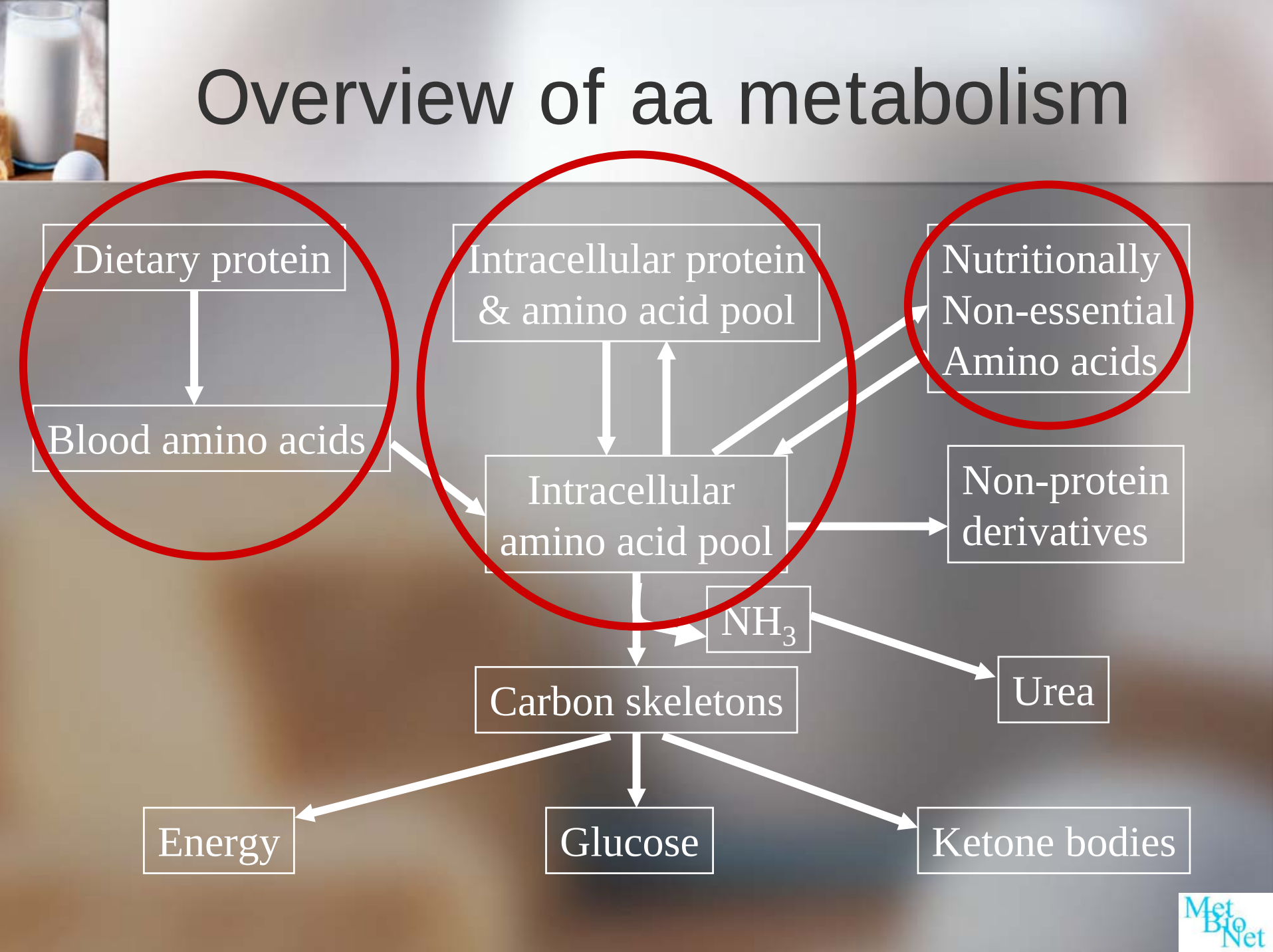
Amino acids: fed or fasted?

- 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 amino acids (aa) via blood
- Muscle – principal reservoir aa supply in absence 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



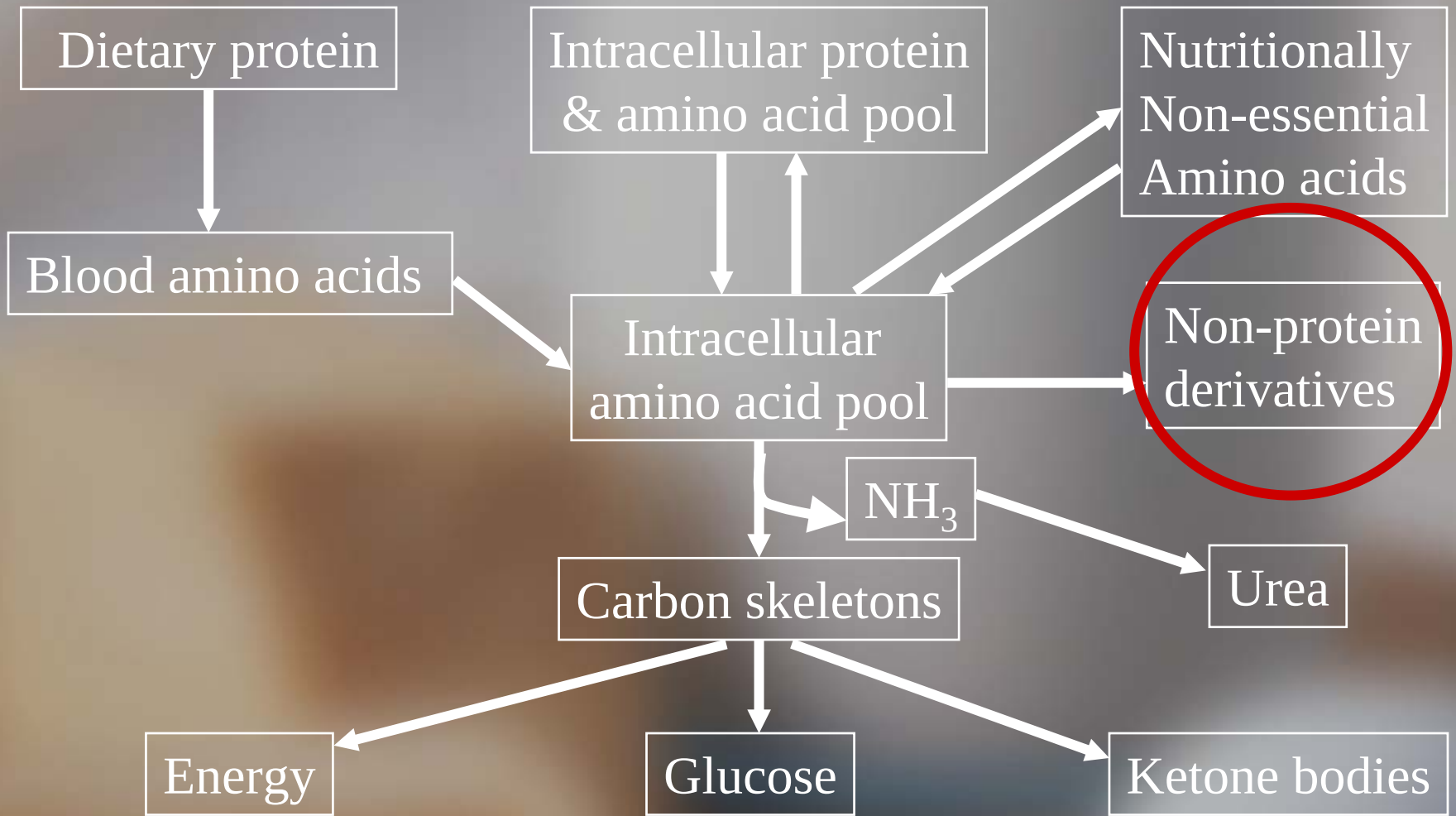
Non-essential amino acids

- 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)

Essential amino acids

- 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

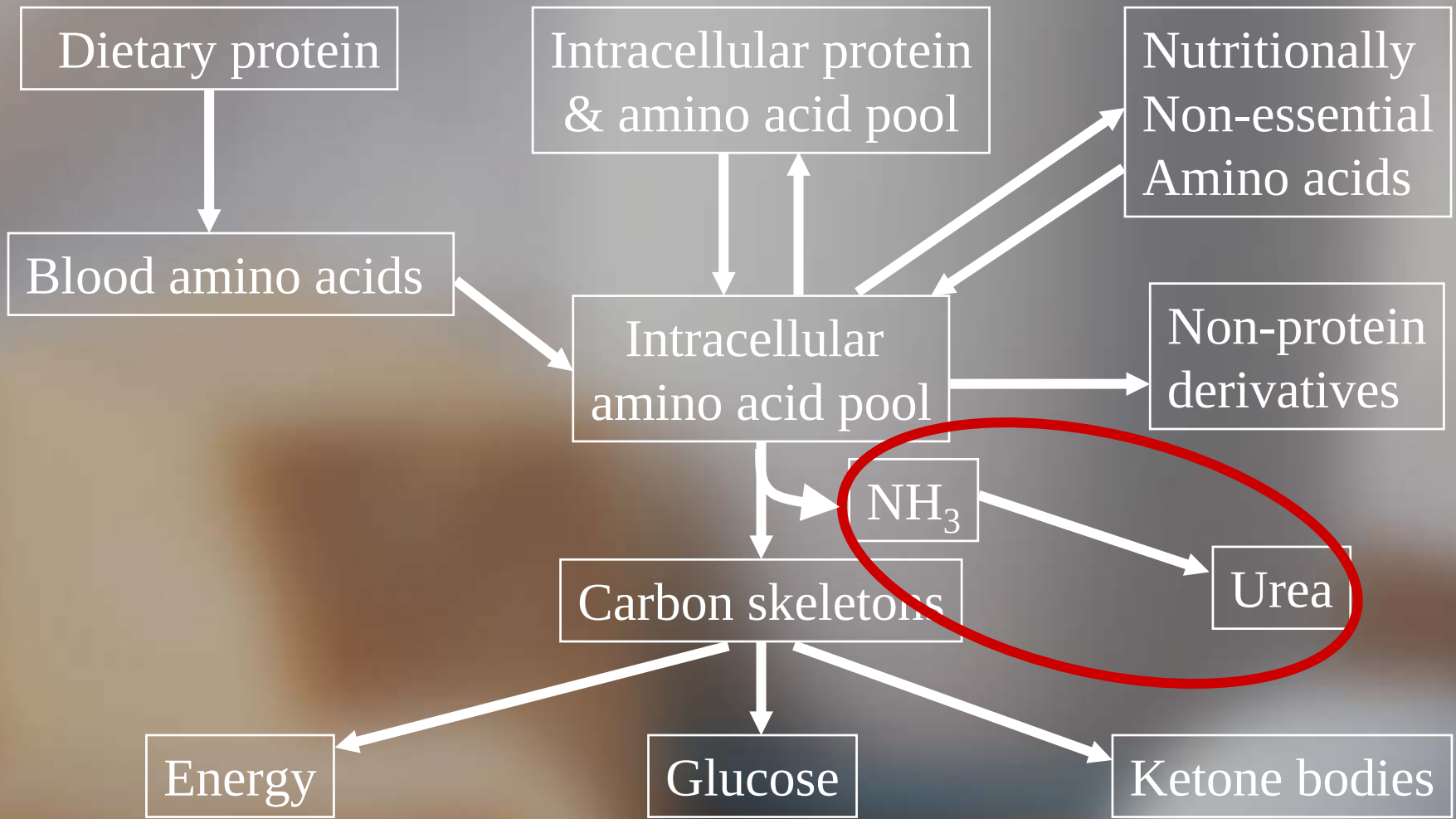
Overview of aa metabolism



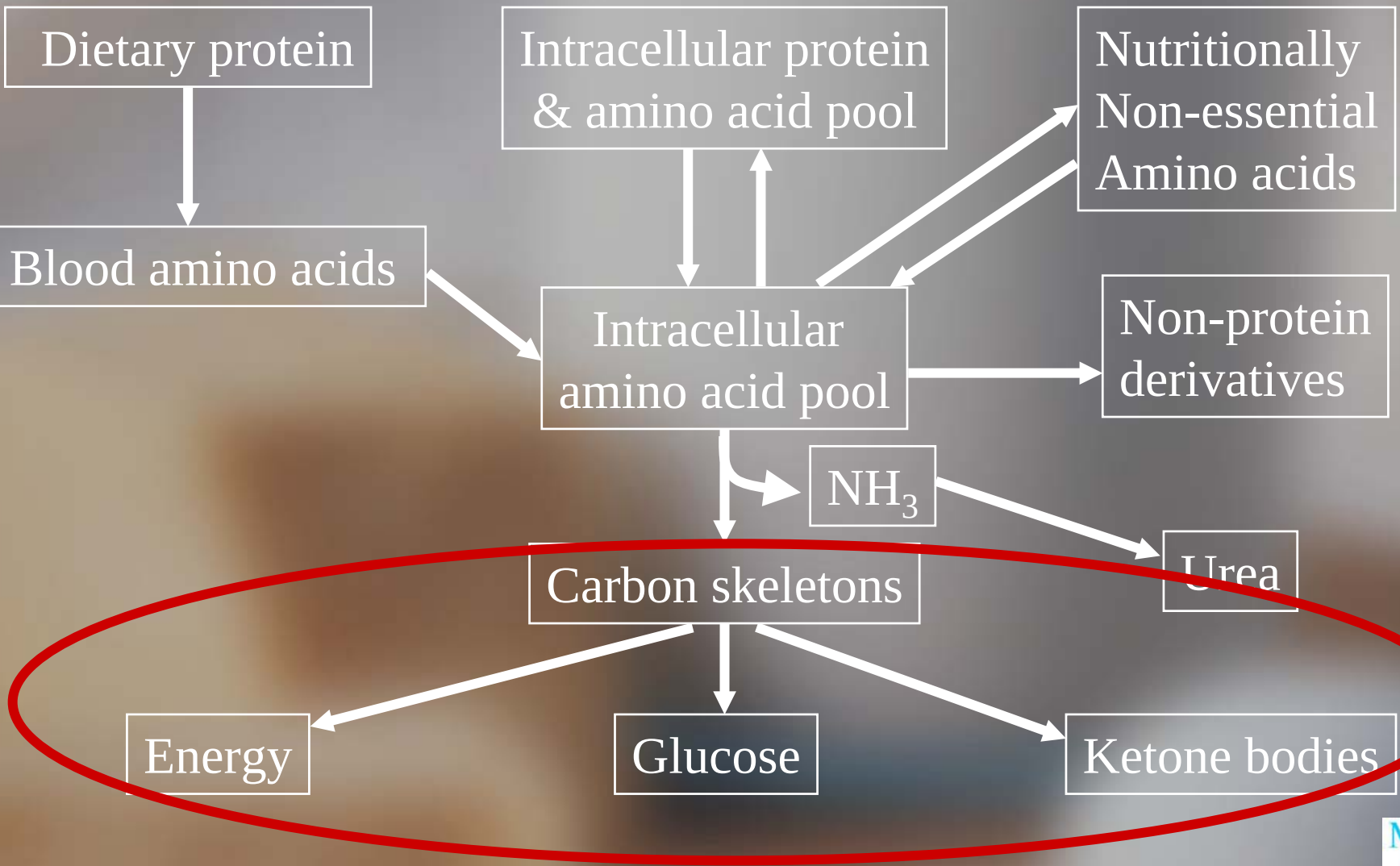
Nitrogen containing compounds

- Purines & Pyrimidines
- Choline
- Creatine
- Niacin
- Bile salts
- Melanin
- Nitric oxide
- Putrescine
- Cadaverine

Overview of aa metabolism



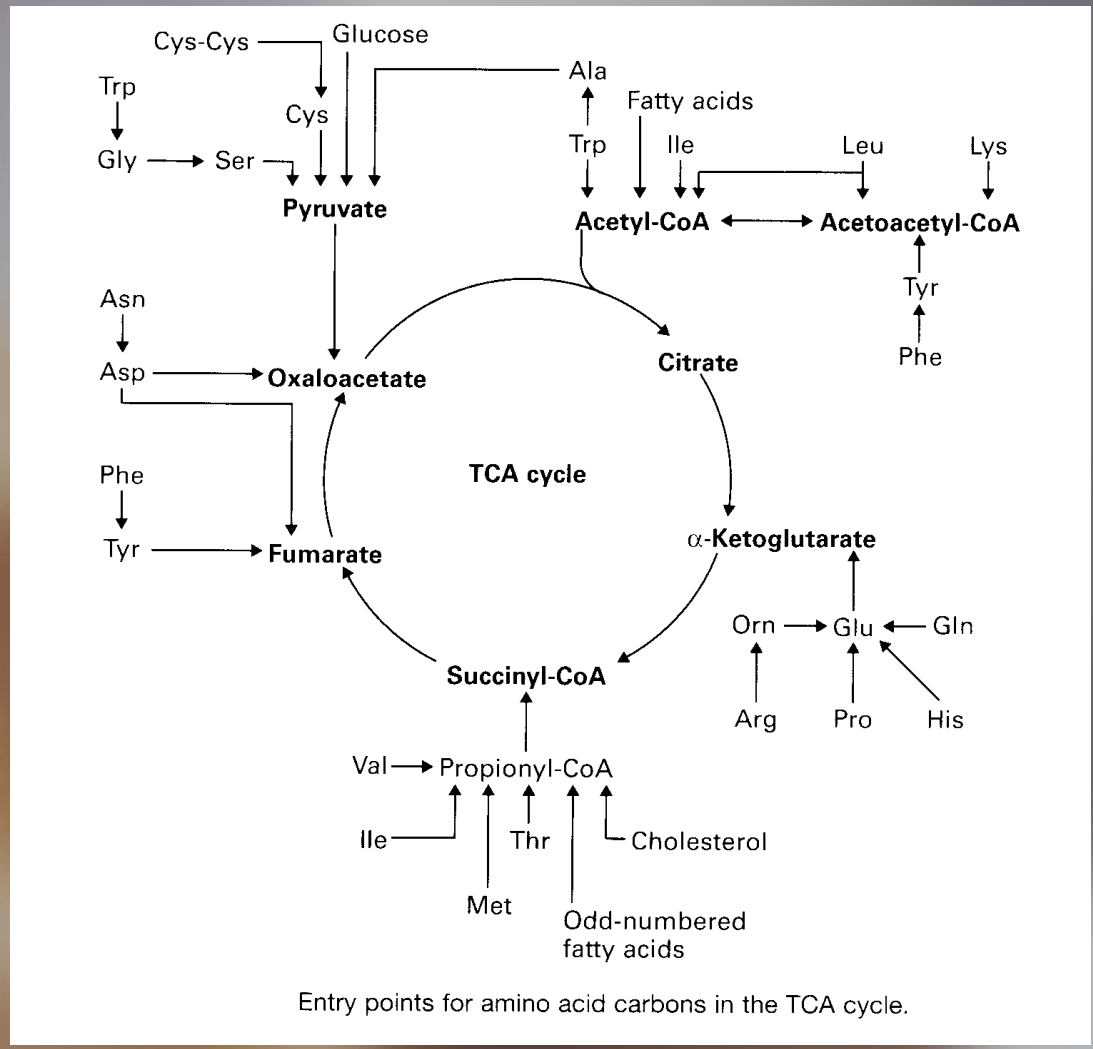
Overview of aa metabolism



7 common metabolic intermediates

- aa - degraded by 20 different pathways
- Converge to 7 metabolites:
  - Pyruvate
  - α-ketoglutarate
  - Succinyl-CoA
  - Fumarate
  - Oxaloacetate
  - Acetyl CoA
  - Acetoacetate

Tricarboxylic acid (TCA) cycle



Entry points for amino acid carbons in the TCA cycle.

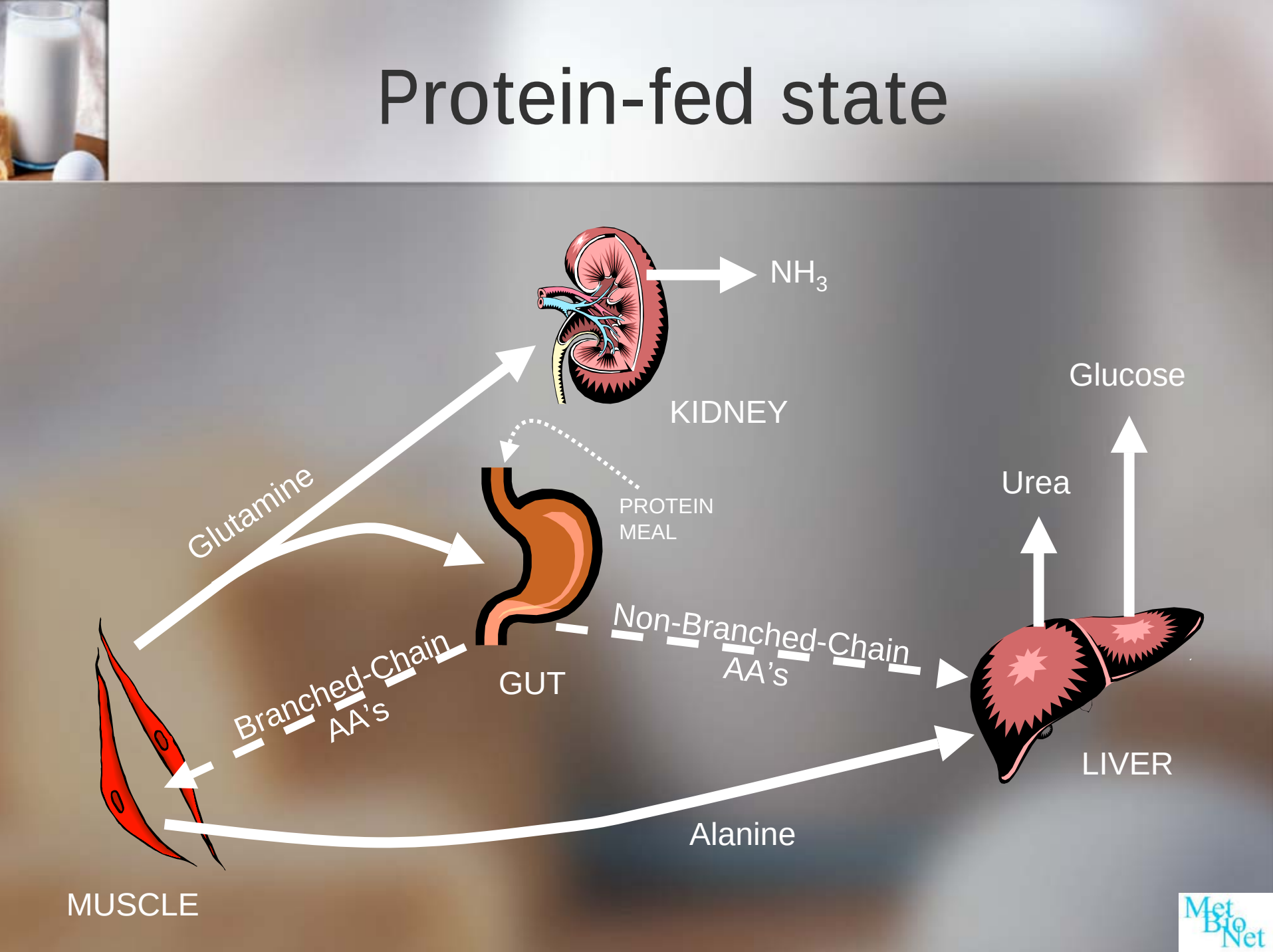
Importance of muscle

- 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)

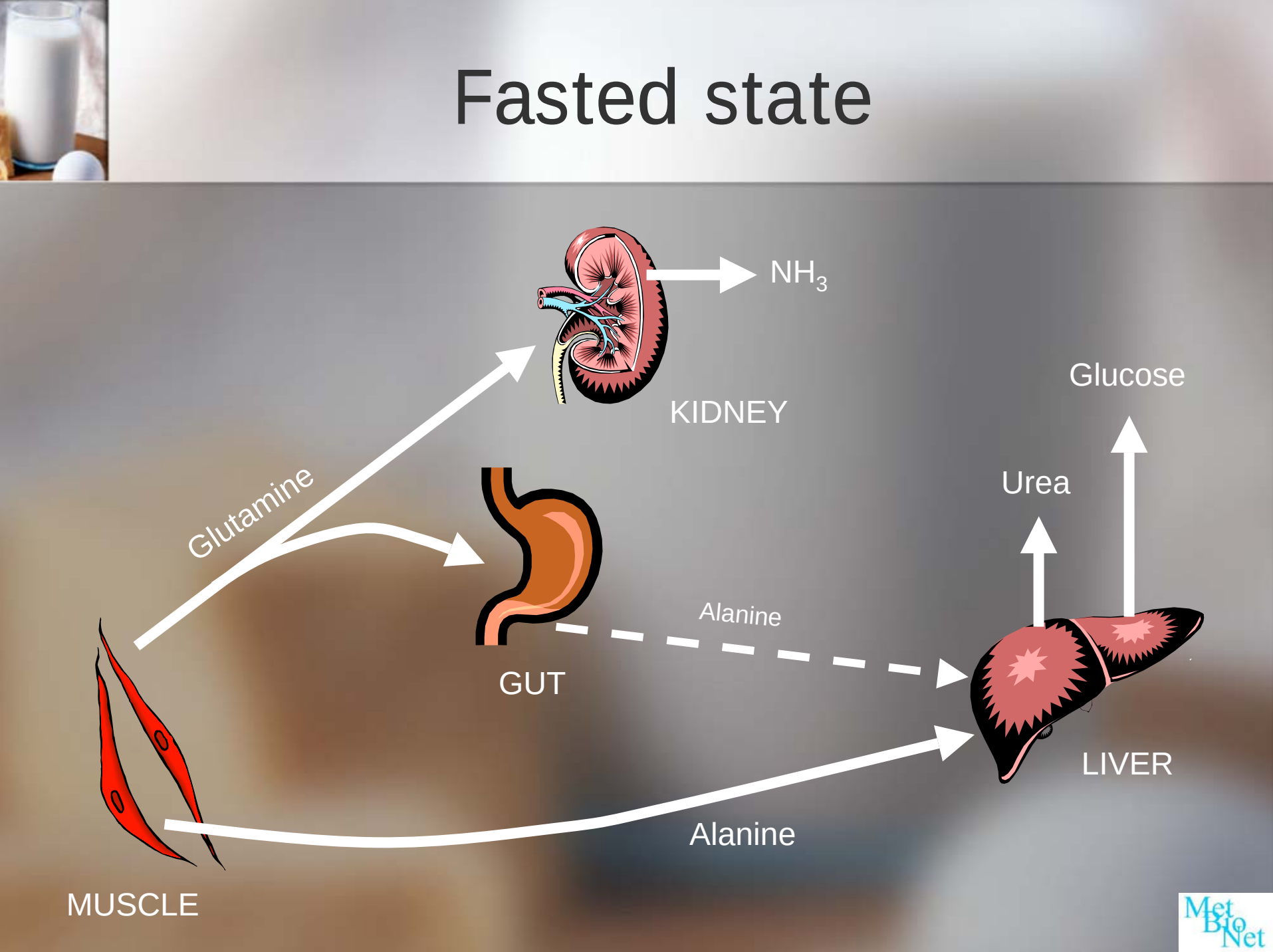
Importance of muscle: continued

- 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

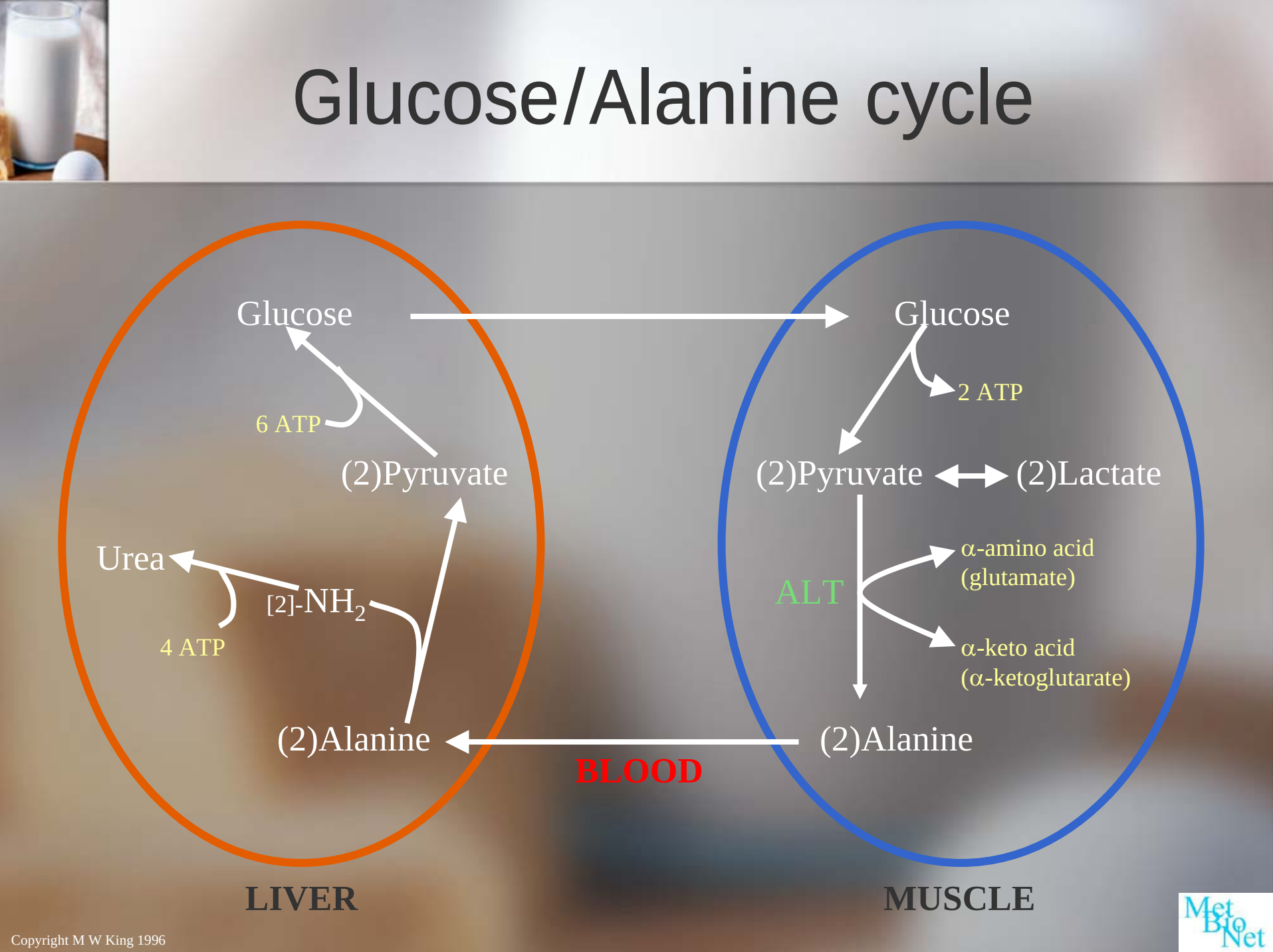
Protein-fed state



Fasted state



Glucose/Alanine cycle



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 NH_3^+ 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

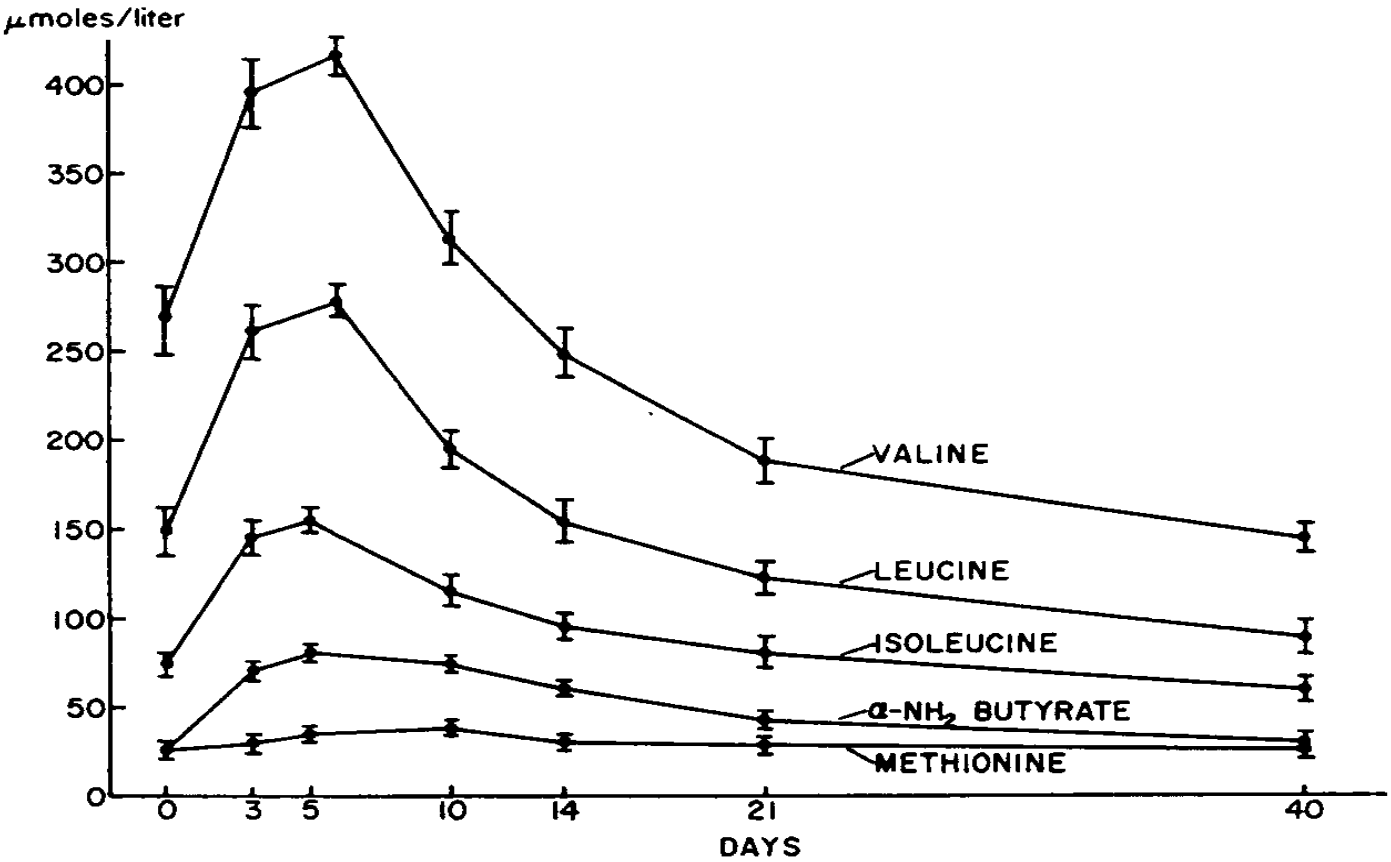


FIGURE 1 Plasma concentration of amino acids demonstrating a transient early increase in starvation. Seven obese subjects were studied during prolonged fasting at the intervals indicated.

aa: delayed increase

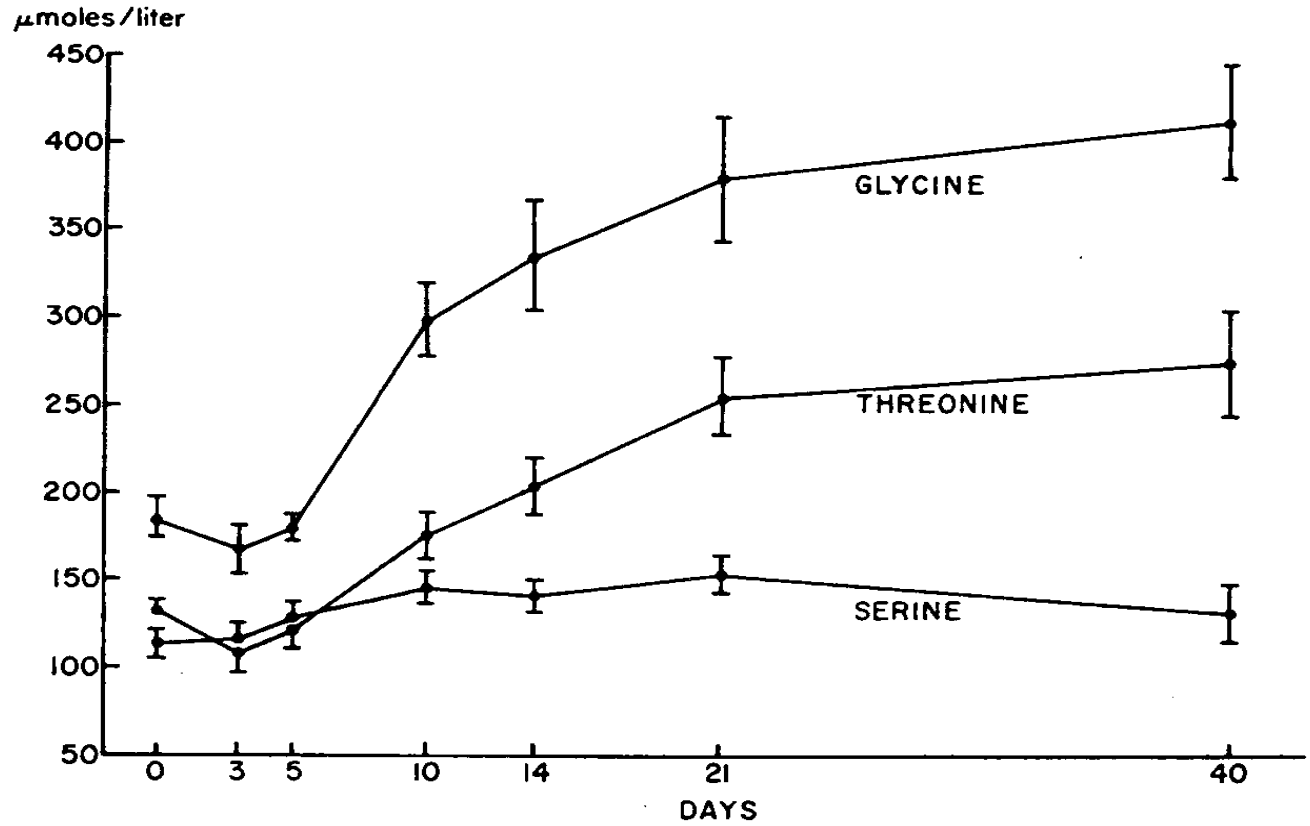


FIGURE 2 Plasma concentration of those amino acids demonstrating a delayed increase in starvation. The early fall in threonine was statistically significant.

aa: ultimately decreased

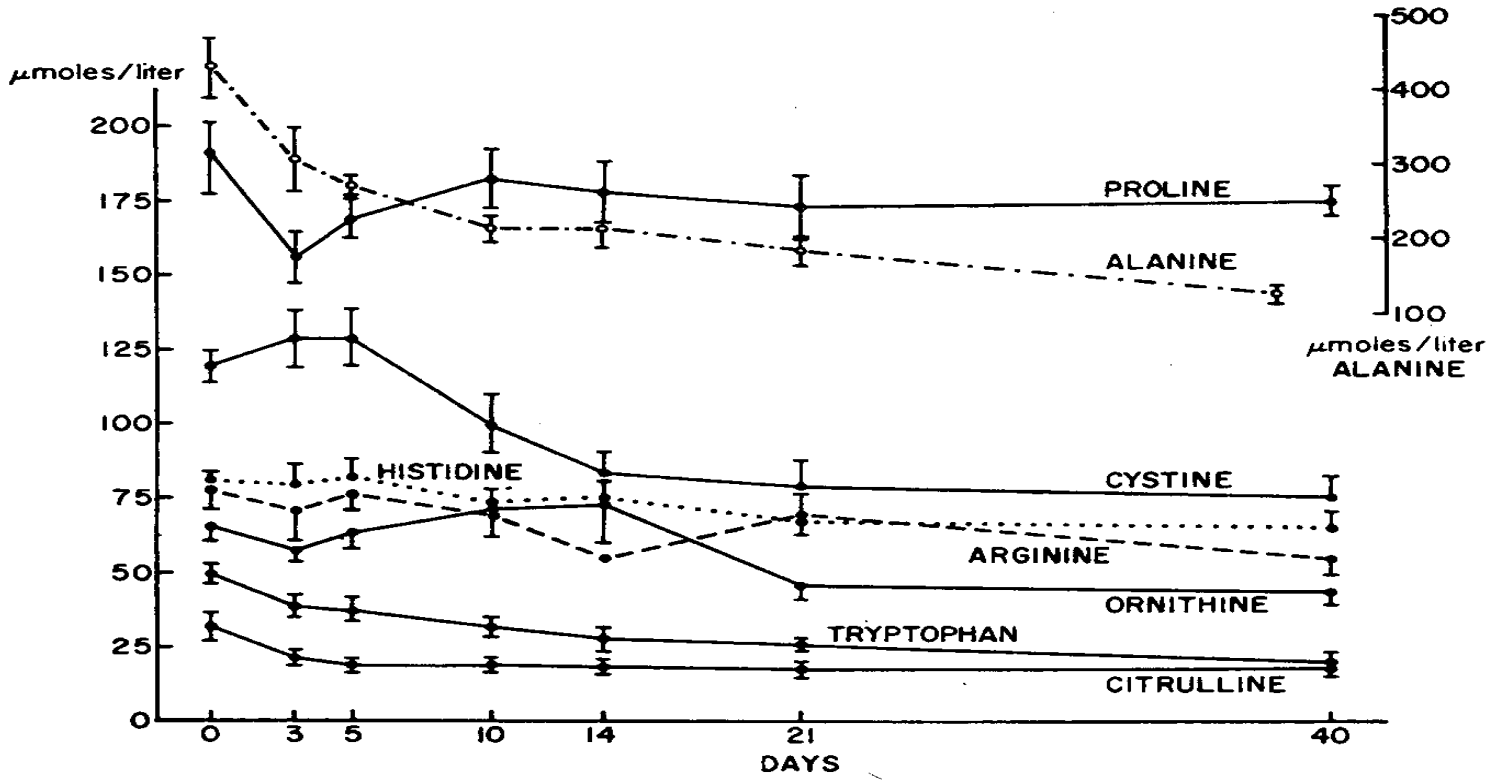
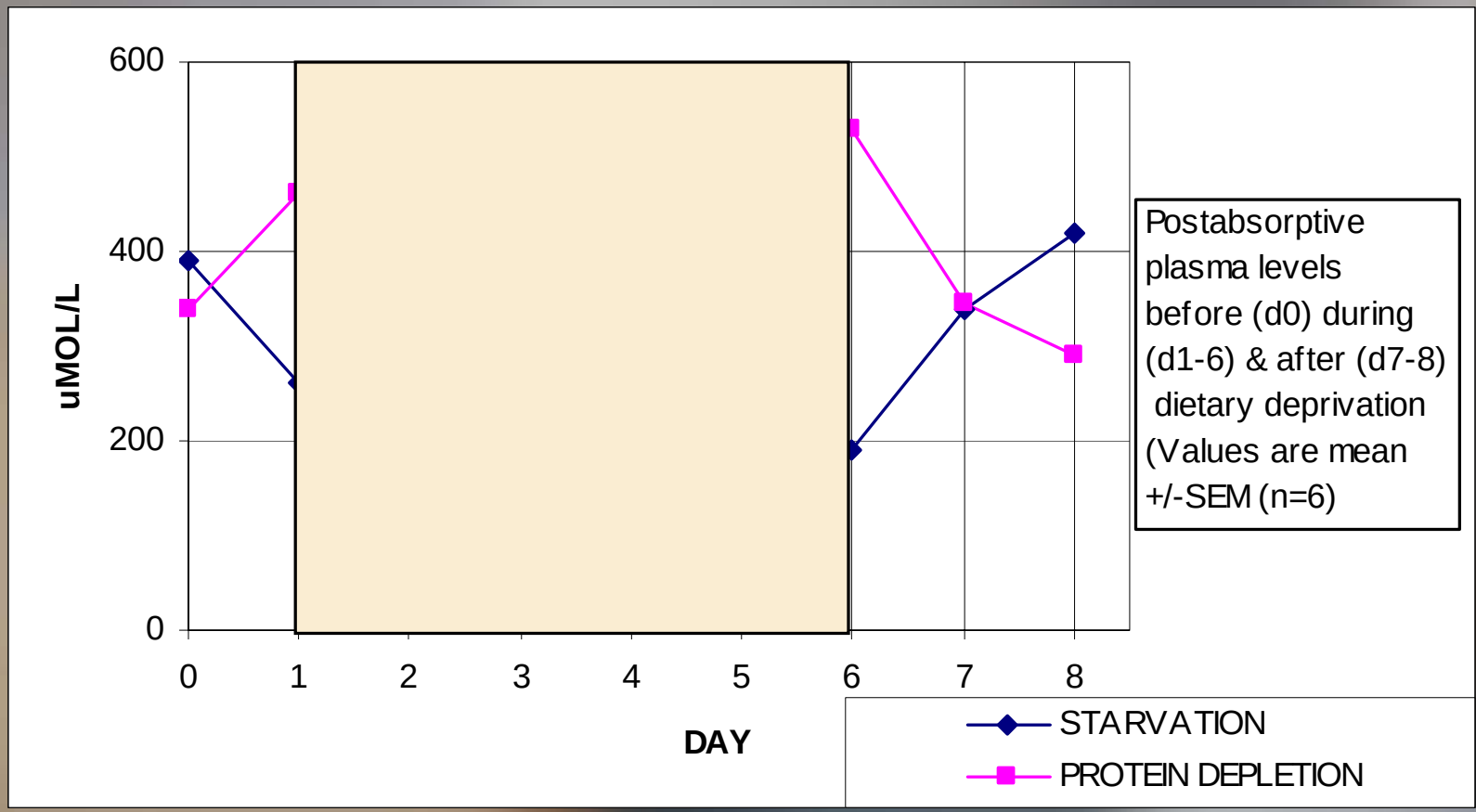


FIGURE 3 Plasma concentration of those amino acids which fell below baseline levels (day 0) during the course of prolonged fasting. Valine, isoleucine, and leucine which ultimately decreased are presented in Fig. 1. Tyrosine and phenylalanine which did not change significantly until day 40 are not shown 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



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 ~ $2^1/_2$ 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

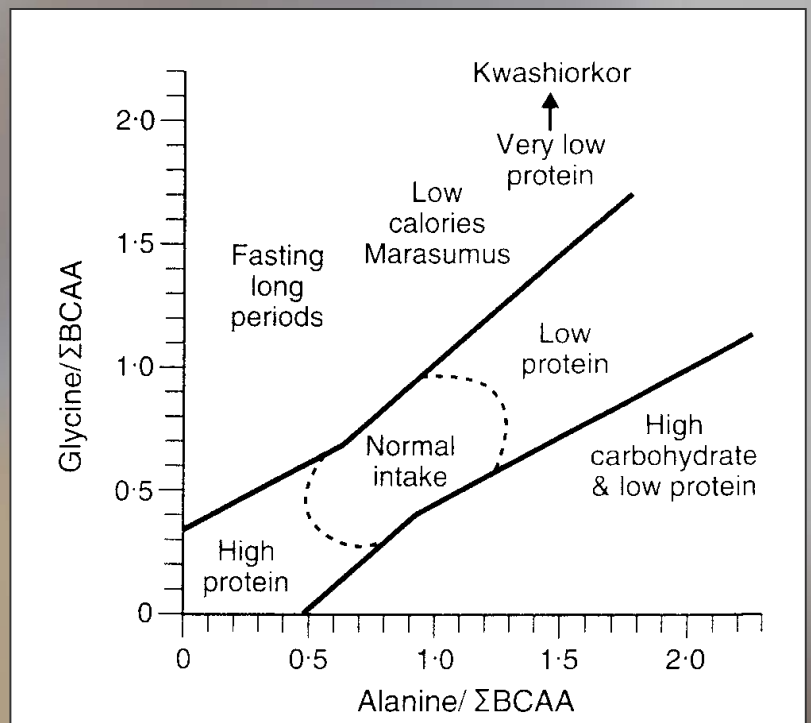


FIGURE 2. *'Ratiogram' of ala/(val + leu + ile) as indicator of calorie intake plotted against gly/(val + leu + ile) as indicator of protein intake. Derived from published and author's own data (see Ref. 4).*[4] *(Reproduced with permission from Amino Acids: Chemistry, Biology and Medicine.* In: Proceedings of 1st International Conference on Amino Acids, Vienna, 1989. *Vienna: Escom Science Publishers, 1990.)*

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

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

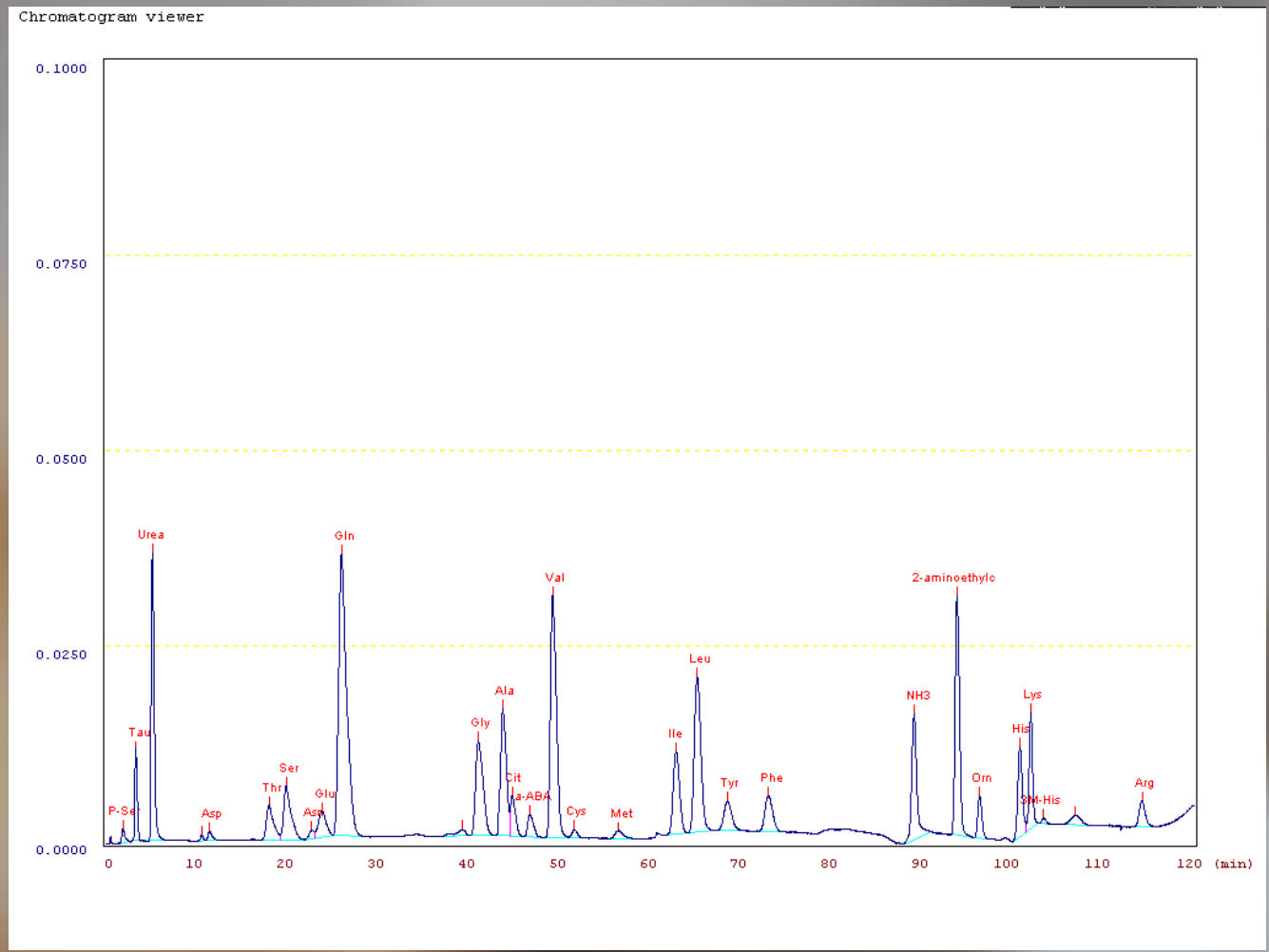
- Whole body physiological mechanisms
- Sample timing
- Availability control data
- Use of ratios
- Fed or fasted?
  - General population fasting samples probably better but not readily available
  - Monitoring pts with IEM may be better taken at a consistent time post feed

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.
Thank you

aa: ketosis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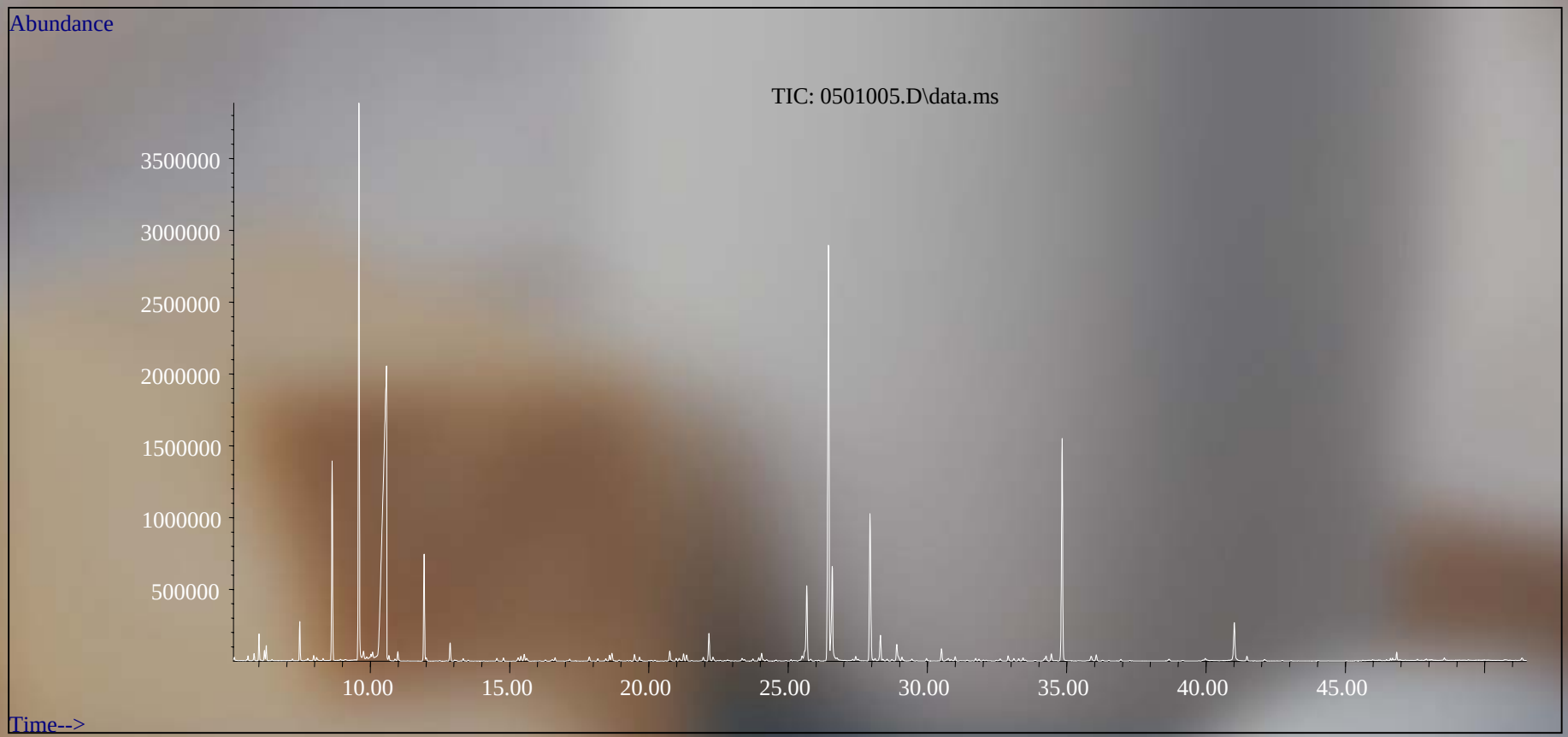
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Protein Turnover and Nitrogen Balance

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 macromolelcular 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

positive nitrogen balance — intake > excretion

negative nitrogen balance — excretion > intake

Positive Nitrogen balance

- growth of children
- pregnancy
- wound healing
- convalescing adult

Negative Nitrogen balance

- starvation
- malnutrition
- disease (burns, trauma, surgery)