Safety of Amino Acids as Essential Building Blocks of Human Nutrition

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A scientific NGO, an observer with CODEX

Our purpose:
• To help establish UPPER LIMITs for amino acid intake from foods or supplements.
• To promote the use of HIGH QUALITY amino acids

We:
• sponsor clinical safety research.
• Organize international Amino Acid Assessment Workshops (J. Nutr.) and other symposia
• interact with national regulatory bodies, WHO/FAO structures and the US/EU Pharmacopeia

Member Companies of ICAAS

1. Ajinomoto Co., Inc. Japan & USA
2. Evonik Rexim Germany
3. Omnichem Belgium
4. Kyowa Bio Co., Ltd. Japan
5. GlaxoSmithKline United Kingdom
6. Real Nutriceuticals Group Ltd. China
7. Otsuka Pharmaceutical Co. Ltd. Japan
8. Suntory Ltd. Japan
9. Meiji Dairies Corporation Japan
10. Kyowa Hakko Europe Germany
1. Prof. D. Bier (Baylor College of Medicine, Nutrition, USA)
2. Prof. S. Morris (Univ. Pittsburgh, Biochemistry, USA)
3. Prof. L. Cynober (Paris 5th Univ., Nutrition, France)
4. Prof. R. Elango (Univ. British Columbia, Canada)
5. Prof. M. Kadowaki (Niigata Univ., Nutrition, Japan)
6. Prof. P. Stover (Cornell Univ., USA)

### Why Amino Acids (1)?

Amino acids = an amino group and a carboxyl group

<table>
<thead>
<tr>
<th>Amino Acids</th>
<th>Essential Amino Acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycine</td>
<td>Valine</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>Tryptophan</td>
</tr>
<tr>
<td>Proline</td>
<td>Isoleucine</td>
</tr>
<tr>
<td>Serine</td>
<td>Arginine</td>
</tr>
<tr>
<td>Aspartic Acid</td>
<td>Histidine</td>
</tr>
<tr>
<td>Cysteine</td>
<td>Leucine</td>
</tr>
<tr>
<td>Glutamate</td>
<td>Threonine</td>
</tr>
<tr>
<td>Alanine</td>
<td>Phenylalanine</td>
</tr>
<tr>
<td>Glutamine</td>
<td>Lysine</td>
</tr>
<tr>
<td>Asparagine</td>
<td>Methionine</td>
</tr>
</tbody>
</table>

**Amino Acids differ from Proteins:**
- No allergenicity
- Endogenous substances present in a diet
- Efficient absorption
- Easy utilization
- Taste properties
- Cost-effective & environmentally-friendly production

### Why Amino Acids (2)?

Approximately 100,000 different proteins are made from 20 amino acids!

**Building the body**
- Keratin (nails, hair), collagen (skin), actin, myosin (muscle)

**Protecting the body**
- Immunoglobulin (IgG, etc.)

**Providing Components in blood**
- Albumin, hemoglobin...

**Controlling reactions**
- Enzymes, neurotransmitters, hormones...
Fermentation Products

- Glucose syrup (Fermentation broth)
- Glucose
- Metabolites (e.g., Amino acids, Organic acids)
- Alcohol (spirits)
- Byproducts
- Purified Crystalized Beer (e.g., Yeast extracts)

Safety risk of nutrients

- Incidence of adverse effects
- Harmfulness
- Breach of specifications
- Low purity
- Incidence of deficiency

Upper limits (UL) for amino acid use

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Classical Toxicology</th>
<th>Alternative Model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leucine</td>
<td>NOAEL (male rat) 3.3 g/kg/D</td>
<td>UL (human, 80 kg BW) 2.68 g/D</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>NOAEL (male rat) 1.6 g/kg/D</td>
<td>UL (human, 80 kg BW) 1.28 g/D</td>
</tr>
<tr>
<td>Valine</td>
<td>NOAEL (male rat) 3.2 g/kg/D</td>
<td>UL (human, 80 kg BW) 2.56 g/D</td>
</tr>
<tr>
<td>Lysine</td>
<td>NOAEL (male rat) 3.4 g/kg/D</td>
<td>UL (human, 80 kg BW) 2.72 g/D</td>
</tr>
<tr>
<td>Glutamine</td>
<td>NOAEL (male rat) 0.8 g/kg/D</td>
<td>UL (human, 80 kg BW) 0.64 g/D</td>
</tr>
<tr>
<td>Arginine</td>
<td>NOAEL (male rat) 3.3 g/kg/D</td>
<td>UL (human, 80 kg BW) Below average dietary intake!</td>
</tr>
</tbody>
</table>

Problems with Applying Classical Toxicology to Amino Acids

- NOAEL: No Observed Adverse Effect Level
- SF: Safety Factor
- ADI: Acceptable Daily Intake = UL

The highest level of daily nutrient intake that is likely to pose no risk of adverse effects to almost all individuals in the general population.
**Leucine intake from a diet**

Mean U.S. Intake: 5.9 g/day

95th Percentile U.S. Intake: 14.1 g/day

1988-1994 NHANES III, for all age/gender groups, food + supplements (DRIs: National Academies Press, 2001)

**Excess leucine intake**

No evidence of harm, no side effects. Max oxidative potential of leucine in a 80 kg human is 44 g/day

Elango et al., AJCN 2012; 96: 759-767

**Leucine target: muscle**

Muscle soreness induced by squat exercise (cm scale)

Protocol:
Subjects: Adult females (non-athletes)
Dose: 5g Leucine (15 min < exercise)
Method: subjective analogue scale

Shimomura et al., J. Nutr. 2006; 136: S529-S562

**Arginine intake from a diet**

Mean U.S. Intake: 4.2 g/day

95th Percentile U.S. Intake: 10.1 g/day

1988-1994 NHANES III, for all age/gender groups, food + supplements (DRIs: National Academies Press, 2001)

**Excess arginine intake (3 months study)**

No evidence of harm, no side effects found up to 30.0 g/day

Marchesini et al, Gastroenterology 2003; 124: 1792-1801

Wu et al., Amino Acids. 2016; 48(7): 1541-52
Tryptophan intake from a diet

Mean U.S. Intake: 0.9 g/day
95\textsuperscript{th} Percentile U.S. Intake: 2.1 g/day

1988-1994 NHANES III, for all age/gender groups, food + supplements
(DRIs: National Academies Press, 2001)

Excess tryptophan intake (3 months study)

No evidence of harm, no side effects found up to 5.0 g/day


Current Phase of ICAAS Clinical Trials

- Methionine ... finished
- Glutamate ... finished
- Glutamine, Lysine ... in preparation

ULTIMATE GOAL:
To establish safety framework for high quality amino acids .... “industry responsibility”


Specifications

<table>
<thead>
<tr>
<th>Breach of specifications</th>
<th>Incidence of deficiency</th>
<th>Incidence of adverse effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low purity</td>
<td>100%</td>
<td>0%</td>
</tr>
</tbody>
</table>

How to address specification issues?

Since 2009; ICAAS has been cooperating with the U.S. Pharmacopoeia Convention on the Food Chemical Codex specifications for amino acids
ICAAS & USP: “insoluble foreign matter”

The number of insoluble matter correlated to the frequency of samples with bacterial contamination.

Case of tryptophan

- A deadly disease (Eosinophilia Myalgia Syndrome) was caused in 1989 by 1 LOT of a tryptophan product from 1 manufacturer with poor quality controls
- Specifications were in compliance with FCC.

Current Stage of ICAAS Effort to Improve Amino Acid Specifications

- Working on USP monographs for BCAA
- Cooperating with Brazil ANVISA on specifications and adulteration issues

ULTIMATE GOAL:
To modernize amino acid monographs in both USP and FCC

Schneider-Helmert D, Spinweber CL. Psychopharmacology 1986; 89: 1-7
Fernstrom JD. J Nutr 2012; 142: 2235-445
Safety risk of nutrients with evolutionary presence in a human diet

- Breach of specifications
- Low purity
- Incidence of deficiency
- Incidence of adverse effects

Harmfulness vs. Intake

Protein & Amino Acid Requirements (WHO/FAO Expert Consultation)

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>1985</th>
<th>2007</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histidine</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>Leucine</td>
<td>14</td>
<td>39</td>
</tr>
<tr>
<td>Valine</td>
<td>10</td>
<td>26</td>
</tr>
<tr>
<td>Lysine</td>
<td>12</td>
<td>30</td>
</tr>
<tr>
<td>Methionine + Cysteine</td>
<td>13</td>
<td>15</td>
</tr>
<tr>
<td>Threonine</td>
<td>7</td>
<td>15</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>3.5</td>
<td>4</td>
</tr>
<tr>
<td>Phenylalanine + Tyrosine</td>
<td>14</td>
<td>20</td>
</tr>
</tbody>
</table>

The most wide-spread amino acid deficiencies

- **Cereal based diets**: Lysine
- **Maize based diets**: Tryptophan, Lysine, Methionine
- **General poor nutrition**: Leucine, Isoleucine, Valine

Results of the Lys fortification trials (3 months) conducted in 2000s

1. Growth improvement in children (Ghana, Syria, China, Pakistan)
2. Improvement of nutritional protein status and immune status (Pakistan, China, Ghana, Syria)
3. Decrease in diarrhoea incidence (Bangladesh, Syria, Ghana)
4. Improvement in mental health (long-term anxiety, Syria)
Ongoing fortification initiative ...

Public Private Partnership (West Africa)

Summary

Amino acids are a “case study” of food ingredients normally present in food/human body.

1. Essential and semi-essential amino acids seem to be safe at very high levels of intake, if specifications are appropriately controlled.

2. Deficiency of essential amino acids is one of the unrecognized nutritional problems. It affects especially growing children in economically underdeveloped regions.

I. Among staple foods, only cereal supply is marginally growing through imports
II. Meat/milk supplies are slightly declining
III. Cassava and yam supply grow only through surface expansion (unsustainable)