INTEGRATED PROTEIN METABOLISM

N. Deutz (US)
Integrated Protein Metabolism

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Integrated Protein Metabolism

• Introduction
• Measure protein metabolism in humans
• Protein anabolism
• Can we improve protein anabolism with specialized components
• Conclusions
The Impact of Dairy Protein Intake in Middle-Aged to Older Adults with or without existing Sarcopenia

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>Dairy protein Mean</th>
<th>SD</th>
<th>Total</th>
<th>Control Mean</th>
<th>SD</th>
<th>Total</th>
<th>Weight</th>
<th>Mean Difference IV, Random, 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alemán–Mateo et al. (20)</td>
<td>0.1</td>
<td>4.04</td>
<td>20</td>
<td>−0.7</td>
<td>4.19</td>
<td>20</td>
<td>9.4%</td>
<td>0.80 [−1.75, 3.35]</td>
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<tr>
<td>Alemán–Mateo et al. (26)</td>
<td>−0.3</td>
<td>5.94</td>
<td>49</td>
<td>−1</td>
<td>5.53</td>
<td>49</td>
<td>10.6%</td>
<td>0.70 [−1.57, 2.97]</td>
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<tr>
<td>Bauer et al. (21)</td>
<td>0.79</td>
<td>3.6</td>
<td>139</td>
<td>0.54</td>
<td>3.2</td>
<td>154</td>
<td>17.9%</td>
<td>0.25 [−0.53, 1.03]</td>
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<tr>
<td>Rondanelli et al. (22)</td>
<td>3.2</td>
<td>4.0379</td>
<td>69</td>
<td>−0.47</td>
<td>2.3427</td>
<td>61</td>
<td>16.3%</td>
<td>0.67 [2.55, 4.79]</td>
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<tr>
<td>Tieland et al. (24)</td>
<td>0.26</td>
<td>4.6</td>
<td>34</td>
<td>2.2</td>
<td>4.1</td>
<td>32</td>
<td>11.1%</td>
<td>−0.20 [−2.35, 1.95]</td>
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<tr>
<td>Verreijen et al. (28)</td>
<td>−1.09</td>
<td>3.93</td>
<td>93</td>
<td>−1.53</td>
<td>3.95</td>
<td>88</td>
<td>16.2%</td>
<td>0.44 [−0.71, 1.59]</td>
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<tr>
<td>Zhu et al. (30)</td>
<td>−1.09</td>
<td>3.93</td>
<td>93</td>
<td>−1.53</td>
<td>3.95</td>
<td>88</td>
<td>16.2%</td>
<td>0.44 [−0.71, 1.59]</td>
</tr>
<tr>
<td>Total (95% CI)</td>
<td></td>
<td></td>
<td>435</td>
<td>435</td>
<td></td>
<td></td>
<td>100.0%</td>
<td>0.84 [−0.24, 1.93]</td>
</tr>
</tbody>
</table>

Heterogeneity: Tau² = 1.55; Chi² = 33.97, df = 6 (P < 0.00001); I² = 82%
Test for overall effect: Z = 1.52 (P = 0.13)

Handgrip Muscle Strength

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>Dairy protein Mean</th>
<th>SD</th>
<th>Total</th>
<th>Control Mean</th>
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<th>Weight</th>
<th>Mean Difference IV, Random, 95% CI</th>
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</thead>
<tbody>
<tr>
<td>Alemán–Mateo et al. (20)</td>
<td>0.3</td>
<td>1.9</td>
<td>20</td>
<td>0.2</td>
<td>1.77</td>
<td>20</td>
<td>1.2%</td>
<td>0.10 [−1.04, 1.24]</td>
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<tr>
<td>Alemán–Mateo et al. (26)</td>
<td>0.26</td>
<td>2.62</td>
<td>49</td>
<td>−0.2</td>
<td>2.59</td>
<td>49</td>
<td>1.4%</td>
<td>0.20 [−0.83, 1.23]</td>
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<tr>
<td>Arnanor et al. (27)</td>
<td>0.6</td>
<td>1.2</td>
<td>66</td>
<td>0.5</td>
<td>0.8</td>
<td>75</td>
<td>11.5%</td>
<td>0.10 [−0.24, 0.44]</td>
</tr>
<tr>
<td>Bauer et al. (21)</td>
<td>0.25</td>
<td>0.68</td>
<td>124</td>
<td>0.08</td>
<td>0.68</td>
<td>135</td>
<td>34.2%</td>
<td>0.17 [0.00, 0.34]</td>
</tr>
<tr>
<td>Norton et al. (33)</td>
<td>0.27</td>
<td>0.59</td>
<td>31</td>
<td>−0.01</td>
<td>0.36</td>
<td>29</td>
<td>19.8%</td>
<td>0.28 [0.03, 0.53]</td>
</tr>
<tr>
<td>Tieland et al. (24)</td>
<td>0.1</td>
<td>1.13</td>
<td>31</td>
<td>0.1</td>
<td>1.13</td>
<td>31</td>
<td>4.6%</td>
<td>0.00 [−0.56, 0.56]</td>
</tr>
<tr>
<td>Verreijen et al. (28)</td>
<td>0.4</td>
<td>1.2</td>
<td>30</td>
<td>−0.5</td>
<td>2.1</td>
<td>30</td>
<td>2.0%</td>
<td>0.90 [0.03, 1.77]</td>
</tr>
<tr>
<td>Zhu et al. (30)</td>
<td>−0.03</td>
<td>0.67</td>
<td>93</td>
<td>0.03</td>
<td>0.75</td>
<td>88</td>
<td>25.4%</td>
<td>−0.06 [−0.27, 0.15]</td>
</tr>
<tr>
<td>Total (95% CI)</td>
<td></td>
<td></td>
<td>444</td>
<td>457</td>
<td></td>
<td></td>
<td>100.0%</td>
<td>0.13 [0.01, 0.26]</td>
</tr>
</tbody>
</table>

Heterogeneity: Tau² = 0.00; Chi² = 8.17, df = 7 (P = 0.32); I² = 14%
Test for overall effect: Z = 2.08 (P = 0.04)
Protein Requirements and Optimal Intakes in older adults: Are We Ready to Recommend More Than the Recommended Daily Allowance?

Increasing RDA/EAR will bring more older adults in a protein deficiency.
Integrated Protein Metabolism

- Introduction
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How to measure human protein metabolism with (stable) tracers

Measure appearance in plasma that relate to

- Essential amino acid → protein synthesis and breakdown

Need Mass Spectrometry to measure enrichment (TTR: tracer(tracee) ratio)

\[
TTR = \frac{\text{# tracer}}{\text{# tracee}} = \frac{\text{red dots}}{\text{black dots}} = 0.2
\]

Measuring protein kinetics after a meal

**Graph:**
- EAA concentration (µM) vs. Time (min)
- Blue dots: 14 g EAA mixture
- Green dots: 14 g TAA mixture

**Timeline:**
- Oral intake of amino acid mixture and L-[15N]-PHE
- Primed continuous IV infusion of L-[ring-2H5]-PHE and L-[ring-2H2]-TYR

**Legend:**
- t = -3 h
- t = 0 h
- t = 3 h

**Blood sampling (min):**
- -180 -60 -30 -15 0 15 30 45 60 90 120 150 180

**Summary:**
Measuring protein kinetics after a meal

Oral intake of amino acid mixture and L-[15N]-PHE

-15

\( t = 3 \text{ h} \)

Primed continuous IV infusion of L-[ring-2H5]-PHE and L-[ring-2H2]-TYR

-3 0 0 3 0 6 0 9 0 1 2 0 1 5 0 1 8 0

\( 0.0 \)

\( 0.06 \)

\( 0.07 \)

\( 0.08 \)

\( 0.09 \)

\( 0.10 \)

Time (min)

cTTR

L-[ring-2H3]-phenylalanine

\( \approx 14 \text{ g TAA mixture} \)

\( \approx 14 \text{ g EAA mixture} \)

\( -30 0 30 60 90 120 150 180 \)

A

L-[ring-2H2]-tyrosine

\( -30 0 30 60 90 120 150 180 \)

B

L-[ring-2H3]-tyrosine

\( -30 0 30 60 90 120 150 180 \)

C

L-[15N]-phenylalanine

\( -30 0 30 60 90 120 150 180 \)

D

Blood sampling (min)

-180 -60 -30 -15 0 15 30 45 60 90 120 150 180

Measuring protein kinetics after a meal

A

L-[^15]N-PHE

cTTR subject A
Integral of cTTR

B

L-[^15]N-PHE

cTTR subject A
Integral of cTTR
Nonlinear fit of integral

Measuring protein kinetics after a meal

**Net protein balance efficiency**

based on PHE hydroxylation derived from intravenous tracer

- Healthy
- COPD

**Net protein balance efficiency**

based on PHE hydroxylation derived from oral tracer

---

Critical analysis of the use of oral tracer/protein to calculate protein synthesis and breakdown

• Underestimation of the suppressive effect of dietary protein on protein breakdown, but possibly correct estimation of protein balance and anabolism

• Classic estimation protein balance (PS+Oxidation = PB+intake) probably acceptable for bolus feeding per 24h, not per meal as it is unclear when the end of anabolic response is present (4h?, 12h?)
Critical analysis of the use of oral tracer/protein to calculate protein synthesis and breakdown

• SIP feeding protocol is more reliable than a bolus feeding protocol to study the quality of protein
  – Bolus feeding protocol show more anabolism of Whey vs Casein protein, while sip feeding protocol and 8 weeks intervention show comparable anabolism (protein quality Whey and Casein is equal)

• Some estimation of protein digestion (and absorption) is necessary
Panel of isotopes measuring gut function (protein digestibility + absorption) and splanchnic extraction

\[
\text{Meal} + [^{15}\text{N}]\text{spirulina protein} + [^{1-13}\text{C}]\text{phenylalanine}
\]

\[\text{Colon}\]

\[\begin{align*}
[^{15}\text{N}]\text{peptides} & \rightarrow [^{15}\text{N}]\text{spirulina protein} \\
[^{15}\text{N}]\text{phenylalanine} & \rightarrow [^{1-13}\text{C}]\text{phenylalanine} \\
[^{15}\text{N}]\text{peptides} & \rightarrow [^{15}\text{N}]\text{phenylalanine} / [^{1-13}\text{C}]\text{phenylalanine} / [^{2}\text{H}_5]\text{phenylalanine} \leftarrow \text{Intravenous}
\end{align*}\]

\[\text{Mucosa cells}\]

\[\text{PEPT1}\]

\[\text{AA transporter}\]

Gut function: ratio \([^{15}\text{N}]\text{phe} / [^{1-13}\text{C}]\text{phe} = \%\text{ appearance} [^{15}\text{N}]\text{phe}\) from spirulina protein into circulation

Splanchnic extraction: ratio \([^{1-13}\text{C}]\text{phe} / [^{2}\text{H}_5]\text{phe} = \%\text{ appearance} [^{1-13}\text{C}]\text{phe}\) into circulation assuming no AA absorption problem

Disturbed gut function in Cystic Fibrosis after improving digestion with pancreatic enzymes

Principle is to use an enriched protein which can measure digestibility of every amino acid. We used mixture of EnsureHP and $^{15}$N-Spirulina protein that was given as 20-min sips.

Impairments in protein digestion and absorption attenuate the anabolic response to feeding in patients with Congestive Heart Failure

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Relation between intake of different types of protein and net protein anabolism

doi: 10.1016/j.clnu.2013.06.014


Jonker et al Metabolism. 2017;69:120-9
doi: 10.1016/j.metabol.2016.12.010


\[ y = -0.7138 + 0.7987 \times x, \ R^2 = 0.711, \ p < 0.001 \]

\[ y = 1.052 + 0.8202 \times x, \ R^2 = 0.83, \ p < 0.001 \]

\[ y = 2.186 + 0.7936 \times x, \ R^2 = 0.893, \ p < 0.001 \]
Apparently no upper limit anabolic response and the role of gut labile protein pool

Protein intake in one meal

\[ y = 13.18x + 0.6743 \]
\[ R^2 = 0.765 \]
\[ p < 0.0001 \]

Intake 30 gram protein in meal

Measure anabolic response at whole body level

The muscle anabolic response is not only determined by protein synthesis but by the balance between protein synthesis and breakdown and the gut anabolic response

Do older adults have anabolic resistance?

Define anabolic resistance as the reduced stimulation of muscle protein synthesis to a given dose of protein/amino acids.


No muscle anabolic resistance to EAA in healthy older adults


Anabolic Threshold and Capacity

y = 0 = anabolic threshold
slope = anabolic capacity

Dietary protein intake

Net protein balance

y-axes

x-axes
Anabolic Threshold and Capacity

A
HEALTHY

-10
0
10
20
30
40
50
PHE intake (μmol PHE × kg ffm⁻¹ × h⁻¹)
Net protein balance (μmol PHE × kg ffm⁻¹ × h⁻¹)

B
COPD

-10
0
10
20
30
40
50
PHE intake (μmol PHE × kg ffm⁻¹ × h⁻¹)
Net protein balance (μmol PHE × kg ffm⁻¹ × h⁻¹)

Anabolic Threshold and Capacity

Anabolic threshold

Anabolic capacity

PHE intake (μmol PHE × kg ffm⁻¹ × h⁻¹)

HEALTHY  COPD

B

1.00

0.95

0.90

0.85

HEALTHY  COPD

24h balance studies in healthy subjects
No effect of pattern, only of amount of protein intake

• 20 healthy older adult subjects (52 - 75 y)
• 4 groups
  – protein intake of 0.8g (1*RDA)
  – protein intake of 1.5g (2*RDA)
  – and uneven [U: 15/20/65%] or even distribution (E: 33/33/33%) of protein intake

Changes in whole body protein turnover (g·750 min⁻¹)
Protein intake distribution pattern does not affect anabolic response, lean body mass, muscle strength or function over 8 weeks in older adults.

There were no differences in any of the functional outcomes between the EVEN and the UNEVEN after 8 weeks of intervention.

EVEN = 33% every meal. ONEVEN = 15/20/65% per meal
The anabolic response to a large protein meal

Protein meal: MP (40g), HP (70g) after X: exercise or R: rest

Increase whole body anabolism, but no change in muscle protein synthesis

Kim et al. AJP. 2016;310(1):E73-E80. doi: 10.1152/ajpendo.00365.2015
Dose-dependent increase of whole body protein balance and muscle protein synthesis with protein intake during exercise recovery in older adult men

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Meal whole body protein anabolism in healthy older adults


Plasma Leucine

Net protein anabolism

30 g hCAS ± 15 g CHO ± 3.5 g LEU
Leucine co-ingestion augments the muscle protein synthetic response to the ingestion of 15 g protein following resistance exercise in older men.
The presence of leucine rather than amount is most crucial for anabolism in older women.

<table>
<thead>
<tr>
<th></th>
<th>LEAA (1.5 g), g</th>
<th>LEAA (6 g), g</th>
</tr>
</thead>
<tbody>
<tr>
<td>l-Leucine</td>
<td>0.6</td>
<td>2.4</td>
</tr>
<tr>
<td>l-Isoleucine</td>
<td>0.16</td>
<td>0.64</td>
</tr>
<tr>
<td>l-Valine</td>
<td>0.165</td>
<td>0.66</td>
</tr>
<tr>
<td>l-Threonine</td>
<td>0.14</td>
<td>0.56</td>
</tr>
<tr>
<td>l-Lysine</td>
<td>0.25</td>
<td>1.0</td>
</tr>
<tr>
<td>l-Methionine</td>
<td>0.05</td>
<td>0.2</td>
</tr>
<tr>
<td>l-Histidine</td>
<td>0.025</td>
<td>0.1</td>
</tr>
<tr>
<td>l-Phenylalanine</td>
<td>0.1</td>
<td>0.4</td>
</tr>
<tr>
<td>l-Tryptophan</td>
<td>0.01</td>
<td>0.04</td>
</tr>
</tbody>
</table>

The presence of leucine rather than amount is most crucial for anabolism in older women. All groups displayed similar MPS in response to feeding (±resistance exercise) suggesting only a small dose of leucine and/or EAA are required.
Excellent relation between intake essential amino acids and net protein Synthesis

1. Quality of Supplement related to amount of EAA
2. Leucine does not seems to make the EAA more anabolic

<table>
<thead>
<tr>
<th>Essential</th>
<th>Conditionally Non-Essential</th>
<th>Non-Essential</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histidine</td>
<td>Arginine</td>
<td>Alanine</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>Asparagine</td>
<td>Aspartate</td>
</tr>
<tr>
<td>Leucine</td>
<td>Glutamine</td>
<td>Cysteine</td>
</tr>
<tr>
<td>Methionine</td>
<td>Glycine</td>
<td>Glutamate</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>Proline</td>
<td>Serine</td>
</tr>
<tr>
<td>Threonine</td>
<td>Tryptophan</td>
<td>Tyrosine</td>
</tr>
<tr>
<td>Valine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lysine</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Acute effect of HMB on muscle protein synthesis and breakdown

Protein Synthesis

Protein breakdown

Change in lean mass after bedrest

HMB or HMB-containing supplements and its impact on skeletal muscle mass and physical function in clinical practice: a systematic review and meta-analysis

Forest plot for the effect of HMB or supplements containing HMB on change in muscle mass

Forest plot showing the effect of HMB or supplements containing HMB on absolute strength

Older (at risk) malnourished adults (mean 78y).
- Intervention HP-HMB: 700 kcal, 40 g protein, 3.0 g HMB vs Placebo
- Nutrition intervention increased energy (22>28 kcal/kg/day) and protein intake (1.0>1.5 g/kg/day)
- At 30 days, 77% of expected intake.
The Effect of early nutritional support on Frailty, Functional Outcomes, and Recovery of malnourished medical inpatients Trial (EFFORT)

Older (at risk) malnourished adults (mean 72y). Nutrition intervention aimed to calculated EE and protein intake of 1.2-1.5 g/day. Day10 - EE (54%→79%), Protein (55→76%)

Comparison of 30 day Mortality of large Nutritional Intervention Trials

<table>
<thead>
<tr>
<th>Trial</th>
<th>Control</th>
<th>Intervention</th>
<th>% Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>NOURISH (2016)</td>
<td>6.2%</td>
<td>2.9%</td>
<td>47%</td>
</tr>
<tr>
<td>EFFORT (2019)</td>
<td>9.9%</td>
<td>7.2%</td>
<td>27%</td>
</tr>
</tbody>
</table>
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Integrated Protein Metabolism

• Stable tracer methods give essential information about the digestion and use of dietary protein

• There is a linear relationship between the amount of protein in the meal and net anabolism
  – Even at high protein content in the meal
Integrated Protein Metabolism

• On a whole body level, anabolic resistance is uncommon in older adults and with disease
  – Reduced protein digestion could play a role

• Specialized components can improve protein anabolism
  – HMB seems to be more promising than Leucine