Effect of Intake of Different Dietary Protein Sources on Plasma Amino Acid Profiles at Rest and After Exercise

Louise M. Burke, Julie A Winter, David Cameron-Smith, Marc Enslen, Michelle Farnfield, and Jacques Decombaz

The authors undertook 2 crossover-designed studies to characterize plasma amino acid (AA) responses to the intake of 20 g of protein. In Study 1, 15 untrained and overnight-fasted subjects consumed 20 g protein from skim milk, soy milk, beefsteak, boiled egg, and a liquid meal supplement. In Study 2, 10 fasted endurance-trained subjects consumed 20 g protein from a protein-rich sports bar at rest and after a 60-min submaximal ride. Plasma AA concentrations were measured immediately before and for 180 min after food ingestion using a gas-chromatography flame-ionization detection technique. A pharmacokinetic analysis was undertaken for profiles of total AAs (TAA), essential AAs, branched-chain AAs (BCAA), and leucine. Although area-under-the-curve values for plasma TAA were similar across protein sources, the pattern of aminoacidemia showed robust differences between foods, with liquid forms of protein achieving peak concentrations twice as quickly after ingestion as solid protein-rich foods (e.g., ~50 min vs ~100 min) and skim milk achieving a significantly faster peak leucine concentration than all other foods (~25 min). Completing exercise before ingesting protein sources did not cause statistically significant changes in the pattern of delivery of key AAs, BCAAs, and leucine apart from a 20–40% increase in the rate of elimination. These results may be useful to plan the type and timing of intake of protein-rich foods to maximize the protein synthetic response to various stimuli such as exercise.

Keywords: leucine, protein consumption, aminoacidemia

Maximizing rates of muscle protein synthesis is an important nutritional goal for athletes. An increase in plasma amino acid (AA) concentrations is a potent stimulator of muscle protein synthesis (Bohé, Low, Wolfe, & Rennie, 2003), while exercise further enhances this effect (Burd, Tang, Moore, & Phillips, 2009). This stimulation of muscle protein synthesis is dose-responsive and saturable; when dietary protein is consumed in excess of the rate at which it can be incorporated into tissue protein, it stimulates oxidation (Moore et al., 2009). Only essential AAs (EAAs) are required for muscle protein synthesis (Tipton, Ferrando, Phillips, Doyle, & Wolfe, 1999; Tipton, Gurkin, Matin, & Wolfe, 1999), with the branched-chain AAs (BCAAs), particularly leucine, acting as regulators of the mTOR-signaling cascade (Kimball & Jefferson, 2006), as well as providing substrate for protein synthesis. Studies of both acute (Tang, Moore, Kujbida, Tarnopolsky, & Phillips, 2009; Tipton et al., 2001) and chronic feeding after resistance exercise (Esmarck et al., 2001; Wilkinson et al., 2007) show different outcomes according to the type and timing of consumption of high-quality protein sources, even when the same amount of protein is consumed. These differences are potentially caused by divergent characteristics of the aminoacidemia caused by different protein-intake protocols in temporal relationship to the exercise stimulus.

The pattern of AA delivery into the plasma after ingestion of a protein-rich food is dependent on its AA composition and rate of digestion. Some researchers have identified the concept of “fast” and “slow” proteins that are characterized by either a rapid and transient increase in plasma AAs or a lower but more sustained response (Fruebeck, 1998). This response mostly reflects differences in the rates of protein digestion and is best demonstrated by the two main milk proteins: The whey subfraction is a quickly digested protein, while casein has a retarded digestion rate due to clotting in the stomach. These properties have been exploited to achieve differences in whole-body protein balance in several populations under resting conditions (Boirie et al., 1997; Dangin et al., 2001; Dangin et al., 2003). Studies that are more recent have attempted to examine differential effects of consuming fast- and slow-type proteins in relation to exercise (Tang et al., 2009; West et al., 2011). Although these studies have used proteins or protein subfractions that are food derived, the findings are hard to apply to the complex mixture of everyday foods or specialized sports...
foods that make up the diets of free-living athletes and other populations.

The glycemic index was developed to rank the effects of different carbohydrate-rich foods on blood glucose concentrations (Wolever, 1990) and has been used to plan meals or diets to achieve a desired blood glucose response across a range of areas of clinical and sports nutrition. Similar information on the postprandial AA responses to a variety of protein-rich foods could be used to plan the type, timing, and amount of foods consumed around training sessions or events, once profiles for the ideal AA response in relation to exercise are established.

Accordingly, the aim of this study was to characterize postprandial AA responses that might influence protein synthesis (timing, peak, and pattern of concentrations) to a standardized amount of protein from a range of common protein-rich foods. We chose a 20-g protein serve, easily provided by a range of common foods and nutritional supplements and known to produce substantial increases in plasma AA concentrations and optimum fractional synthetic rates of muscle protein after resistance exercise (Moore et al., 2009). Because this information might be used to plan meals around exercise activities, a secondary question addressed by this study was whether AA responses to the consumption of protein-rich foods could be influenced by a prior bout of exercise, which may alter gastric-emptying or blood-flow responses. Since there has been some recent research on the effect of resistance exercise on aminoadenemia after intake of AAs (Mero, Leikas, Knuutinen, Hulim, & Kovanen, 2009), and because intake of protein after endurance exercise has also been shown to promote superior protein synthesis (Levenhagen et al., 2002; Levenhagen et al., 2001), we chose to investigate endurance exercise.

Methods

We undertook two studies, one involving sedentary subjects under resting conditions and the other involving endurance-trained subjects who undertook an exercise trial and a resting trial. Sample sizes were chosen after power estimation of the likely difference in area-under-the-curve (AUC) calculations for plasma AA concentrations from previous studies (van Loon, Saris, Verhagen, & Wagenmakers, 2000). Experimental procedures were approved by the Deakin University Human Research Ethics Committee, and each subject provided written consent after being informed of the purpose, protocols, and risks of the relevant study.

Study 1 Protocol

Subjects and Study Overview. We recruited 15 healthy untrained male and female subjects (24 ± 4 years, 69 ± 13 kg, BMI = 22.6 ± 2.0; M ± SD). We used a single-shot crossover design to characterize plasma AA responses to five different protein-containing foods representing solid and liquid forms, animal and vegetable protein, and everyday foods versus specialized sports foods. Each subject received the same portion of the test foods (equivalent to 20 g protein), and treatments were received in a counterbalanced order, with at least 6 days separating trials. Although each food provided an equivalent protein serve, the type of protein and its AA composition varied, as did the fat, carbohydrate, and water content and food texture. Consequently, postprandial AA responses were expected to differ as a result of these variations and differences in protein digestion.

Food Treatments. Serving sizes that supplied 20 g of protein for each food source were calculated using a dietary-analysis computer package (Foodworks Professional v3.01, Xyris Software, Sydney, Australia) based on information from the Australian database of food composition sources (Nuttab 95, Canberra, Australia), as well as specific information from food manufacturers. The foods, obtained from standardized sources and prepared with a standardized protocol on the morning of the trial, are summarized in Table 1.

Experimental Protocol. Subjects undertook each trial at the same time of the day, having fasted overnight and abstained from alcohol, caffeine, and exercise for the 24 hr beforehand. A 22G indwelling catheter was placed into a hand vein for blood collection; samples were arterialized by having subjects rest their hand in a heat blanket. After a 5-ml blood sample was collected (fasting), subjects were instructed to consume their test food in less than 15 min. To standardize gastric emptying, water was not available until 60 min after food consumption. Further samples were taken at 20-min intervals after the commencement of the meal for 180 min, with the cannula being flushed after each occasion with saline. Withdrawn blood was transferred into 5-ml lithium heparin blood-collection tubes and placed on ice. Within 30 min of final sample collection, blood samples were centrifuged at 4,500 rpm for 5 min at 4 °C. The plasma supernatant was separated, and samples stored in 2-ml Eppendorf tubes at –80 °C until further analysis.

Study 2 Protocol

Subjects. We recruited 10 healthy endurance-trained men and women from a local pool of cyclists and triathletes (25 ± 6 years, 67 ± 6 kg, BMI = 21.6 ± 0.9 kg/m², VO2max = 61.9 ± 6.3 ml · kg⁻¹ · min⁻¹, peak power output = 327 ± 54 W). Inclusion criteria included engagement in regular cycling exercise (>5 hr/week) and the ability to cycle for 1 hr at ~70% VO2max.

Study Overview. We used a single-shot crossover design to compare the plasma AA responses to a single protein source providing 20 g of protein, at rest or after 1 hr of submaximal exercise. Treatments were received in a counterbalanced order, with at least 6 days separating trials. The 20-g protein serve was provided in the form of a protein-rich sports bar (PowerBar Protein Plus bar, Nestle Australia), of AA composition similar to that of the liquid meal supplement summarized in Table 1, but
with a macronutrient composition of 27 g carbohydrate, 6 g fat, and 24 g protein in a 78-g bar.

**Preliminary Testing.**
Before the experimental trials, each subject undertook an incremental cycling test to exhaustion on an electronically braked cycle ergometer (Lode, Groningen, The Netherlands) to determine peak power output as described previously by Hawley and Noakes (1992). This value was used to determine the power output corresponding to ~70% of each subject’s VO₂peak (63% of peak power output) for use in the experimental trials. Subjects then completed a 30-min familiarization bout at this power output to confirm their ability to undertake prolonged cycling on this ergometer and to determine an approximation of their sweat rate.

**Experimental Protocol.** Subjects undertook two trials, each following the same preparation and presentation at the laboratory as described in Study 1, while also refraining from exercise for 24 hr pretrial. On the rest trial (Trial A), once the fasting blood sample had been
collected, they consumed the test meal (Protein Plus Sports Bar) within 10–15 min and were monitored for the subsequent 180 min as previously described. For the exercise trial (Trial B), subjects arrived an hour earlier to complete their cycling task. They voided their bladder and weighed themselves in minimal clothing before completing a 60-min bout of exercise on the cycling ergometer, at the workload corresponding to 63% of their peak power output. To avoid substantial differences in fluid status at the commencement of the test meal, they drank a volume of water equivalent to their predicted hourly sweat rate predicted during the bout. After completing the cycling, subjects reweighed (towel-dried and clothed as for the pretrial weigh-in), provided a blood sample, and commenced the test meal. Blood collection and provision of fluids were undertaken as for Study 1.

**Analytical Protocols**

Plasma AA concentrations were measured using gas-chromatography analysis with the EZ: Fast Free (Physiological) AA gas chromatography analysis kit (Phenomenex Inc., Torrance, CA). Briefly, samples were prepared according to the manufacturer’s specifications with the addition of an internal standard (norvaline, prepared according to the manufacturer’s specifications (Phenomenex Inc., Torrance, CA)). Prepared samples were loaded onto an 8200 Autosampler (Varian Analytical Instruments, CA), from which 2.0 μL of each sample was injected and analyzed on a CP-3800 gas chromatograph, using a Zebron ZB-PACC column (10 × 0.25 mm). Plasma AA concentrations were expressed as increases above baseline values (at 0 min) as per a pharmacokinetics approach. Absolute values for baseline of plasma leucine are provided, to allow further analysis should a threshold concentration for triggering muscle protein synthesis be identified (Tang et al. 2009).

Results were obtained for plasma concentrations of valine, leucine, isoleucine, threonine, methionine, phenylalanine, lysine, histidine, alanine, glycine, serine, proline, aspartate/aspartic acid, glutamic acid, tyrosine, and cystine. Measurement of concentrations of ornithine, asparagine, glutamine, arginine, and tryptophan were not usable. These concentrations were expressed in terms of total AA (TAA; all AAs above), EAAs (valine, leucine, isoleucine, threonine, methionine, phenylalanine, lysine, and histidine), BCAAs (valine, leucine, and isoleucine), and leucine alone. Plasma glucose concentrations were measured using an automated method (EML-105, Radiometer, Copenhagen, Denmark). Insulin concentrations were determined using a solid-phase, two-site chemiluminescent immunometric assay (Immulite Diagnostic Products Corp., Los Angeles, CA).

**Statistical Procedures**

A pharmacokinetic analysis was done with Kinetics software (Version 4.1). A noncompartmental analysis was made of the following kinetics parameters: partial and total AUC (AUC$_{0-180\text{ min}}$ and AUC$_{0-\text{inf}}$), observed maximum concentration (C$_{\text{max obs}}$), time corresponding to maximal concentration (T$_{\text{max obs}}$), the elimination rate constant (Ke), and half-life time of elimination (T$_{\text{half}}$). This analysis was carried out for TAAs, EAAs, and BCAAs, as well as leucine. The effects in Study 1 were differences in parameters due to the five dietary protein sources, while in Study 2, the effects were differences in parameters due to exercise or rest. Parameters of secondary outcome were the C$_{\text{max obs}}$, T$_{\text{max obs}}$ and AUC from plasma kinetics of glucose and insulin. The AUC was calculated as area over baseline, where the baseline was subtracted from subsequent values and negative values were taken as zero.

Statistical analyses were undertaken using SAS software (version 8.2). According to normality of data distribution (skewness and kurtosis tests), the nonparametric test (Wilcoxon’s signed-rank test) was used for nonnormal distribution and the linear mixed model was used for normal distribution. A few data were normalized with logarithm or square-root transformation. A linear mixed model was applied, using treatment (Study 1) or group (Study 2) and period as fixed effects and subject as random effect. Differences in absolute leucine concentrations at baseline between treatment groups were assessed within each study using a standard two-way (Subject × Treatment) analysis of variance. In Study 1, Tukey-Kramer adjustments were applied to the p values for differences between the two treatments. Otherwise, the rejection level in statistical tests was equal to 5%.

**Results**

**Study 1**

Substantial differences in blood AA responses to the intake of the various protein-rich foods were observed (Figure 1). The plasma kinetics of TAA, EAA, BCAA, and leucine profiles are presented in Tables 2–5. With regard to TAAs, post hoc testing failed to detect differences in partial and total AUC values between the five treatments (Table 1). Maximal TAA concentrations with skim milk and liquid meal supplement were higher than with soy milk, steak, and eggs (p < .05). Time to reach C$_{\text{max obs}}$ was faster with liquid treatments (skim milk, soy milk, and liquid meal) than with solid foods (eggs and steak; p < .05). Ke of the liquid meal was higher than that of steak (p = .01). T$_{\text{half}}$ did not differ between treatments. AUC$_{0-180\text{ min}}$ and AUC$_{0-\text{inf}}$ for plasma EAA did not differ between the five treatments (Table 3). The maximal EAA concentration achieved by skim milk was higher than that of soy milk and eggs (p = .002 and p = .02, respectively). T$_{\text{max obs}}$ of liquid treatments was lower than with treatments with solid food (p < .0001). Ke of the liquid meal was higher than that of steak (p = .007). T$_{\text{half}}$ was not different between treatments.

In the case of BCAAs, AUC$_{0-180\text{ min}}$ of soy milk was lower than that of the liquid meal (p = .03). AUC$_{0-\text{inf}}$ of eggs was higher than that of soy milk and liquid meal treatments (p = .01; Table 4). Maximal BCAA...
Burke et al. concentration (Cmax obs) achieved by skim milk was higher than with soy milk or steak (p = .0002 and p = .008, respectively). Time to reach maximal concentration (Tmax obs) of liquid treatments was more rapid than that of solid treatments (p < .05). Ke and Thalf were not different between treatments.

Plasma kinetics of leucine profiles are presented in Table 5, along with the absolute values for baseline concentrations. Plasma leucine concentration at baseline did not differ between treatments (p = .30) and was equal to 13.6 ± 2.8 μg/ml (CV 21%, n = 70) overall. AUC0–180 min and AUC0–inf are not significantly different between the 5 treatments. Cmax obs: A > C (p < .0001); A > D (p = .0003); E > C (p = .0001); E > D (p = .002); Tmax obs: A < C and A < D (p < .0001); B < C (p = .004); B < D (p = .001); E < C (p = .002); E < D (p = .0005). Ke: E > C (p = .01).

**Figure 1** — Plasma amino acid (AA) concentration changes (μg/ml) over 3 hr after the intake of portions of skim milk, soy milk, grilled beef, boiled eggs, and a liquid meal supplement providing 20 g protein, with individual profiles for total AAs (TAA), essential AAs (TAA), branched-chain AAs (BCAA), and leucine. Pharmacokinetics of these profiles are presented in Tables 2–5.

**Table 2** Kinetics Parameters of Total Amino Acid Concentrations After Ingestion of Different Protein-Rich Foods (M ± SD), N = 13–15

<table>
<thead>
<tr>
<th>Treatment</th>
<th>AUC0–180 min</th>
<th>AUC0–inf</th>
<th>Cmax obs (μg/ml)</th>
<th>Tmax obs (min)</th>
<th>Ke (1/min)</th>
<th>Thalf (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A: skim milk</td>
<td>12,196 ± 4,127</td>
<td>13,911 ± 4,917</td>
<td>168 ± 59</td>
<td>43 ± 33</td>
<td>0.071 ± 0.208</td>
<td>29 ± 23</td>
</tr>
<tr>
<td>B: soy milk</td>
<td>9,717 ± 2,823</td>
<td>15,986 ± 10,748</td>
<td>97 ± 28</td>
<td>56 ± 21</td>
<td>0.014 ± 0.008</td>
<td>92 ± 114</td>
</tr>
<tr>
<td>C: steak</td>
<td>8,877 ± 4,285</td>
<td>11,826 ± 8,819</td>
<td>88 ± 32</td>
<td>96 ± 31</td>
<td>0.012 ± 0.006</td>
<td>54 ± 64</td>
</tr>
<tr>
<td>D: eggs</td>
<td>8,082 ± 3,132</td>
<td>8,845 ± 3,626</td>
<td>101 ± 36</td>
<td>101 ± 33</td>
<td>0.016 ± 0.005</td>
<td>30 ± 18</td>
</tr>
<tr>
<td>E: liquid meal</td>
<td>12,355 ± 6,716</td>
<td>14,474 ± 8,246</td>
<td>159 ± 52</td>
<td>55 ± 19</td>
<td>0.021 ± 0.011</td>
<td>35 ± 29</td>
</tr>
</tbody>
</table>

Treatment effect p = .02 .03 <.0001 <.0001 <.05 .06

Note. AUC = area under the curve; AUC0–180 min = partial AUC; AUC0–inf = total AUC; Cmax obs = observed maximum concentration; Tmax obs = time corresponding to maximal concentration; Ke = elimination-rate constant; Thalf = half-life time of elimination. AUC0–180 min and AUC0–inf are not significantly different between the 5 treatments. Cmax obs: A > C (p < .0001); A > D (p = .0003); E > C (p = .0001); E > D (p = .002); Tmax obs: A < C and A < D (p < .0001); B < C (p = .004); B < D (p = .001); E < C (p = .002); E < D (p = .0005). Ke: E > C (p = .01).
concentration (T_{max \ obs}) of liquid treatments was shorter than with solid treatments (p < .001), with the liquid meal achieving a faster maximal concentration than soy milk, steak, and eggs (p < .05) and skim milk being faster than all other treatments (p < .05). Ke and T_{half} were not different between treatments.

Plasma glucose and insulin responses to the different protein-rich foods are presented in Figure 2. The AUC_{0–180 min} of glucose concentrations after the intake of the liquid meal was higher than with other treatments (p = .001). The AUC_{0–inf} of the liquid meal was significantly higher than that of steak (p = .009). The maximal plasma glucose concentrations after liquid treatments were higher than that achieved by solid foods (skim milk > steak, p = .002, and skim milk > eggs, p = .0002; soy milk > steak, p = .04, and soy milk > eggs, p = .004; liquid meal > steak, p = .02).

### Table 3  Kinetics Parameters of Essential Amino Acid Concentrations After Ingestion of Different Protein-Rich Foods (M ± SD), N = 13–15

<table>
<thead>
<tr>
<th>Treatment</th>
<th>AUC_{0–180 min}</th>
<th>AUC_{0–inf}</th>
<th>C_{max \ obs} (μg/ml)</th>
<th>T_{max \ obs} (min)</th>
<th>Ke (1/min)</th>
<th>T_{half} (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A: skim milk</td>
<td>6,800 ± 1,986</td>
<td>7,861 ± 2,772</td>
<td>93 ± 28</td>
<td>31 ± 17</td>
<td>0.017 ± 0.011</td>
<td>38 ± 24</td>
</tr>
<tr>
<td>B: soy milk</td>
<td>5,599 ± 1,217</td>
<td>8,506 ± 5,508</td>
<td>54 ± 12</td>
<td>51 ± 15</td>
<td>0.014 ± 0.009</td>
<td>80 ± 75</td>
</tr>
<tr>
<td>C: steak</td>
<td>6,517 ± 1,743</td>
<td>9,748 ± 3,616</td>
<td>75 ± 26</td>
<td>100 ± 29</td>
<td>0.010 ± 0.007</td>
<td>69 ± 51</td>
</tr>
<tr>
<td>D: eggs</td>
<td>6,504 ± 1,542</td>
<td>7,835 ± 1,501</td>
<td>64 ± 13</td>
<td>104 ± 32</td>
<td>0.015 ± 0.013</td>
<td>41 ± 15</td>
</tr>
<tr>
<td>E: liquid meal</td>
<td>6,903 ± 3,340</td>
<td>8,152 ± 4,259</td>
<td>80 ± 29</td>
<td>47 ± 27</td>
<td>0.016 ± 0.006</td>
<td>46 ± 21</td>
</tr>
</tbody>
</table>

Treatment effect | p   | NS | <.05 | <.05 | <.05 | NS |

Note. AUC = area under the curve; AUC_{0–180 min} = partial AUC; AUC_{0–inf} = total AUC; C_{max \ obs} = observed maximum concentration; T_{max \ obs} = time corresponding to maximal concentration; Ke = elimination-rate constant; T_{half} = half-life time of elimination. AUC_{0–180 min} and AUC_{0–inf} are not significantly different between the 5 treatments. C_{max \ obs}: A > B (p = .002); A > D (p = .02); T_{max \ obs}: A, B, E < C, D (p < .0001); Ke: E > C (p = .007).

### Table 4  Kinetics Parameters of Branched-Chain Amino Acid Concentrations After Ingestion of Different Protein-Rich Foods (M ± SD), N = 13–15

<table>
<thead>
<tr>
<th>Treatment</th>
<th>AUC_{0–180 min}</th>
<th>AUC_{0–inf}</th>
<th>C_{max \ obs} (μg/ml)</th>
<th>T_{max \ obs} (min)</th>
<th>Ke (1/min)</th>
<th>T_{half} (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A: skim milk</td>
<td>3,771 ± 1,129</td>
<td>4,295 ± 1,667</td>
<td>49 ± 15</td>
<td>46 ± 32</td>
<td>0.014 ± 0.010</td>
<td>28 ± 114</td>
</tr>
<tr>
<td>B: soy milk</td>
<td>2,840 ± 657</td>
<td>3,679 ± 1,424</td>
<td>30 ± 6</td>
<td>56 ± 19</td>
<td>0.016 ± 0.007</td>
<td>38 ± 27</td>
</tr>
<tr>
<td>C: steak</td>
<td>3,141 ± 1,061</td>
<td>5,080 ± 1,962</td>
<td>35 ± 11</td>
<td>94 ± 34</td>
<td>0.013 ± 0.006</td>
<td>89 ± 49</td>
</tr>
<tr>
<td>D: eggs</td>
<td>3,820 ± 823</td>
<td>5,865 ± 1,769</td>
<td>38 ± 7</td>
<td>110 ± 35</td>
<td>0.013 ± 0.007</td>
<td>80 ± 39</td>
</tr>
<tr>
<td>E: liquid meal</td>
<td>3,185 ± 796</td>
<td>3,846 ± 1,495</td>
<td>39 ± 10</td>
<td>49 ± 20</td>
<td>0.016 ± 0.005</td>
<td>38 ± 19</td>
</tr>
</tbody>
</table>

Treatment effect | p   | <.05 | <.05 | <.05 | NS | NS |

Note. AUC = area under the curve; AUC_{0–180 min} = partial AUC; AUC_{0–inf} = total AUC; C_{max \ obs} = observed maximum concentration; T_{max \ obs} = time corresponding to maximal concentration; Ke = elimination-rate constant; T_{half} = half-life time of elimination. AUC_{0–180 min}: B < D (p = .03); AUC_{0–inf}: D > B, E (p = .01); C_{max obs}: A > B (p = .0002); A > C (p = .008); T_{max obs}: A < C (p = .002); A < D (p < .0001); B < C (p = .009); B < D (p < .0001); E < C (p = .001); E < D (p < .0001).

### Table 5  Absolute Baseline Concentrations and Kinetics Parameters of Leucine Concentrations After Ingestion of Different Protein-Rich Foods (M ± SD), N = 13–15

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Absolute baseline value (μg/ml)</th>
<th>AUC_{0–180 min}</th>
<th>AUC_{0–inf}</th>
<th>C_{max obs} (μg/ml)</th>
<th>T_{max obs} (min)</th>
<th>T_{half} (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A: skim milk</td>
<td>13.1 ± 2.7</td>
<td>1,146 ± 412</td>
<td>1,181 ± 420</td>
<td>20 ± 6</td>
<td>24 ± 8.5</td>
<td>24 ± 10</td>
</tr>
<tr>
<td>B: soy milk</td>
<td>13.5 ± 2.2</td>
<td>895 ± 288</td>
<td>956 ± 330</td>
<td>10 ± 3</td>
<td>56 ± 22</td>
<td>22 ± 14.5</td>
</tr>
<tr>
<td>C: steak</td>
<td>13.8 ± 2.2</td>
<td>1,375 ± 728</td>
<td>1,729 ± 1,005</td>
<td>15 ± 6</td>
<td>91 ± 38</td>
<td>43 ± 26</td>
</tr>
<tr>
<td>D: eggs</td>
<td>14.1 ± 3.6</td>
<td>1,295 ± 245</td>
<td>1,723 ± 410</td>
<td>12 ± 3</td>
<td>113 ± 35</td>
<td>57 ± 38</td>
</tr>
<tr>
<td>E: liquid meal</td>
<td>13.5 ± 3.7</td>
<td>1,243 ± 363</td>
<td>1,332 ± 372</td>
<td>16 ± 5</td>
<td>40 ± 15</td>
<td>30 ± 18.5</td>
</tr>
</tbody>
</table>

Treatment effect | p   | NS | <.05 | <.05 | <.05 | NS |

Note. AUC = area under the curve; AUC_{0–180 min} = partial AUC; AUC_{0–inf} = total AUC; C_{max obs} = observed maximum concentration; T_{max obs} = time corresponding to maximal concentration; Ke = elimination-rate constant; T_{half} = half-life time of elimination. AUC_{0–inf}: B < C (p = .003); B < D (p = .018); A < D (p = .010); C_{max obs}: A > B, D (p < .0001); A > C (p = .002); A > E (p = .007); B < E (p = .003); B < C (p = .012); T_{max obs}: A < B, C, D (p < .0001); A < E (p = .02); B < C (p = .0009); B < D (p < .0001); B > E (p = .048); E < C, D (p < .0001); C < D (p = .046).
The time to reach maximal glucose concentrations was faster with liquid ingestion than with solid foods (skim milk > steak, \( p = .004 \); skim milk > eggs, \( p = .001 \); liquid meal > steak, eggs, \( p < .0001 \)).

The AUC\textsubscript{0–180 min} of plasma insulin concentrations after intake of liquid treatments was higher than treatments involving solid food (skim milk, soy milk, liquid meal > steak, eggs, \( p < .0001 \)). The AUC\textsubscript{0–inf} of plasma insulin concentrations with liquids was higher than with treatments with solid food (skim milk vs. steak vs. eggs, \( p < .0004 \) and \( p = .0006 \), respectively; soy milk vs. steak vs. eggs, \( p < .0001 \); and liquid meal vs. steak vs. eggs, \( p < .0001 \)). Maximal insulin concentrations after ingestion of liquids were than higher than after solid-food ingestion (skim milk, soy milk, liquid meal > steak, eggs, \( p < .0000 \)). Time to reach maximal insulin concentrations with steak was slower than with liquid meals (\( p < .0001 \)), while skim milk was significantly faster than eggs (\( p < .0001 \)).

**Study 2**

Figure 3 summarizes plasma AA concentrations over 3 hr after the intake of a protein-rich sports bar at rest or after 60 min submaximal cycling exercise, with individual profiles for TAAs, EAAs, BCAAs, and leucine.

Plasma leucine concentrations at baseline did not differ between conditions (rest 118 ± 16 μmol/L, exercise 108 ± 20 μmol/L; \( p = .07 \)). There were no between-trials differences in the pharmacokinetics of BCCAs or leucine. Exercise significantly affected the kinetics of plasma values for EAAs and TAAs; in each case, plasma concentrations in the exercise trial achieved lower AUC\textsubscript{0–180 min} and AUC\textsubscript{0–inf}, with a lower C\textsubscript{max obs} and a higher (20–40%) Ke.

Plasma glucose and insulin responses to the intake of the sports bar at rest or after cycling are presented in Figure 4. Differences in kinetics (AUCs, maximal concentrations, and time to reach maximal concentrations) did not reach significance between treatments.

**Discussion**

This study provides evidence of characteristic patterns of aminoacidemia associated with the intake of different types of commonly consumed protein-rich foods (skim milk, soy milk, eggs, and steak) and a specialized sports food (a liquid meal product). Although a standard amount of high-biological-value protein from different dietary sources produced similar total AA availability as...
Figure 3 — Plasma amino acid (AA) concentration changes (μg/ml) over 3 hr after the intake of a protein-rich sports bar providing 20 g protein, at rest and after 60 min of moderate-intensity cycling, with individual profiles for total AAs (TAA), essential AAs (TAA), branched-chain AAs (BCAA), and leucine. Pharmacokinetics of these profiles are presented in the text.

Figure 4 — Plasma glucose (mmol/L) and insulin (pmol/L) concentration changes over 3 hr after the intake of a protein-rich sports bar providing 20 g protein, at rest or after 60 min of moderate-intensity cycling. Pharmacokinetics of these profiles are presented in the text.
measured by the AUC of the plasma AA responses, the shape of the curves revealed robust differences between foods, with liquid forms of protein achieving peak concentrations twice as quickly after ingestion than solid protein-rich foods (e.g., ~50 min vs. ~100 min) and skim milk achieving a significantly faster peak leucine concentration than all other foods (~25 min). These clear differences justify, as in the concept of the glycemic index (Wolever, 1990), the use of standard portions of protein regardless of subject body mass to characterize the postprandial response. The limitations of the analytical techniques used in the current study are acknowledged in that we were unable to measure several AAs including tryptophan and glutamine. While future work of this kind might benefit from improved methodologies for quantification of blood AAs (Kvitvang,Andreassen, Adam, Villas-Bôas, & Bruheim, 2011), we are at least confident that the AAs most important for muscle protein synthesis were included in the current analyses.

Our study found differences in peak AA concentrations after the intake of different protein foods, with skim milk promoting consistently higher peak values for TAAs, EAAs, BCAAs, and leucine than the other sources. Characteristics of aminoacidemia after soy milk intake were generally lower than with other protein sources, perhaps reflecting its lower content of leucine and BCAAs. Although differences in the rates of decay of AA concentrations were not significant, postprandial AA concentrations remained elevated 180 min after ingestion of the solid protein-rich foods. The effects of protein-rich foods on plasma glucose and insulin responses were affected by the presence of other macronutrients (especially carbohydrate) in the food. Meanwhile, undertaking a prior bout of moderate endurance exercise reduced peak concentrations and AUC values of postprandial aminoacidemia after the intake of a protein-rich sports bar, but these effects were minimal in the case of key AAs (BCAAs and leucine). These findings can help explain the variability in muscle responses to different types of protein reported in previous studies. Furthermore, they may help athletes and other people undertaking exercise organize their protein intake in relation to a workout to maximize their adaptive response.

AA delivery into plasma after the intake of a dietary protein source reflects the rate of digestion and absorption of the food and its AA composition. The aminoacidemia resulting from the intake of a dietary AA source can be manipulated by changing the amount and type of the source and the timing and pattern of its intake. The amount of protein required to optimize the fractional synthetic rate of muscle protein in response to a bout of resistance exercise has been systematically determined (Moore et al., 2009); however, the benefits of achieving other manipulations have been more randomly investigated. Nevertheless, there is evidence that manipulating aminoacidemia around the time of exercise by changing the type or timing of intake of the AA source alters both acute and chronic outcomes. For example, the ingestion of EAAs before a bout of resistance exercise achieved better muscle protein balance than consuming it immediately after the bout (Tipton et al., 2001). Longitudinal investigations of resistance training have found that individuals who consumed a protein supplement immediately after each resistance bout achieved greater improvements in muscle mass and strength after 10–12 weeks than control groups who consumed the same supplement or protein amount 2 hr after each session (Esmarck et al., 2001) or at other times of the day (Cribb & Hayes, 2006; Wilkinson et al., 2007). Varying the source of the same dose of a high-biological-value protein leads to changes in the acute fractional synthetic rate of muscle protein after exercise (Tang et al., 2009) and the functional outcomes to a diet and resistance-training program (Wilkinson et al., 2007). Specifically, milk protein has been shown to be superior to soy protein (Wilkinson et al., 2007), while the whey subfraction is better than casein (Tang et al., 2009).

It is clearly unfeasible to make direct comparisons of the protein synthetic response with the intake of each dietary protein source in combination with exercise to identify the best or better choices. However, it might be helpful, once the ideal aminoacidemic profile in relation to exercise has been established, to have an index of the typical aminoacidemia produced by the intake of different protein-rich foods, or groups of foods. Recent projects have tried to more systematically investigate the optimal delivery of plasma AAs to stimulate muscle protein synthesis after exercise, at least in the short term (up to 5 hr) after an exercise bout (Burke et al., 2012; Koopman et al., 2009; West et al., 2011). Strategies to compare the rate of digestion of different protein sources independently of their AA composition have included comparing a fast protein fed as a bolus with the same amount fed as a series of small doses (pulse pattern; Burke et al., 2012; West et al., 2011) or comparing a slow intact protein (casein) with a hydrolysate of that protein (Koopman et al., 2009).

Studies using these strategies have shown that the achievement of a rapid and pronounced plasma concentration of EAAs and leucine is associated with increased rates of muscle protein synthesis at rest (Koopman et al., 2009) and after resistance exercise (West et al., 2011). This occurred in the face of identical extracellular AA availability, as shown by area under the plasma AA curve (West et al., 2011). Further studies are needed to investigate the best AA pattern to achieve via eating patterns over the rest of the day, since it is known that muscle protein synthesis is increased above resting levels for 24–48 hr after a single bout of exercise (see Burd et al., 2009), but there is also a refractory period after protein feeding (Atherton et al., 2010).

Although the concept is simple, there are surprisingly few studies characterizing plasma AA patterns after the intake of a range of different intact protein-rich foods. Previous investigations include the identification of different times to reach peak plasma AA concentration after the consumption of cottage cheese and egg whites (Nuttall & Gannon, 1990) and differences in plasma AA profiles with the consumption of fish, beef, and chicken (Uhe, Collier, & O’Dea, 1992). The results of the cur-
rent study suggest that the aminoacidemia achieved by eating protein-rich foods may be characteristic of each protein-rich food or group of food; however, further research is required to see how robust these findings are. For example, although the current study showed that a bout of moderate endurance exercise had only a small effect on the plasma delivery of key AAs, other studies of intake of AA sources around resistance exercise have identified substantial changes or delays in the aminoacidic response (Burke et al., 2012; Mero et al., 2009). This may be due to the reduction in gastric emptying and intestinal absorption associated with the limb hyperemia of high-intensity exercise (Durham et al., 2004).

In addition, there is evidence that the form of a food affects postprandial AA concentrations. Conley et al. (2011) reported that the intake of a liquid meal replacement (“shake”) produced greater AUC values for plasma concentrations of TAAs, EAAs, BCAAs, and nonessential AAs than a solid of very similar composition (bar). These findings are in agreement with the current study, in which we observed differences in the plasma AA kinetics of the liquid and solid forms of protein-rich foods. Other factors that may alter the pattern of aminoacidemia after protein-rich meals include the addition of substantial amounts of fat or fiber in mixed meals and the water content of foods or meals, which alters bolus size and intragastric pressure. Changing the timing or increasing the amount of intake of slowly digested protein sources may allow optimal AA concentrations to be achieved at important times related to exercise.

In summary, we investigated the effect of consuming standard portions of different high-quality dietary protein sources on the plasma patterns of TAAs and different AA subfractions. We found that although the TAA plasma availability was similar, there were characteristic differences in the timing and value of peak concentrations of total and individual AAs between different dietary protein sources. These characteristics, at least for the key AAs involved in stimulating muscle protein synthesis, were maintained when the protein was consumed after a moderate bout of endurance exercise. As we increase our knowledge of the aminoacidemia associated with optimal protein synthesis, further investigations of this kind may provide valuable information to inform athletes and other active people on how to choose the timing, amount, and type of their protein intake to maximize their combined response to diet and exercise.

Acknowledgments

This study was supported by Nestec Ltd., Vevey, Switzerland. We acknowledge the technical assistance of Dr. Andrew Garnham and the oversight of Choo Hoffman.

References


