The Postprandial Plasma Amino Acid Response Does Not Differ Following the Ingestion of a Solid Versus a Liquid Milk Protein Product in Healthy Adult Females

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Dietary protein digestion and amino acid absorption rates are modulated by numerous factors such as the food matrix. It has been speculated that protein ingested in liquid form is more rapidly digested and absorbed when compared with ingestion in solid form. Here, we assessed the postprandial plasma amino acid availability following ingestion of a single bolus of protein provided in either liquid or solid form. Twelve healthy, young females were included in this randomized cross-over study. On two separate test days, participants ingested 20-g milk protein concentrate in solid form (protein bar) or in liquid form (protein drink). Products were composed of matched ingredients and, thereby, had the same macro- and micronutrient composition. On both test days, arterialized blood samples were collected at regular time intervals for up to 4 hr following protein ingestion to assess the postprandial rise in plasma amino acid concentrations. Protein ingestion robustly elevated circulating plasma amino acid concentrations (p < .001), with no significant differences between treatments (p = .088). The incremental area under the curve of the postprandial rise in total plasma amino acid concentrations did not differ following bar versus drink consumption (160 ± 73 vs. 160 ± 71 mmol·L⁻¹·240 min⁻¹, respectively; 95% confidence interval [−37, 37]; Cohen’s dₙ = 0.003; p = .992). Ingestion of protein in liquid or solid form does not modulate postprandial amino acid availability in healthy, female adults. Any differences in protein digestion and amino acid absorption due to differences in food matrix are not attributed to the protein being consumed as a bar or as a drink.

Keywords: digestibility, protein quality, protein supplementation

It has been well-established that protein ingestion increases muscle protein synthesis rates (Biolo et al., 1997; Trommelen et al., 2019). The postprandial rise in muscle protein synthesis plays a key role in muscle maintenance and is instrumental in supporting the skeletal muscle adaptive response to exercise training (Cermak et al., 2012). The muscle protein synthetic response to protein ingestion is attributed to the postprandial increase in circulating plasma amino acid (AA) concentrations, with leucine being of particular relevance (Devries et al., 2018; Katsanos et al., 2006). The postprandial increase in AA availability not only provides ample precursors for de novo protein synthesis, but also directly activates molecular pathways that stimulate muscle protein synthesis and inhibit proteolysis (Groen et al., 2015; Holowaty et al., 2022). The plasma AA response to protein ingestion is modulated by various factors, including the amount of protein ingested (Moore et al., 2009; Witard et al., 2014), the type of protein (Gorissen et al., 2020), the protein matrix (Churchward-Venne et al., 2015; Weijzen et al., 2022), and nutrient co-ingestion (Churchward-Venne et al., 2015; Gorissen et al., 2014; Kashyap, Shivakumar, et al., 2019). Furthermore, processing and preparation of the food product and/or derived protein can modulate protein digestion and AA absorption kinetics and, as such, impact postprandial plasma AA kinetics (Nyakayiru et al., 2019; Oberli et al., 2015; Trommelen et al., 2020).

Athletes often consume protein supplements to support muscle maintenance and maximize the skeletal muscle adaptive response to a bout of exercise (Gillen et al., 2017). Such protein supplements are mostly consumed as protein isolates or protein concentrates dissolved in a drink. However, the market for protein bars is increasing and is expected to grow even more with the increasing popularity among athletes (Arenas-Jal et al., 2020). Most studies investigating protein digestion and AA absorption kinetics apply the ingestion of protein isolates or concentrates provided in a drink (Gorissen et al., 2015). Based on previous work, it could be speculated that protein digestion and AA absorption kinetics differ substantially following ingestion of protein in either liquid or solid form. The latter may result in marked differences in postprandial AA availability. Previously, comparisons of postprandial AA responses following ingestion of cheese versus milk ingestion (de Hart et al., 2021; Hermans et al., 2022; Horstman et al., 2021), as well as a meal replacement bar versus drink (Conley et al., 2011), have reported an attenuated rise in circulating AA concentrations when protein was provided in a solid versus liquid form. However, such comparisons did not only include differences...
in the food form, but also in their ingredient(s), resulting in differences in macro- and micronutrient composition, and/or protein composition. Consequently, the impact of ingesting protein in the form of a bar or drink on postprandial plasma AA concentrations has never been directly assessed. We hypothesized that ingestion of 20-g protein provided in solid form leads to an attenuated postprandial rise in circulating plasma AAs when compared with the ingestion of the same amount and type of protein provided in a drink.

In the present study, we assessed postprandial plasma AA responses following ingestion of 20-g milk protein provided as a protein bar or as a protein drink in 12 healthy young women. Both the protein bar and drink were specifically produced for this study to allow matching of ingredients and, thereby obtain an equivalent macro- and micronutrient composition.

**Methods**

**Subjects**

A total of 12 healthy, young women were recruited to participate in this randomized cross-over study. Potential subjects were included if they were nonsmoking, recreationally active (exercise at least once per 2 weeks and with a maximum of four times per week), and not pregnant. Subject characteristics are presented in Table 1. The subjects were fully informed about the experimental procedures and possible risks of participation before signing an informed consent. The study was approved by the Medical Ethical Committee of Maastricht University Medical Center, The Netherlands, and was registered at the Nederlands Trial Register (https://trialsearch.who.int/Trial2.aspx?TrialID=NL9694). All procedures were carried out in accordance with the standards stated in the most recent version of the Helsinki Declaration. The study was independently monitored by the Clinical Trial Center Maastricht.

**Table 1** Subjects’ Characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>12</td>
</tr>
<tr>
<td>Age (years)</td>
<td>25 ± 2</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>68.0 ± 10.3</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.70 ± 0.05</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>23.4 ± 2.8</td>
</tr>
<tr>
<td>Lean body mass (kg)</td>
<td>45.6 ± 5.9</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>30 ± 5</td>
</tr>
</tbody>
</table>

*Note. All values are mean ± SD.*

**Study Design**

This randomized cross-over study assessed the postprandial plasma AA concentrations following the ingestion of 20-g milk protein in the form of a protein bar or a protein drink. During two separate test days, subjects ingested a protein bar or protein drink, followed by a 4 hr postprandial assessment period during which multiple blood samples were collected. Randomization for the treatment order was balanced and random allocation sequence was generated using www.randomization.com. To control for the menstrual cycle, test days were conducted in the follicular phase by planning the tests in the first 10 days after start of the menses. Test days were separated by a minimum of 3 days and a maximum of 10 weeks.

**Pretesting**

During screening, before inclusion into the study, body weight and height were assessed, and body composition was determined by a dual-energy X-ray absorptiometry scan (Discovery A; Hologic). All subjects were deemed healthy based on their responses to a routine medical screening questionnaire.

**Standardization of Physical Activity and Diet**

Subjects refrained from strenuous physical activity in the 48 hr leading up to the trial days. Physical activity and dietary intake were recorded during 48 hr prior to the first test day. Subjects were provided with copies of their physical activity and dietary intake, and instructed to maintain a similar pattern in the 48 hr prior to the second test. Subjects did not consume alcohol for 24 hr and, did not consume caffeine for 12 hr before each test day. On the evening prior to the test days, all subjects consumed the same standardized dinner (1.71 MJ/405 kcal), providing 53% energy as carbohydrate, 26% energy as fat, and 21% energy as protein. Thereafter, subjects were only allowed to drink water.

**Protein Products**

The protein bar and protein drink were specifically produced by FrieslandCampina Ingredients to match the ingredient profiles. All ingredients in the protein drink were in powder form: protein powder (Nutri Whey™ Isolate and Excellion® Calcium Caseinate), dried glucose syrup, and medium chain triglyceride powder. The protein bar was composed of the same protein powders (Nutri Whey Isolate and Excellion Calcium Caseinate), but carbohydrate was in (glucose–fructose) syrup form, and fat was in (medium chain triglyceride) oil form. Consequently, the products had similar macronutrients and AA composition (Tables 2 and 3). The protein drink was...
Table 3  AA Composition of the Investigational Products

<table>
<thead>
<tr>
<th>AA (% of total)</th>
<th>Bar</th>
<th>Drink</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine</td>
<td>7.4</td>
<td>6.5</td>
</tr>
<tr>
<td>Arginine</td>
<td>1.7</td>
<td>1.8</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>7.2</td>
<td>8.1</td>
</tr>
<tr>
<td>Cystine</td>
<td>0.8</td>
<td>0.9</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>16.7</td>
<td>16.5</td>
</tr>
<tr>
<td>Glycine</td>
<td>2.3</td>
<td>2.9</td>
</tr>
<tr>
<td>Histidine</td>
<td>1.6</td>
<td>1.5</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>6.3</td>
<td>6.1</td>
</tr>
<tr>
<td>Leucine</td>
<td>10.9</td>
<td>10.7</td>
</tr>
<tr>
<td>Lysine</td>
<td>8.1</td>
<td>8.3</td>
</tr>
<tr>
<td>Methionine</td>
<td>2.1</td>
<td>1.8</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>3.0</td>
<td>2.9</td>
</tr>
<tr>
<td>Proline</td>
<td>9.4</td>
<td>8.8</td>
</tr>
<tr>
<td>Serine</td>
<td>6.0</td>
<td>6.8</td>
</tr>
<tr>
<td>Threonine</td>
<td>7.1</td>
<td>7.3</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>2.6</td>
<td>2.6</td>
</tr>
<tr>
<td>Valine</td>
<td>6.8</td>
<td>6.6</td>
</tr>
<tr>
<td>EAA</td>
<td>45.8</td>
<td>45.2</td>
</tr>
<tr>
<td>NEAA</td>
<td>54.2</td>
<td>54.8</td>
</tr>
<tr>
<td>TAA</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Note. AA = amino acid; EAA = essential amino acid; NEAA = nonessential amino acid; TAA = total amino acid.

Experimental Procedures

An outline of the study protocol is provided in Figure 1. At 08.00 hr, subjects reported to the laboratory after an overnight fast. A Teflon catheter was placed in a dorsal hand vein for arterialized blood sampling. Before each blood sample, the hand was placed in a hot box (60 °C) for 10 min to allow sampling of arterialized blood. After collection of a basal blood sample, the subjects consumed their supplement within 8 min (t=0 min), followed by a 4 hr postprandial period. During the postprandial period, blood samples were collected at t = 15, 30, 45, 60, 90, 120, 150, 180, 210, and 240 min while subjects remained in a seated position. With each blood sample subjects rated their hunger, desire to eat, and fullness according the visual analog scale.

Plasma Analyses

Blood samples were collected in tubes containing EDTA and centrifuged at 1,000g for 10 min at 4 °C. Aliquots of plasma were frozen in liquid nitrogen and stored at −80 °C. Plasma glucose and insulin concentrations were analyzed using commercially available kits (ABX Pentra Glucose HK CP, Horiba ABX, ref: A11A01667 and Human Insulin kit, Meso Scale Discovery, ref: K151BZC). Quantification of plasma AA concentrations was performed using ultra-performance liquid chromatography mass spectrometry (ACQUITY UPLC H-Class with QDa; Waters), as described previously (Nyakayiru et al., 2019).

Statistical Analyses

A sample size of 12 subjects was calculated based on a 0.09-mmol/L difference in peak plasma EAA concentration and a SD of 0.1 mmol/L, following ingestion of cheese (solid) versus milk (liquid; Horstman et al., 2021). Sample size was calculated for a two-tailed, paired Student’s t test with a power of 80% and a significance level of .05. Time-dependent variables (i.e., plasma glucose, insulin, and AA concentrations) were analyzed by two-factor repeated-measures analysis of variance with time and treatment as within-subjects factors. In case of significant interactions, separate paired t tests were performed for each time point to detect differences between treatments. Time-independent variables (i.e., incremental area under the curve [AUC] for the t = 0–240 min period, maximal AA concentration C_max, time to peak T_max) were analyzed using a paired t test. 95% confidence interval (CI) of the difference and Cohen’s d, effect size (calculated as MD/SD of difference) are presented for the within-subject assessments where appropriate. Statistical significance was set at p < .05. All calculations were performed using SPSS Statistics (version 27) and are presented as mean values with their SDs.

Results

Plasma Glucose and Insulin Concentrations

Plasma glucose and insulin concentrations are shown in Figure 2. Plasma glucose concentrations were increased at t = 30 min for both treatments (time effect: p < .001). Changes in plasma glucose concentrations over time did not differ between the protein bar and protein drink (Time × Treatment effect: p = .081). Similarly, plasma insulin concentrations increased briefly following protein ingestion (time effect: p < .001) with no significant differences between treatments over time (Time × Treatment effect: p = .062).

Plasma AA Concentrations

Protein ingestion resulted in an increase in total plasma AA concentrations (TAA; time effect: p < .001), as depicted in Figure 3a. No differences in TAA concentrations over time were observed between the two treatments (Time × Treatment effect: p = .088). Accordingly, incremental AUC (iAUC) for
plasma TAA concentrations over the full 4 hr postprandial period did not differ between the bar and drink treatment (160 ± 73 vs. 160 ± 71 mmol·L⁻¹·240 min⁻¹, respectively; 95% CI [−37, 37]; Cohen $d_z = 0.003; p = .992$; Figure 3b). In addition, no differences were observed in peak TAA concentrations (3,872 ± 481 vs. 3,833 ± 867 μmol/L; 95% CI [−658, 580]; Cohen $d_z = −0.040; p = .891$) and time to peak (56 ± 20 vs. 53 ± 20 min; 95% CI [−15, 10]; Cohen $d_z = −0.125; p = .674$) between the bar and drink treatment, respectively. No difference in TAA concentrations over time was observed between the first and second test (Time × Treatment effect: $p = .126$), indicating that the order of treatment did not affect the results.

Plasma essential AA (EAA) concentrations and nonessential AA (NEAA) concentrations are presented in Figure 4. Plasma EAA and NEAA concentrations increased following protein ingestion (time effect: $p < .001$ for both EAA and NEAA). No differences were observed over time between the bar and drink for EAA (Time × Treatment effect: $p = .119$) and NEAA (Time × Treatment effect: $p = .075$) concentrations. Likewise, plasma leucine concentrations increased following protein ingestion (time effect: $p < .001$), with no significant differences between treatments (Time × Treatment effect: $p = .067$; Figure 5). Furthermore, iAUC, maximal concentration, and time to peak did not differ between treatments for EAA, NEAA, and leucine (Supplementary Table S1 [available online]). An overview of the postprandial responses of the individual AAs is provided in Supplementary Figure S1 (available online).

**Hunger, Desire to Eat, and Fullness**

Visual analog scale scores for hunger, desire to eat, and fullness are displayed in Figure 6. Hunger and desire to eat decreased after protein ingestion (time effect: $p < .001$; Figure 6a and 6b), while visual analog scale scores for fullness increased after protein ingestion (time effect: $p < .001$; Figure 6c). No differences over time were observed for hunger, desire to eat, and fullness between treatments (Time × Treatment effect: $p = .503$, $p = .476$, and $p = .732$, respectively).

**Discussion**

The present study assessed the postprandial plasma AA responses after ingestion of 20-g dairy protein provided as a solid and liquid milk protein product in healthy adult females. Plasma AA concentrations increased following protein ingestion, reaching peak levels 45–60 min after consumption. Postprandial plasma AA responses did not differ between the ingestion of protein in either solid or liquid form.
Ingestion of protein increased plasma AA concentrations, resulting in peak concentrations at ∼55 min after protein ingestion with levels 89 ± 31% higher when compared with baseline values (Figure 3). The observed magnitude and timeline of the plasma AA response to the ingestion of 20-g milk protein is in line with previous studies (Alcock et al., 2019; de Hart et al., 2021). Such strong postprandial increases in plasma AA availability have previously been shown to result in a robust increase in myofibrillar protein synthesis rates (Gorissen et al., 2014; Moore et al., 2009; Witard et al., 2014). Consequently, athletes are typically advised to consume 20-g high-quality protein to increase plasma AA availability and, as such, to support skeletal muscle reconditioning.

Previous studies have observed an attenuated rise in plasma AA concentrations following ingestion of various solid protein sources (e.g., cheese) when compared with liquid protein sources (e.g., milk; Conley et al., 2011; de Hart et al., 2021; Hermans et al.,
In the present study, the protein bar and drink were specifically produced to match their ingredients, and, thereby obtain an equivalent macro- and micronutrient composition. As a result, no differences were observed between the solid and liquid food form on postprandial plasma TAA, EAA, NEAA, and leucine concentrations (Figures 3–5). These data demonstrate that the food form of a protein supplement per se does not modulate its postprandial plasma AA response. However, the food matrix of commercial protein bars and drinks typically varies in more factors than only the food form. For example, the specific processing (i.e., heating) and storage conditions of a protein product modulate the plasma AA response to its ingestion (Nyakayiru et al., 2019; van Lieshout et al., 2019). Furthermore, protein bars typically contain added ingredients that contribute to the desired taste and texture of the product (e.g., fibers, a chocolate outer layer). Moreover, protein bars typically also contain some collagen protein (i.e., gelatin) to stabilize the structure of the product. However, this results in an altered AA composition and subsequent plasma AA response (Alcock et al., 2019; Brook et al., 2021). The addition of nonprotein ingredients such as fat and carbohydrates can delay protein digestion and AA absorption, while the addition of fibers can attenuate protein digestion and AA absorption and even lower protein digestibility (Churchward-Venne et al., 2015; Gorissen et al., 2014, 2017; Kashyap, Varkey, et al., 2019). Hence, it can be speculated that the previously observed differences in protein digestion and AA absorption between solid and liquid protein products were primarily the result of differences in ingredients and/or nutrient composition rather than the solid versus liquid food form.

In the present study, the protein bars were co-ingested with water to match the water volume of the drink treatment. In practice, protein bars are typically co-ingested with water because of their dry texture and to ensure (re)hydration during or following exercise. It is well-established that a larger gastric volume stimulates gastric emptying rates (Kwiatek et al., 2009; Okabe et al., 2017). Therefore, it could be speculated that water co-ingestion may result in more rapid protein digestion and absorption kinetics. However, various observations suggest that co-ingested water does not mix effectively with more caloric dense matter in the stomach and results in gastric sieving by which low caloric water leaves the stomach relatively quickly while the more caloric dense material is retained (Camps et al., 2017; Marciani et al., 2012). Therefore, water co-ingestion is unlikely to substantially impact the plasma AA response to protein ingestion.

In the present study, ingestion of a solid and liquid food did not result in different appetite ratings. This is in contrast to an extensive meta-analysis that reported a significant decrease in hunger following consumption of solid compared with liquid foods (Stribiçaia et al., 2020). However, most studies that assess appetite ratings of solid and liquid foods use a bigger meal size (i.e., a complete meal) and include more participants. The small meal size and relatively small number of participants in the current study may explain why no differences in appetite ratings were observed between treatments.

Our data suggests that protein drinks and bars are equally effective to increase AA availability and thereby support postprandial protein balance. This observation allows product choice to be determined by other practical considerations. Protein bars may be more convenient for athletes due to their ready-to-eat format and they are more compact and lighter than ready-to-drink shakes (Arenas-Jal et al., 2020). The lower fluid intake of a protein bar may also be beneficial for the purpose of presleep feeding to avoid overnight urination and for clinical populations that need to restrict their fluid intake like haemodialysis patients and heart failure patients (Meade, 2007; Vest et al., 2019). Conversely, protein drinks may be preferred for populations that experience difficulties with dysphagia and surgical patients in their acute postoperative period (Lobo et al., 2020).

This study presents several limitations. First, the plasma AA response to feeding is only a proxy marker for protein digestion and AA absorption (Trommelen et al., 2021). However, postprandial plasma AA concentrations generally reflect exogenous AA kinetics and are indicative of AA availability. Second, the protein bars and drinks were consumed in rested conditions. In practice, these products are typically consumed immediately after a bout of exercise. As dietary protein digestion and absorption are impaired during postexercise recovery (van Wijck et al., 2013), it cannot be excluded that potential difference between solid versus liquid protein products may become apparent under such conditions. Finally, the current study was limited to healthy young females. We designed the study to exclude the possibility of sex-based differences and selected females because they are greatly underrepresented in sports nutrition research (Cowley et al., 2021).

In conclusion, ingestion of protein in liquid or solid form does not modulate postprandial AA availability in healthy, female adults. Differences in protein digestion and AA absorption due to differences in food matrix are not attributed to the protein being consumed as a bar or a drink.
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