

Jejunal Casein Feeding Is Followed by More Rapid Protein Digestion and Amino Acid Absorption When Compared with Gastric Feeding in Healthy Young Men^{1,2}

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Abstract

Background: Dietary protein is required to attenuate the loss of muscle mass and to support recovery during a period of hospitalization. Jejunal feeding is preferred over gastric feeding in patients who are intolerant of gastric feeding. However, the impact of gastric vs. jejunal feeding on postprandial dietary protein digestion and absorption kinetics in vivo in humans remains largely unexplored.

Objective: We compared the impact of gastric vs. jejunal feeding on subsequent dietary protein digestion and amino acid (AA) absorption in vivo in healthy young men.

Methods: In a randomized crossover study design, 11 healthy young men (aged 21 ± 2 y) were administered 25 g specifically produced intrinsically L-[1-¹³C]phenylalanine-labeled intact casein via a nasogastric and a nasojejunal tube placed ~30 cm distal to the ligament of Treitz. Protein was provided in a 240-mL solution administered over a 65-min period in both feeding regimens. Blood samples were collected during the 7-h postprandial period to assess the increase in plasma AA concentrations and dietary protein-derived plasma L-[1-¹³C]phenylalanine enrichment.

Results: Jejunal feeding compared with gastric feeding resulted in higher peak plasma phenylalanine, leucine, total essential AA (EAA), and total AA concentrations (all $P < 0.05$). This was attributed to a more rapid release of dietary protein-derived AAs into the circulation, as evidenced by a higher peak plasma L-[1-¹³C]phenylalanine enrichment concentration (2.9 ± 0.2 vs. 2.2 ± 0.2 mole percent excess; $P < 0.05$). The total postprandial plasma AA incremental area under the curve and time to peak did not differ after jejunal vs. gastric feeding. Plasma insulin concentrations increased to a greater extent after jejunal feeding when compared with gastric feeding (275 ± 38 vs. 178 ± 38 pmol/L; $P < 0.05$).

Conclusions: Jejunal feeding of intact casein is followed by more rapid protein digestion and AA absorption when compared with gastric feeding in healthy young men. The greater postprandial increase in circulating EAA concentrations may allow a more robust increase in muscle protein synthesis rate after jejunal vs. gastric casein feeding. This trial was registered at trialregister.nl as NTR2801. *J Nutr* 2015;145:2033–8.

Keywords: enteral nutrition, malnutrition, protein, casein, gastric feeding, jejunal feeding

Introduction

Malnutrition during hospitalization is associated with greater morbidity, mortality, length of hospital stay, and medical costs (1). It is essential to provide adequate amounts of calories and

protein to minimize catabolism, diminish the suppression of immune competence, and reduce the risk of septic complications in patients (2–5). Enteral nutrition (EN)¹⁰ is preferred over parenteral nutrition as the general route of feeding for critically ill patients who require nutritional support therapy (6). Compared with parenteral nutrition, EN is associated with a lower

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¹⁰ Abbreviations used: AA, amino acid; AE, adverse event; EAA, essential amino acid; EN, enteral nutrition; iAUC, incremental AUC; MPE, mole percent excess; NGT, nasogastric tube; NJT, nasojejunal tube.

risk of infectious complications and mortality (7). Moreover, EN allows preservation of intestinal integrity and prevents mucosal atrophy and bacterial translocation (8, 9).

Delayed gastric emptying occurs in ~50% of all mechanically ventilated, critically ill patients and limits the administration of EN (10). It leads to high gastric residues, higher risk of aspiration and pneumonia, and the inability to administer required nutrients. To avoid malnutrition and aspiration, patients should be fed via an enteral tube placed in the small intestine (6, 11). Although jejunal feeding is generally well tolerated, whether it modulates and impairs proper dietary protein digestion and absorption is unknown. It has been suggested that jejunal feeding requires a predigested rather than a polymeric diet (12–14).

The fact that there are few *in vivo* data on the impact of gastric vs. jejunal feeding on dietary protein digestion and absorption is likely attributed to the obvious methodologic limitations of *in vivo* human research (15). To allow *in vivo* assessment of dietary protein digestion and absorption kinetics we applied intrinsically L-[1-¹³C]phenylalanine-labeled protein, which was produced by collecting milk protein from lactating cows that had been infused with large amounts of L-[1-¹³C]phenylalanine (16). We added [6,6-²H₂]glucose to compare gastric-emptying rates between regimens, because glucose is not modulated by the gastric enzymes and acidity in the stomach.

The aim of this study was to compare the impact of gastric vs. jejunal protein feeding on casein digestion and subsequent amino acid (AA) absorption in healthy young men. Subjects received (micellar) intact casein protein in this study because it is commonly used in EN for its high essential AA (EAA) content. Casein protein coagulates in an acidic environment such as in the stomach, which may slow down the availability of the protein in the digestive tract. Therefore, jejunal casein feeding may result in more rapid digestion and subsequent absorption of dietary protein-derived plasma AAs. Consequently, we hypothesized that jejunal casein feeding leads to more rapid protein digestion and AA absorption when compared with gastric feeding. In this study we used intrinsically L-[1-¹³C]phenylalanine-labeled casein protein (16, 17) to assess differences in protein digestion and AA absorption after gastric vs. jejunal casein feeding *in vivo* in healthy young men.

Methods

Subjects. Twelve healthy young men (aged 21 ± 2 y) participated in the present study. Women were excluded because of the perceived confounding effect of the menstrual cycle on gastrointestinal function. Subjects were randomly assigned to either treatment sequence: gastric-jejunal or jejunal-gastric. Inclusion criteria were as follows: age between 18 and 45 y; a BMI (in kg/m²) between 18 and 27, which was considered a healthy weight; not using medication; nonsmoking; no abnormalities on general physical examination; and basic blood results within the respective reference ranges. One subject dropped out before the start of the study because of a vasovagal reaction on blood withdrawal. The subjects' characteristics are presented in **Table 1**.

This randomized crossover clinical trial was carried out at a university-based hospital (Kennemer Gasthuis, Haarlem, Netherlands) to evaluate the effects of 2 regimens of nutritional support on subsequent protein digestion and AA absorption of intact casein. All subjects were informed of the nature and possible risk of the experimental procedures before their written informed consent was obtained. The study was carried out after international ethical approval by the Medical Ethical Committee of Noord-Holland, Alkmaar, Netherlands. This trial was registered at trialregister.nl as NTR2801.

Diet and physical activity before testing. The day before the experiment all of the subjects consumed a standardized diet providing 50% of

TABLE 1 Baseline characteristics of study participants¹

	Value
Age, y	21.7 ± 2.1
Height, m	1.87 ± 0.06
Weight, kg	80.2 ± 9.3
BMI, kg/m ²	23.0 ± 1.9
Basal plasma glucose, mmol/L	5.6 ± 0.4
Basal plasma insulin, pmol/L	63 ± 24
HOMA-IR	2.3 ± 0.9

¹ Values are means ± SDs; n = 11.

energy as carbohydrate, 16% as protein, and 34% as fat. All volunteers were instructed to refrain from alcohol consumption, not to perform any exhaustive physical activity, and to maintain a consistent diet 3 d before the trial.

Experiment. According to a randomized crossover design each subject received 240 mL fluid containing 25 g intrinsically L-[1-¹³C]phenylalanine-labeled intact casein with 1 g [6,6-²H₂]glucose through a nasogastric tube (NGT) and a nasojejunal tube (NJT). A 4-wk period separated both experimental trials. Abdominal X-ray was used to confirm that the NGT was positioned in the stomach and the NJT ~30 cm distal to the ligament of Treitz. Flow rate was set at 220 mL/h, resulting in a total administration time of 65 min.

Protocol. After an overnight fast, a polyurethane catheter was placed in a dorsal hand vein for frequent blood sampling. The administration of the fluid containing intrinsically L-[1-¹³C]phenylalanine-labeled casein protein through a NGT or NJT was started directly after basal blood sampling. Venous blood samples were collected frequently during a 7-h postprandial period, at 15, 30, 45, 60, 75, 90, 120, 150, 180, 240, 300, 360, and 420 min. Venous blood glucose analysis was performed immediately after collection. Blood samples were collected in EDTA-containing tubes and serum tubes and centrifuged at 1770 × g for 12 min at 4°C. Plasma and serum aliquots were frozen and stored at -80°C.

Preparation of the study fluid. Intrinsically L-[1-¹³C]phenylalanine-labeled casein protein was obtained by infusing a Holstein cow with large quantities of L-[1-¹³C]phenylalanine, collecting the milk, and purifying the casein fraction as described previously (16). The L-[1-¹³C]phenylalanine enrichment of the intact casein protein used was 6.1 mole percent excess (MPE). The casein protein met all chemical and bacteriologic specifications for human consumption. Subjects received a volume of 240 mL through an NJT or an NGT, providing 25 g intrinsically labeled casein protein to which 1 g [6,6-²H₂]-labeled glucose was added.

Plasma analysis. Plasma glucose concentrations were analyzed with the HemoCue Glucose 201 DM Analyzer (HemoCue Diagnostics BV). Insulin was analyzed by luminescence immunometric assay (Advia Centaur; Siemens Medical Solutions Diagnostics). After precipitation of proteins and polypeptides with perchloric acid, the plasma samples were centrifuged, and the clear supernatant was collected. Plasma AA concentrations were measured by HPLC after precolumn derivatization with o-phthalaldehyde and fluorimetry (Nutricia Research).

For plasma phenylalanine enrichment measurements, plasma phenylalanine was derivatized to its t-butyl-dimethyl-silyl derivative, and its ¹³C enrichment was determined by electron impact ionization gas chromatography–mass spectrometry (model 6890N GC/5973N MSD; Agilent) by using selected ion monitoring of masses 336 and 337 for unlabeled and [1-¹³C]-labeled phenylalanine, respectively (18). We applied standard regression curves in all isotopic enrichment analyses to assess the linearity of the mass spectrometer and to control for the loss of tracer. Enrichments were corrected for the presence of [1-¹³C]phenylalanine isotopes in baseline samples (19). After derivatization of the plasma samples, plasma [6,6-²H₂]glucose enrichment was measured

by electron impact ionization gas chromatography–mass spectrometry (Finnigan INCOS-XL; Finnigan Mat).

Statistical analysis. Baseline characteristics are expressed as means \pm SDs. *P* values are based on Student's *t* test for independent samples. Efficacy parameters *P* value are based on repeated measures mixed model ANOVA with fixed factors: treatment, period, sequence, and random-factor subject. The *P* value of “within time analysis” to compare differences between treatments over time is based on repeated measures mixed model ANOVA with fixed factors: treatment, period, sequence, time and time \cdot treatment interaction, and random-factor subject. For variables with ordered or ordinal categories, the Wilcoxon's signed rank test was used and binomial variables were analyzed by using McNemar's test. Significance was set at *P* < 0.05. All calculations were performed by Nutricia Research Utrecht with the use of SAS (SAS Enterprise Guide 4.3 or higher) for Windows (SAS Institute).

Results

Plasma glucose and insulin. Plasma glucose concentrations did not change significantly during casein administration and averaged 5.3 ± 0.1 mmol/L after gastric feeding and 5.2 ± 0.1 mmol/L after jejunal feeding (Figure 1A). After the onset of casein administration, plasma insulin concentrations showed a rapid but short-lived increase in both feeding regimens (Figure 1B). Peak plasma insulin concentrations were higher after jejunal (275 ± 38 pmol/L) than after gastric (178 ± 38 pmol/L) feeding (*P* < 0.05). The incremental AUC (iAUC) was also significantly higher after jejunal (9880 ± 1690 pmol/L \cdot 7 h) than after gastric (6180 ± 1760 pmol/L \cdot 7h) feeding (*P* < 0.05). Within-time analysis showed a significantly higher concentration at 45, 60, and 75 min after jejunal feeding than after gastric feeding (Figure 1B).

Plasma AAs. Plasma phenylalanine, leucine, and the sum of all EAA and AA concentrations over time are shown in Figure 2. Baseline and peak values, time to peak, and the iAUC of plasma

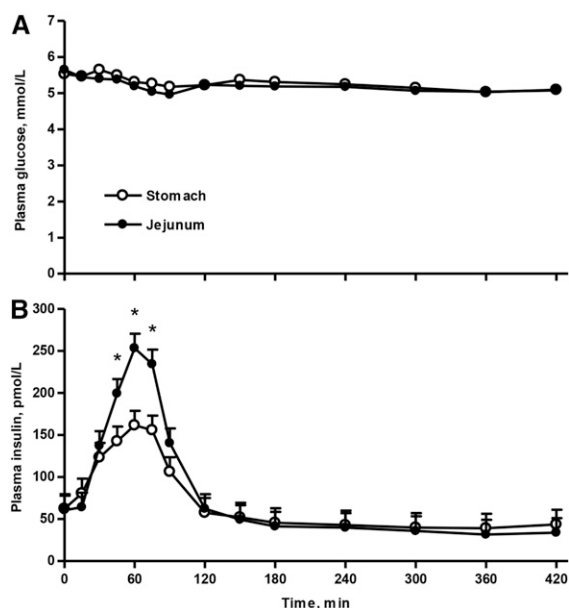


FIGURE 1 Plasma glucose (A) and insulin (B) concentrations after either gastric or jejunal casein protein feeding in healthy young men. Values are means \pm SEMs, *n* = 11. Data were analyzed with repeated-measures mixed-model ANOVA. *Different from jejunal feeding, *P* < 0.05.

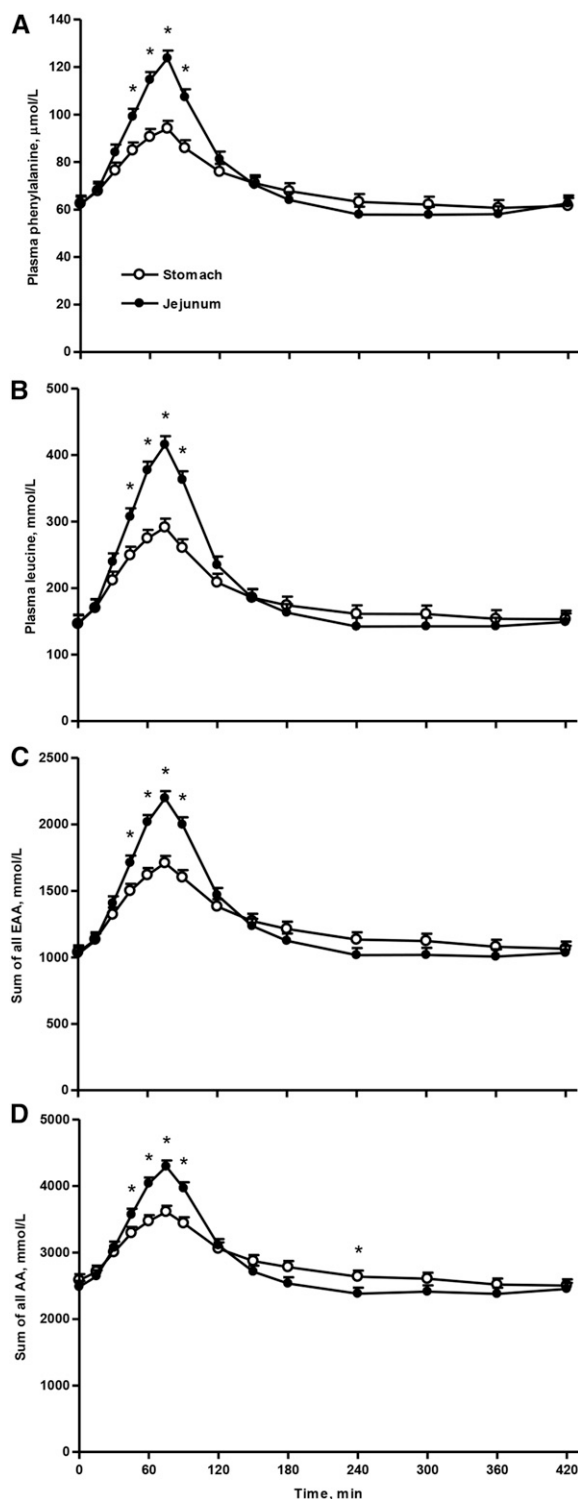


FIGURE 2 Plasma phenylalanine (A), leucine (B), and the sum of all EAA (C) and AA (D) concentrations after either gastric or jejunal casein protein feeding in healthy young men. Values are means \pm SEMs, *n* = 11. Data were analyzed with repeated-measures mixed-model ANOVA. *Different from jejunal feeding, *P* < 0.05. AA, amino acid; EAA, essential amino acid.

phenylalanine, leucine, and the sum of all EAA and AA concentrations are presented in Table 2.

Peak plasma phenylalanine concentrations were significantly higher after jejunal feeding than after gastric feeding (Table 2). Within-time analysis showed significantly higher concentrations

TABLE 2 Baseline and peak values, time to peak, and iAUC of phenylalanine, leucine, and all EAAs and AAs after gastric or jejunal casein protein feeding in healthy young men¹

	Gastric feeding	Jejunal feeding
Phenylalanine		
Baseline, $\mu\text{mol/L}$	63 \pm 3	62 \pm 3
Peak value, $\mu\text{mol/L}$	100 \pm 6	124 \pm 6*
Time to peak, min	90 \pm 17	74 \pm 17
iAUC, $\text{mmol/L} \cdot 7 \text{ h}$	3 \pm 1	4 \pm 1
Leucine		
Baseline, $\mu\text{mol/L}$	146 \pm 13	147 \pm 13
Peak value, $\mu\text{mol/L}$	313 \pm 22	420 \pm 22*
Time to peak, min	90 \pm 17	77 \pm 17
iAUC, $\text{mmol/L} \cdot 7 \text{ h}$	16 \pm 2	19 \pm 2
Sum of all EAAs		
Baseline, $\mu\text{mol/L}$	1040 \pm 54.0	1020 \pm 54.0
Peak value, $\mu\text{mol/L}$	1770 \pm 93.0	2210 \pm 93.0*
Time to peak, min	92 \pm 17	79 \pm 17
iAUC, $\text{mmol/L} \cdot 7 \text{ h}$	81 \pm 9	88 \pm 8
Sum of all AAs		
Baseline, $\mu\text{mol/L}$	2580 \pm 92.0	2480 \pm 92.0
Peak value, $\mu\text{mol/L}$	3720 \pm 150	4300 \pm 150*
Time to peak, min	98 \pm 17	78 \pm 17
iAUC, $\text{mmol/L} \cdot 7 \text{ h}$	109 \pm 13	126 \pm 12

¹ Values are means \pm SEMs, $n = 11$. Data were analyzed with repeated-measures mixed-model ANOVA. *Different from gastric feeding, $P < 0.05$. AA, amino acid; EAA, essential amino acid; iAUC, incremental AUC.

at 45, 60, 75, and 90 min after jejunal vs. gastric feeding (Figure 2A). Peak plasma leucine concentrations were significantly higher after jejunal feeding than after gastric feeding (Table 2). Within-time analysis showed significantly higher concentrations at 45, 60, 75, and 90 min after jejunal vs. gastric feeding (Figure 2B). Peak plasma EAA concentrations were significantly higher after jejunal feeding than after gastric feeding (Table 2). Within-time analysis showed significantly higher concentrations at 45, 60, 75, and 90 min after jejunal vs. gastric feeding (Figure 2C). Peak plasma AA concentrations were significantly higher after jejunal feeding than after gastric feeding (Table 2). Within-time analysis showed significantly higher concentrations at 45, 60, 75, and 90 min after jejunal vs. gastric feeding (Figure 2D).

Plasma tracer enrichments. Plasma L-[1-¹³C]phenylalanine enrichments increased immediately after casein administration in both groups (Figure 3). Peak plasma L-[1-¹³C]phenylalanine enrichments were significantly higher after jejunal feeding (2.9 ± 0.2 MPE) than after gastric feeding (2.2 ± 0.2 MPE) ($P < 0.05$). Time to peak and the iAUC did not differ significantly between groups. Within-time analysis showed significantly higher concentrations at 45, 60, 75, and 90 min after jejunal feeding than after gastric feeding, although at 240, 300, and 360 min concentrations were significantly higher after gastric feeding than after jejunal feeding.

Plasma [6,6-²H₂]glucose enrichment increased immediately after casein administration in both groups (Figure 3). The increase in [6,6-²H₂]glucose enrichment did not differ significantly between feeding regimens. Time to peak, peak value, and the iAUC of plasma [6,6-²H₂]glucose enrichments did not differ significantly between regimens.

Safety and tolerance. A data safety monitoring board was installed before the first subject was enrolled to ensure an

ongoing evaluation of the serious adverse events (AEs) that might occur during the study. Data on tolerance of the study product were collected every 4 h with a visual analog scale. No serious AEs were reported. A total of 3 AEs were possibly related to the administration of intact casein, of which 1 was reported with gastric feeding (occurring in 1 subject: nausea) and 2 with jejunal feeding (occurring in 2 subjects: 1 with nausea, 1 with diarrhea). The number of AEs was not significantly different between groups. Blood safety variables all remained within the respective reference ranges, and no clinically relevant changes in liver and kidney function were observed (data not shown).

Discussion

In the present study, we compared the impact of gastric vs. jejunal casein protein feeding on protein digestion and subsequent AA absorption in healthy young men. Jejunal feeding of intact micellar casein protein was followed by a more rapid release of dietary protein-derived AAs in plasma when compared with gastric feeding. This implies that jejunal casein feeding is followed by more rapid protein digestion and AA absorption than with gastric feeding.

The apparent differences in dietary protein digestion and AA absorption after gastric and jejunal feeding may be attributed to various factors, ranging from gastric emptying to protein hydrolysis and mucosal AA absorption (21). In the present study, we also added [6,6-²H₂]glucose to the solutions to compare the appearance rates of both exogenous glucose and protein-derived phenylalanine after gastric vs. jejunal feeding. It is evident from the data presented in Figure 3B that there is an

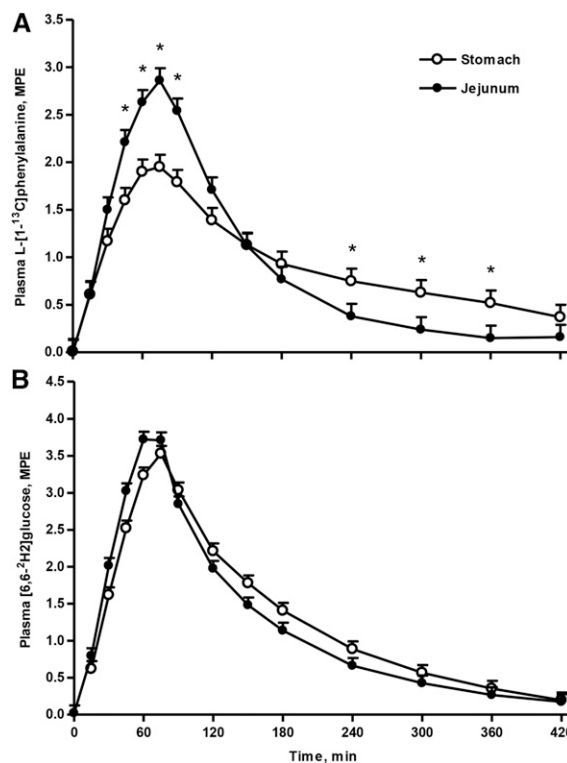


FIGURE 3 Plasma L-[1-¹³C]phenylalanine (A) and [6,6-²H₂]glucose (B) enrichment after either gastric or jejunal casein protein feeding in healthy young men. Values are means \pm SEMs, $n = 11$. Data were analyzed with repeated-measures mixed-model ANOVA. *Different from jejunal feeding, $P < 0.05$. MPE, mole percent excess.

earlier increase in $[6,6\text{-}^2\text{H}_2]$ glucose appearance after jejunal feeding than after gastric feeding. This is not surprising because glucose does not need to travel from the stomach first after jejunal feeding. As was evident from the SDs and the variance in the measurements between subjects, there was much less variation in the postprandial increase in plasma $[6,6\text{-}^2\text{H}_2]$ glucose when compared with L-[1- ^{13}C]phenylalanine enrichments after jejunal vs. gastric feeding. The apparent differences in micellar casein protein digestion and AA absorption rate after gastric vs. jejunal feeding are likely attributable to the coagulation of micellar casein in the acidic environment of the stomach (20). The clotting of the casein in the stomach attenuates gastric emptying and changes in micellar structure render the casein less accessible to luminal digestion, both of which result in slower mucosal AA absorption after gastric vs. jejunal feeding. These findings indicate that predigestion of micellar casein by gastric acid is not required and may even reduce the capacity to degrade intact casein into peptides and AAs. Our results indicate that jejunal feeding with micellar casein allows more rapid protein digestion and AA absorption than does gastric feeding.

Postprandial protein kinetics are affected by the rate of dietary protein digestion and subsequent absorption of dietary protein-derived AAs. Dietary proteins are commonly classified as “fast” or “slow,” because it is well recognized that their structure affects their rate of digestion and absorption, which strongly modulates the postprandial hormonal and metabolic response as well as postprandial protein accretion (22). Intact casein is generally classified as a slowly digestible protein and is commonly used in EN because of its high EAA content (23). As shown in Figure 3, we observed a more rapid and greater increase in L-[1- ^{13}C]phenylalanine enrichment after jejunal vs. gastric feeding. In line with this finding, we observed a more rapid increase in circulating plasma phenylalanine, leucine, total EAA, and total AA concentrations after jejunal casein feeding when compared with gastric casein feeding (Figure 2). This suggests that jejunal casein feeding results in more rapid protein digestion and AA absorption when compared with gastric feeding, turning the “slow” casein into a “fast” casein protein.

In the present study we used specifically produced intrinsically L-[1- ^{13}C]phenylalanine-labeled casein protein to allow an appropriate comparison of in vivo dietary protein digestion and subsequent AA absorption between both feeding regimens. The ingestion of more rapidly digestible protein results in a more rapid increase in circulating AAs and greater postprandial plasma AA availability, thereby increasing postprandial muscle protein accretion (22, 24, 25). Because we did not apply continuous intravenous infusions with a secondary phenylalanine tracer, we cannot quantify the absolute amount of dietary protein-derived AAs that were released in the circulation after protein feeding. However, because we observed no differences in the time to peak or iAUC of phenylalanine between trials, there is no indication that there were substantial differences in the overall postprandial release of dietary protein-derived AAs in the circulation between feeding groups during the evaluated postprandial period. Previously, Koopman et al. (26) compared dietary protein digestion and absorption kinetics after the ingestion of a single bolus of intact and hydrolyzed casein and found that the ingestion of casein hydrolysate resulted in more rapid protein digestion and AA absorption from the gut, augmented postprandial plasma AA availability, and tended to increase the incorporation rate of dietary protein-derived AAs into de novo skeletal muscle protein. The jejunal feeding of the casein seems to mimic the response we previously observed after

the ingestion of hydrolyzed casein as opposed to intact (micellar) casein. The greater postprandial increase in circulating EAA concentrations, and plasma leucine concentrations in particular (27, 28), may allow a more robust increase in muscle protein synthesis rate after jejunal vs. gastric casein feeding, which may be particularly relevant in conditions of anabolic resistance (29).

The development of insulin resistance during hospitalization is an unwanted phenomenon, because it may lead to more infectious complications and a prolonged length of hospital stay (30). Elevated postprandial insulin concentrations have been shown to inhibit proteolysis, stimulate AA uptake, and/or augment muscle protein synthesis (31, 32). Increases in plasma insulin concentrations were observed after the intravenous infusion of free AAs (33). However, the insulinotropic properties of protein when given either via gastric or jejunal routes have not been investigated previously. As shown in Figure 1, we observed a greater increase in insulin concentrations after jejunal when compared with gastric feeding, implying that a more rapid release of AAs in the circulation stimulates endogenous insulin release. In accordance, it has been well established that a more rapid increase in circulating plasma AA concentrations stimulates endogenous insulin release, thereby attenuating postprandial blood glucose excursions (34).

The current study is the first, to our knowledge, to compare in vivo dietary protein digestion and absorption kinetics after jejunal vs. gastric casein feeding in healthy young men. We conclude that jejunal casein protein feeding allows a more rapid dietary protein digestion and subsequent AA absorption when compared with gastric feeding. The present findings show that feeding strategy can have a distinct impact on dietary protein digestion and AA absorption kinetics and, as such, may also modulate postprandial muscle protein accretion.

Acknowledgments

JL, KvN, PAMvL, and LJCvL designed the research; JL, MA, ACH, JG, and HR conducted the research; JL and KvN analyzed the data and performed statistical analysis; JL, KvN, NB, HR, PAMvL, and LJCvL wrote the manuscript; and LJCvL had primary responsibility for final content. All authors read and approved the final manuscript.

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