

# CHAPTER FIVE

Jejunal casein feeding is followed by more rapid protein digestion and amino acid absorption when compared with gastric feeding in healthy young men

Joanna Luttikhoud, Klaske van Norren, Nikki Buijs, Marjolein Ankersmit, Annemieke C Heijboer, Jeannette Gootjes, Herman Rijna, Paul AM van Leeuwen, and Luc JC van Loon

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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 for gastric feeding. However, the impact of gastric versus jejunal feeding on post-prandial dietary protein digestion and absorption kinetics *in vivo* in humans remains largely unexplored.

**Objective** We compared the impact of gastric versus jejunal feeding on subsequent dietary protein digestion and amino acid absorption *in vivo* in healthy young men.

**Methods** Utilizing a randomized cross-over study design, eleven healthy young men ( $21 \pm 2$  y) were administered 25 g specifically produced intrinsically L-[1- $^{13}\text{C}$ ]phenylalanine labeled intact casein via a nasogastric and a nasojejunal tube placed  $\sim 30$  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 post-prandial period to assess the rise in plasma amino acid concentrations and dietary protein derived plasma L-[1- $^{13}\text{C}$ ]phenylalanine enrichment.

**Results** Jejunal feeding compared to gastric feeding resulted in higher peak plasma phenylalanine, leucine, total EAA, and total AA concentrations (all  $P < 0.05$ ). This was attributed to a more rapid release of dietary protein derived amino acids into the circulation, as evidenced by a higher peak plasma L-[1- $^{13}\text{C}$ ]phenylalanine enrichment level ( $2.9 \pm 0.2$  vs.  $2.2 \pm 0.2$  MPE;  $P < 0.05$ ). The total post-prandial plasma amino acid areas under the curve (iAUC) and time to peak did not differ following jejunal versus gastric feeding. Plasma insulin concentrations increased to a greater extent following jejunal feeding when compared with gastric feeding ( $275 \pm 38$  vs.  $178 \pm 38$  pmol/L;  $P < 0.05$ ).

**Conclusions** Jejunal feeding of intact casein is followed by more rapid protein digestion and amino acid absorption when compared with gastric feeding in healthy young men. The greater post-prandial rise in circulating essential amino acids concentrations, may allow a more robust increase in muscle protein synthesis rate following jejunal as opposed to gastric casein feeding.

*This trial was registered at TrialRegister.nl as NTR2801.*

## 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) is preferred over parenteral nutrition as the general route of feeding for the critically ill patient who requires nutritional support therapy (6). Compared to parenteral nutrition, EN is associated with a lower risk of infectious complications and mortality (7). Moreover, EN allows preservation of the intestinal integrity, prevents mucosal atrophy and bacterial translocation (8, 9).

Delayed gastric emptying occurs in approximately 50% of all mechanically ventilated critically ill patients and limits the administration of EN (10). It leads to high gastric residues, higher risk for 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 is few *in vivo* data on the impact of gastric versus jejunal feeding on dietary protein digestion and absorption is likely attributed to the obvious methodological limitations of *in vivo* human research (15). To allow *in vivo* assessment of dietary protein digestion and absorption kinetics we applied intrinsically L-[1-<sup>13</sup>C]phenylalanine-labeled protein that was produced by collecting milk protein from lactating cows that was infused with large amounts of L-[1-<sup>13</sup>C]phenylalanine (16). We added [6,6-<sup>2</sup>H<sub>2</sub>]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 versus jejunal protein feeding on casein digestion and subsequent amino acid absorption in healthy young men. Subjects received (micellar) intact casein protein in this study since it is commonly used in EN for its high essential amino acid 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 amino acids. Consequently, we hypothesized that jejunal casein feeding leads to more rapid protein digestion and amino acid absorption when compared with gastric feeding. In this study we used intrinsically L-[1-<sup>13</sup>C]phenylalanine labeled casein protein (16, 17) to assess differences in protein digestion and amino acid absorption following gastric versus jejunal casein feeding *in vivo* in healthy young men.

## METHODS

### Subjects

Twelve healthy young men (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: aged between 18 and 45 y, a body mass index (BMI) between 18 and 27 was considered as a healthy weight, not using medication, non smoking, 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 cross-over clinical trial was carried out at a university-based hospital (Kennemer Gasthuis, Haarlem, The Netherlands) to evaluate the effects of 2 regimens of nutritional support on subsequent protein digestion and amino acid 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, The Netherlands. This trial was registered at TrialRegister.nl as NTR2801.

**TABLE 1.** *Subjects' characteristics.*

<b>Baseline characteristics</b>	<b>n = 11</b>
Age (y)	21.7 ± 2.1
Body height (m)	1.87 ± 0.06
Body weight (kg)	80.2 ± 9.3
Body mass index (kg/m <sup>2</sup> )	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

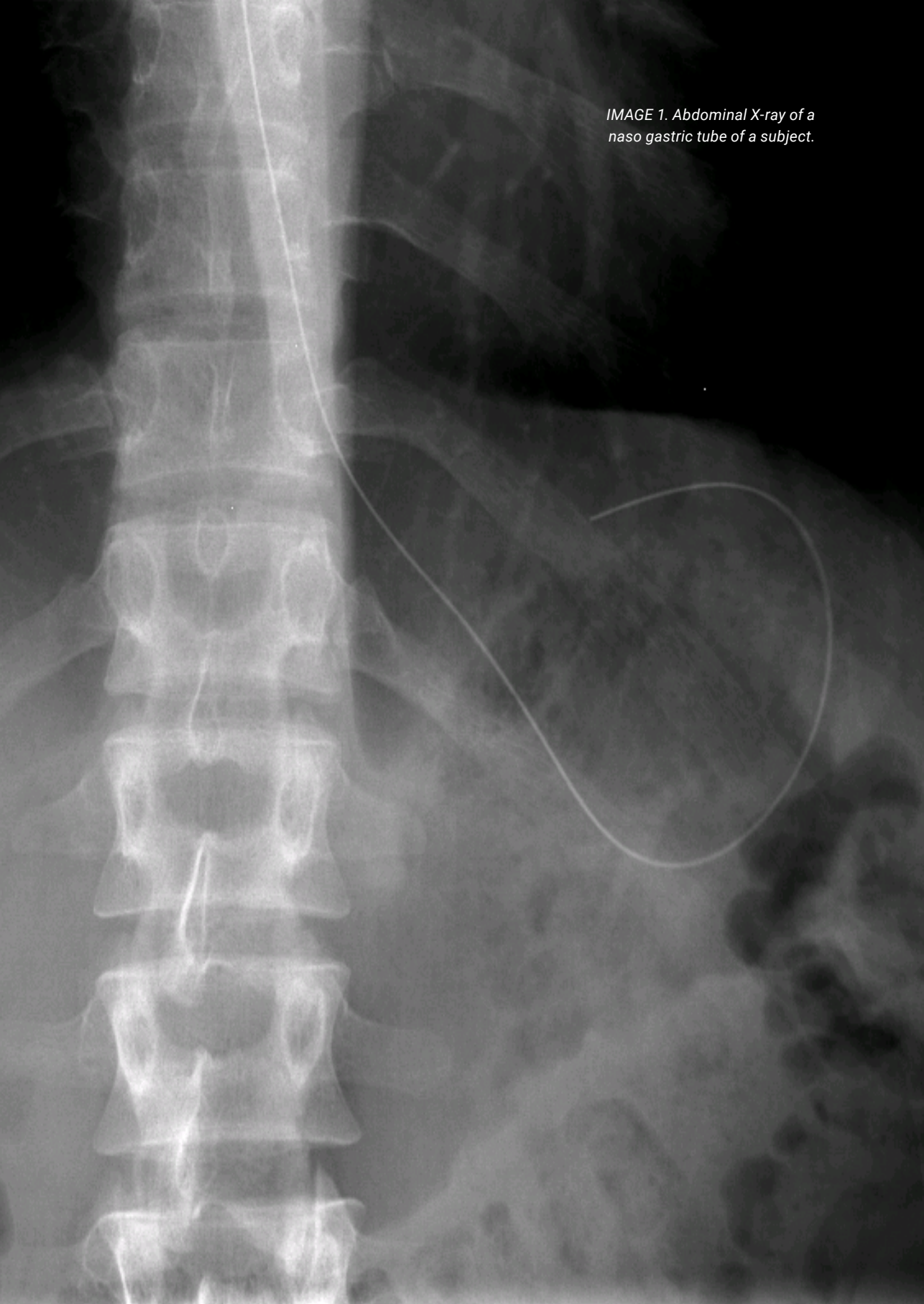
All values are means ± SDs. HOMA-IR, Homeostatic Model Assessment – Insulin Resistance.

## Diet and physical activity before testing

The day before the experiment all subjects consumed a standardized diet providing 50% of the 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 keep a constant diet 3 days before the trial.

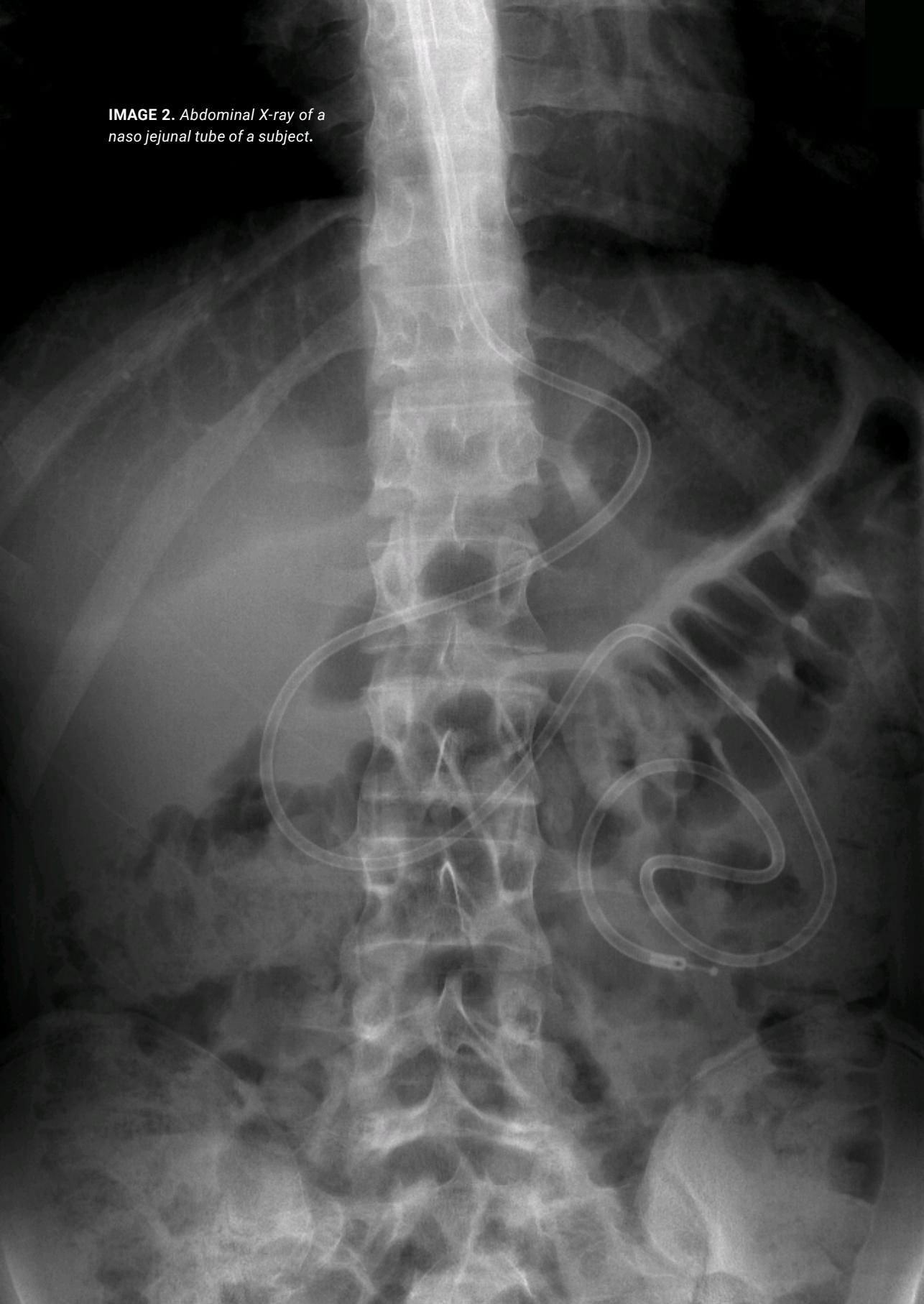
## Experiment

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



*IMAGE 1. Abdominal X-ray of a nasogastric tube of a subject.*

**IMAGE 2.** Abdominal X-ray of a naso jejunal tube of a subject.



## Protocol

Following an overnight fast, a polyurethane catheter was placed in a dorsal hand vein for frequent blood sampling. Administration of the fluid containing intrinsically L-[1-<sup>13</sup>C] 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 post-prandial period, at 15, 30, 45, 60, 75, 90, 120, 150, 180, 240, 300, 360, and 420 min. Venous blood glucose analysis were performed immediately. Blood samples were collected in EDTA-containing tubes and serum tubes and centrifuged at 1770g 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-<sup>13</sup>C]phenylalanine-labeled casein protein was obtained by infusing a Holstein cow with large quantities of L-[1-<sup>13</sup>C]phenylalanine, collecting milk, and purifying the casein fraction as described previously (16). The L-[1-<sup>13</sup>C]phenylalanine enrichment of the intact casein protein used was 6.1 mole percent excess (MPE). The casein protein met *all* chemical and bacteriological specifications for human consumption. Subjects received a volume of 240 mL through a NJT or a NGT, providing 25 g intrinsically labeled casein protein to which 1 g of [6,6-<sup>2</sup>H<sub>2</sub>] labeled glucose was added.

## Plasma analysis

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

For plasma phenylalanine enrichment measurements, plasma phenylalanine was derivatized to its t-butyltrimethylsilyl (TBDMS) derivative, and its <sup>13</sup>C enrichment determined by electron impact ionization GC-MS (model 6890N GC/5973N MSD; Agilent, Little Falls, DE) by using selected ion monitoring of masses 336 and 337 for unlabeled and [1-<sup>13</sup>C] 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-<sup>13</sup>C]phenylalanine isotopes in baseline samples (19). After derivatization of the plasma samples, plasma [6,6-<sup>2</sup>H<sub>2</sub>]glucose enrichment was measured by electron impact ionization gas chromatography-mass spectrometry (Finnigan INCOS-XL; Finnigan Mat, Ilemel, Hemstead, United Kingdom).

## Statistics

Baseline characteristics are expressed as means ± SD, P-value are based on Student's t-test for independent samples. All efficacy data are expressed as means ± SEMs. Efficacy parameters P-value are based on repeated measures mixed model analysis of variance (ANOVA) with

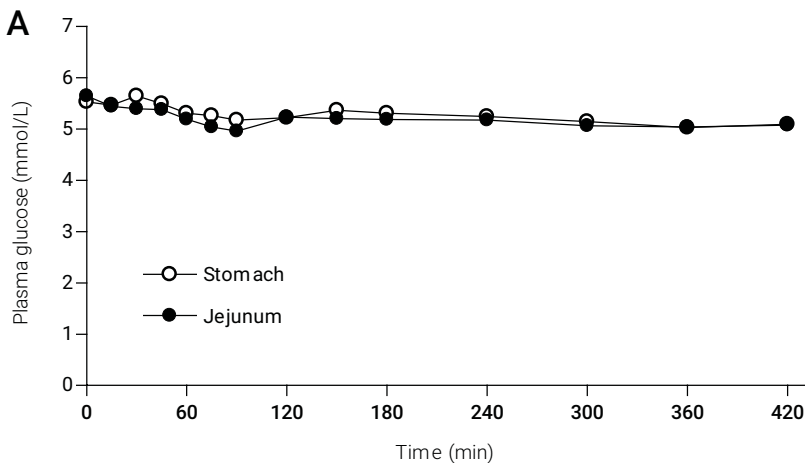
fixed factors: treatment, period, sequence, and random-factor subject. The P-value of 'within time analysis' to compare differences between treatments over time is based on repeated measures mixed model ANOVA with fixed factors: treatment, period, sequence, time and time-treatment interaction, and random-factor subject. For variables with ordered or ordinal categories, the Wilcoxon signed ranked test was used and binomial variables were analysed using the McNemar's test. Statistical significance was set at  $P < 0.05$ . All calculations were performed by Nutricia Research Utrecht using SAS (SAS Enterprise Guide 4.3 or higher) for Windows (SAS Institute Inc, Cary, NC).

## RESULTS

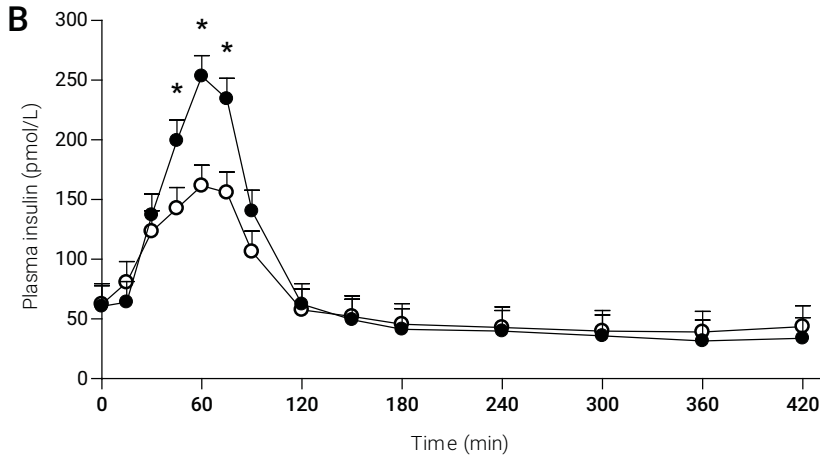
### Plasma glucose and insulin (Figure 1)

Plasma glucose concentrations did not change significantly during casein administration, and averaged  $5.3 \pm 0.1$  mmol/L following gastric feeding and  $5.2 \pm 0.1$  mmol/L following jejunal feeding. Following the onset of casein administration plasma insulin concentrations showed a rapid, but short-lived increase in both feeding regimens. Peak plasma insulin concentrations were higher following jejunal ( $275 \pm 38$  pmol/L) when compared with gastric ( $178 \pm 38$  pmol/L;  $P < 0.05$ ) feeding. The incremental area under the curve (iAUC) was also significantly higher following jejunal ( $9880 \pm 1690$  pmol/L·7h) when compared with gastric ( $6180 \pm 1760$  pmol/L·7h;  $P < 0.05$ ) feeding. Within time analysis showed a significantly higher level at 45, 60 and 75 min following jejunal when compared with gastric feeding.

**FIGURE 1.** Plasma glucose (A), and insulin (B) concentrations following 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. \*Significantly different from jejunal feeding,  $P < 0.05$ .







### Plasma amino acids (Table 2 and Figure 2)

Peak plasma phenylalanine concentrations were significantly higher following jejunal when compared with gastric feeding. Within time analysis showed significantly higher levels at 45, 60, 75, and 90 min following jejunal when compared with gastric feeding.

Peak plasma leucine concentrations were significantly higher following jejunal when compared with gastric feeding. Within time analysis showed significantly higher levels at 45, 60, 75, and 90 min following jejunal when compared with gastric feeding.

Peak plasma EAA concentrations were significantly higher following jejunal when compared with gastric feeding. Within time analysis showed significantly higher levels at 45, 60, 75, and 90 min following jejunal when compared with gastric feeding.

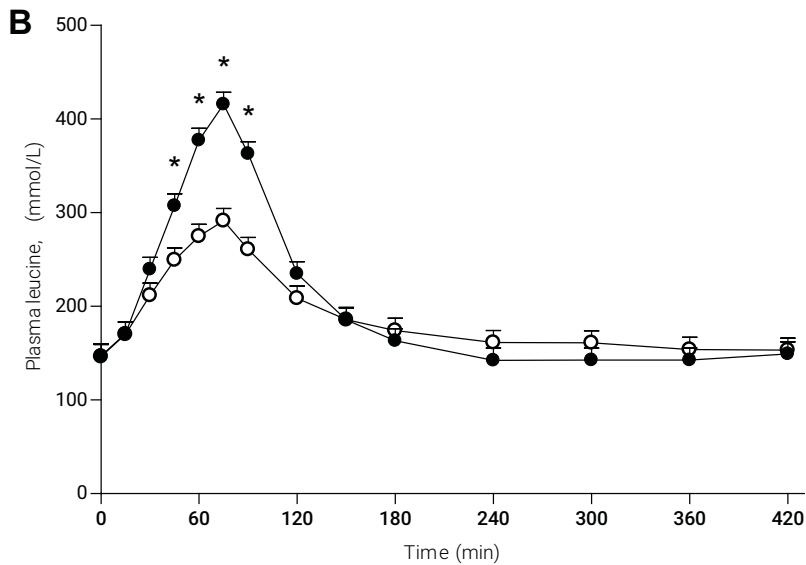
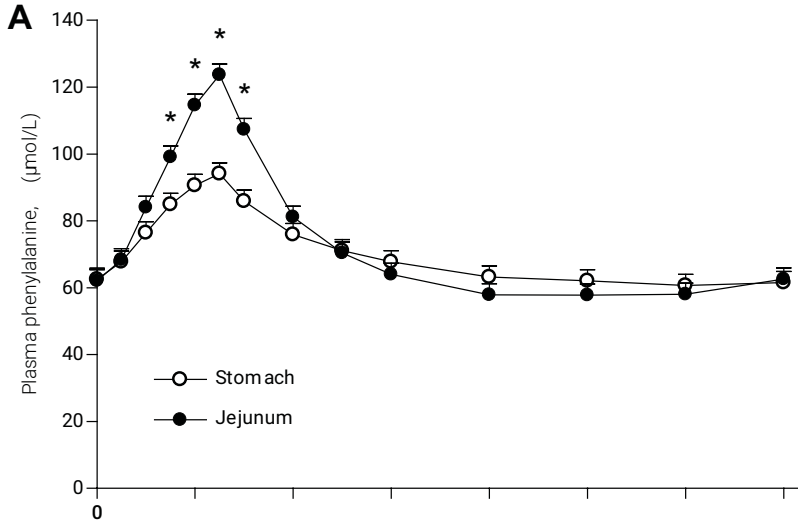
Peak plasma AA concentrations were significantly higher following jejunal when compared with gastric feeding. Within time analysis showed significantly higher levels at 45, 60, 75, and 90 min following jejunal when compared with gastric feeding.

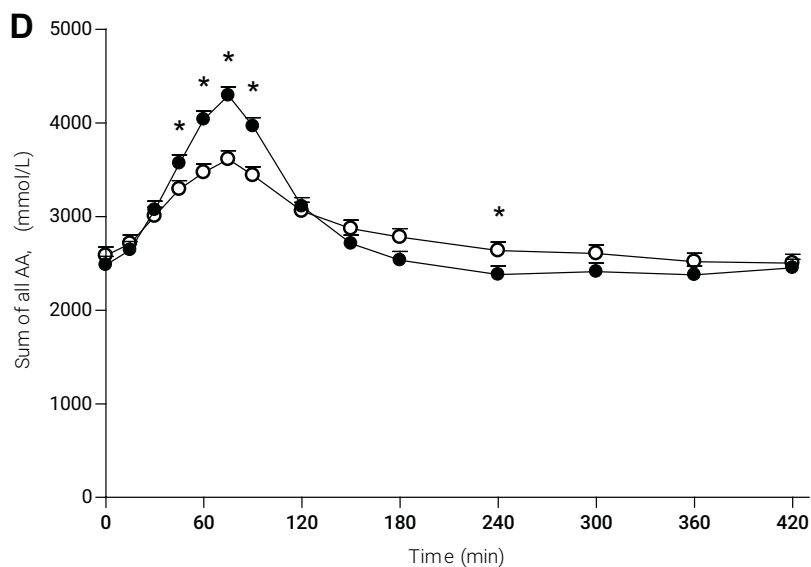
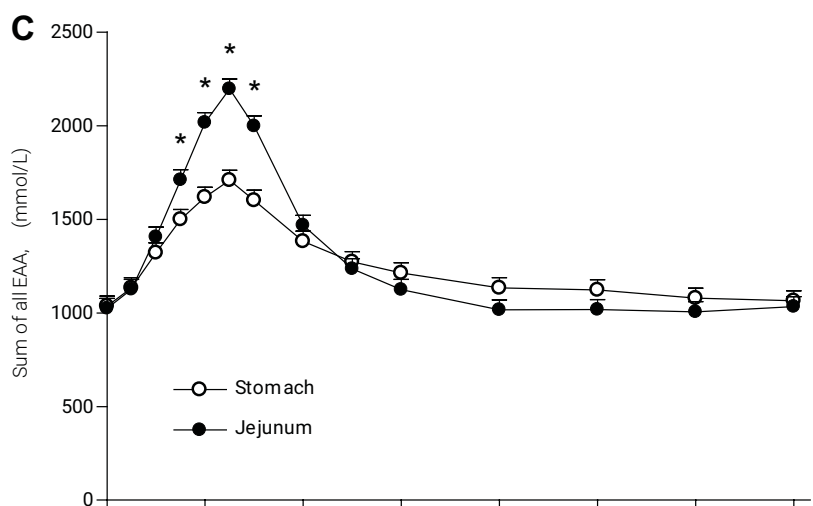
**TABLE 2.** Plasma amino acid concentrations. Baseline, peak value, time to peak, and iAUC following either gastric or jejunal feeding (n = 11).

	Phenylalanine		Leucine		Sum of all EAA		Sum of all AA	
	NGT	NJT	NGT	NJT	NGT	NJT	NGT	NJT
Baseline (μmol/L)	63 ± 3	62 ± 3	146 ± 13	147 ± 13	1040 ± 54	1020 ± 54	2580 ± 92	2480 ± 92
Peak value (μmol/L)	100 ± 6	124 ± 6*	313 ± 22	420 ± 22*	1779 ± 92	2210 ± 93*	3720 ± 150	4300 ± 150
Time to peak (min)	90 ± 17	74 ± 17	90 ± 17	77 ± 17	92 ± 17	79 ± 17	98 ± 17	78 ± 17
iAUC (mol/L*420)	3 ± 1	4 ± 1	16 ± 2	19 ± 2	81 ± 9	88 ± 8	109 ± 13	126 ± 12

All values are means ± SEMs. \*Significantly different (P < 0.05) compared with gastric feeding. AA, amino acids; EAA, essential amino acids; iAUC, incremental area under the curve; NGT, nasogastric tube; NJT, nasojejunal tube.

**FIGURE 2.** Plasma phenylalanine (A), leucine (B), sum of all EAA (C), and sum of all AA (D) concentrations following 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. \*Significantly different from jejunal feeding,  $P < 0.05$ . EAA, essential amino acids; AA, amino acids.





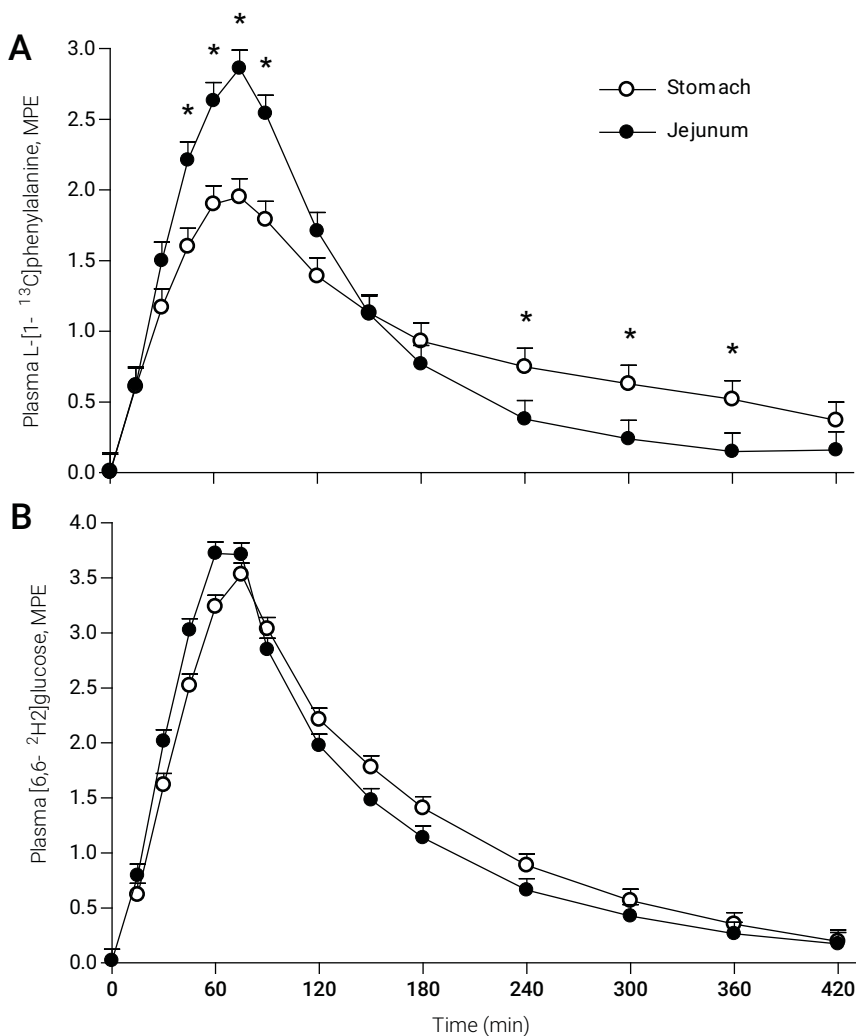
### Plasma tracer enrichments (Figure 3)

Plasma L-[1-<sup>13</sup>C]phenylalanine enrichments increased immediately following casein administration in both groups. Peak plasma L-[1-<sup>13</sup>C]phenylalanine enrichments were significantly higher following jejunal ( $2.9 \pm 0.2$  MPE) when compared with gastric ( $2.2 \pm 0.2$  MPE;  $P < 0.05$ ) feeding. Time to peak and iAUC did not differ significantly between groups. Within time analysis showed significantly higher levels at 45, 60, 75, and 90 min following jejunal when compared with gastric feeding. Whereas at 240, 300, and 360 min levels were significantly higher following gastric when compared with jejunal feeding.

Plasma g [6,6-<sup>2</sup>H<sub>2</sub>]glucose enrichment increased immediately following casein administration in both groups. The rise in [6,6-<sup>2</sup>H<sub>2</sub>]glucose enrichment did not differ significantly between

feeding regimens. Time to peak, peak value, and iAUC of plasma [6,6-<sup>2</sup>H<sub>2</sub>]glucose enrichments did not differ significantly between regimens.

**FIGURE 3.** Plasma L-[1-<sup>13</sup>C]phenylalanine (A), and [6,6-<sup>2</sup>H<sub>2</sub>]glucose enrichment (B) expressed as MPE following either gastric or jejunal casein protein feeding in healthy young men. Values are means ± SEMs, n = 11. Data were analyzed with repeated-measures mixed model ANOVA. \*Significantly different from jejunal feeding, P < 0.05. MPE, mole percentage excess.



## 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 that might occur during the study. Data on tolerance of the study product were collected every 4 hours with a Visual Analogue Scale. No serious adverse events were reported. A total of 3 adverse events (AEs) were possibly related to the administration of intact casein, of which 1 was reported with gastric feeding (occurring in 1 subject: 1 nausea) and 2 with jejunal feeding (occurring in 2 subjects: 1 nausea, 1 diarrhoea). The number of AEs was not significantly different between groups. Blood safety parameters 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 versus jejunal casein protein feeding on protein digestion and subsequent amino acid absorption in healthy young men. Jejunal feeding of intact micellar casein protein is followed by a more rapid release of dietary protein derived amino acids in plasma when compared to gastric feeding. This implies that jejunal casein feeding is followed by more rapid protein digestion and amino acid absorption when compared with gastric feeding.

The apparent differences in dietary protein digestion and amino acid absorption following gastric and jejunal feeding may be attributed to various factors, ranging from gastric emptying (20) to protein hydrolysis and mucosal amino acid absorption. In the present study, we also added [6,6-<sup>2</sup>H<sub>2</sub>]glucose to the solutions to compare the appearance rates of both exogenous glucose and protein derived phenylalanine following gastric versus jejunal feeding. It is evident from the data presented in Figure 3B that there is an earlier rise in [6,6-<sup>2</sup>H<sub>2</sub>]glucose appearance following jejunal when compared with gastric feeding. This is not surprising as the glucose does not need to travel from the stomach first following jejunal feeding. As evident from the standard deviation and the variance in the measurements between subjects, there is much less variation in the post-prandial rise in plasma [6,6-<sup>2</sup>H<sub>2</sub>]glucose when compared with L-[1-<sup>13</sup>C]phenylalanine enrichments following jejunal versus gastric feeding. The apparent differences in micellar casein protein digestion and amino acid absorption rate following gastric versus jejunal feeding are likely attributed to the coagulation of micellar casein in the acidic environment of the stomach (21). The clotting of the casein in the stomach attenuates gastric emptying and changes in micellar structure renders the casein less accessible to luminal digestion, both resulting in slower mucosal amino acid absorption following gastric as opposed to jejunal feeding. These findings indicate that pre-digestion of micellar casein by gastric acid is not required, and may even reduce the capacity to degrade intact casein into peptides and amino acids. Our results indicate that jejunal feeding with micellar casein allows more rapid protein digestion and amino acid absorption when compared with gastric feeding.

Post-prandial protein kinetics are affected by the rate of dietary protein digestion and subsequent absorption of dietary protein derived amino acids. 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 post-prandial hormonal and

metabolic response, as well as post-prandial protein accretion (22). Intact casein is generally classified as a slowly digestible protein and is commonly used in EN because of its high essential amino acid content (23). As shown in Figure 3, we observed a more rapid and greater rise in [1-<sup>13</sup>C]phenylalanine enrichment following jejunal compared with gastric feeding. In line with this finding, we observed a more rapid rise in circulating plasma phenylalanine, leucine, total EAA, and total AA concentrations following jejunal casein feeding when compared with gastric casein feeding (Figure 2). This suggests that jejunal casein feeding results in more rapid protein digestion and amino acid 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-<sup>13</sup>C]phenylalanine labeled casein protein to allow an appropriate comparison of *in vivo* dietary protein digestion and subsequent amino acid absorption between both feeding regimens. Ingestion of more rapidly digestible protein results in a more rapid rise in circulating amino acids and greater post-prandial plasma amino acid availability, thereby increasing post-prandial muscle protein accretion (22, 24, 25). As we did not apply continuous intravenous infusions with a secondary phenylalanine tracer, we cannot quantify the absolute amount of dietary protein derived amino acids that were released in the circulation following protein feeding. However, as 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 post-prandial release of dietary protein derived amino acids in the circulation following gastric versus jejunal feeding during the evaluated post-prandial period. Previously, Koopman *et al.* compared dietary protein digestion and absorption kinetics following the ingestion of a single bolus of intact casein and hydrolysed casein. They found that ingestion of casein hydrolysate results in more rapid protein digestion and amino acid absorption from the gut, it augments post-prandial plasma amino acid availability, and it tends to increase the incorporation rate of dietary protein derived amino acids into *de novo* skeletal muscle protein (26). The jejunal feeding of the casein seems to mimic the response we previously observed following the ingestion of hydrolysed casein as opposed to intact (micellar) casein. The greater post-prandial rise in circulating essential amino acids concentrations, and plasma leucine concentrations in particular (27, 28), may allow a more robust increase in muscle protein synthesis rate following jejunal as opposed to gastric casein feeding, which may be particularly relevant in conditions of anabolic resistance (29).

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

prandial blood glucose excursions (34).

In conclusion, the current study is the first to compare *in vivo* dietary protein digestion and absorption kinetics following jejunal versus gastric casein feeding in healthy young men. We conclude that jejunal casein protein feeding allows a more rapid dietary protein digestion and subsequent amino acid absorption when compared with gastric feeding. The present findings show that feeding strategy can have a distinct impact on dietary protein digestion and amino acid absorption kinetics and, as such, may also modulate post-prandial muscle protein accretion.

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