CHAPTER EIGHT

Jejunal feeding is followed by a greater rise in plasma CCK, PYY, GLP-1 and GLP-2 concentrations when compared with gastric feeding in vivo in humans; a randomized trial

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ABSTRACT

Background Jejunal feeding is preferred over gastric feeding in patients who are intolerant to gastric feeding or at risk for aspiration. However, the impact of gastric versus jejunal feeding on post-prandial circulating plasma glucose and amino acids concentrations and the associated endocrine response ***in vivo*** in humans remains largely unexplored.

Objective We compared the impact of administering enteral nutrition either gastric or jejunal on endocrine responses ***in vivo*** in humans.

Methods In a randomized cross-over study design, 12 healthy young males (21±2 y) received continuous enteral nutrition containing non-coagulating proteins (Nutrison Multi Fibre, Nutricia N.V., Zoetermeer, The Netherlands) for 12 h via a nasogastric tube or a nasojejunal tube placed 30 to 40 cm post Treitz. Blood samples were collected during the 12 h post-prandial period to assess the rise in plasma glucose, amino acid, and gastrointestinal hormone concentrations.

Results No differences were observed in the post-prandial rise in circulating plasma amino acid and glucose concentrations between regimens. Jejunal feeding resulted in higher peak plasma insulin concentrations when compared with gastric feeding (392±53 vs. 326±54 pmol/L; $P<0.05$). The post-prandial rise in plasma CCK, PYY, GLP-1, and GLP-2 levels was greater following jejunal versus gastric feeding, with higher peak concentrations and a greater post-prandial iAUC for GLP-1 and CCK (all $P<0.05$). Plasma ghrelin concentrations did not differ between regimens.

Conclusions Enteral nutrition with gastric or jejunal feeding in healthy young males results in similar post-prandial plasma amino acid and glucose concentrations. The endocrine response, however, differs substantially, with higher peak plasma CCK, PYY, GLP-1, and GLP-2 concentrations being attained following jejunal feeding. This may result in an improved anabolic response, greater insulin sensitivity, and an improved intestinotropic effect. Nevertheless, it may also lead to delayed gastric emptying.

This trial was registered at TrialRegister.nl as NTR2801.

INTRODUCTION

Malnutrition has a reported prevalence rate as high as 50% in hospitalized patients (1). The negative impact caused by malnutrition in the hospital setting has been shown to increase morbidity, mortality, length of hospital stay and, as a consequence, associated costs (2, 3). Patients have metabolic and immune neuro-endocrine derangements that are exacerbated by energy and protein deficits occurring during the early stages of admission to the intensive care unit (4). Recently the treatment of critically ill has become more focused on nutrition therapy, specifically attempting to attenuate the metabolic response to stress, to prevent oxidative cellular injury, and to favorably modulate the immune response (5).

As parenteral feeding has been associated with a greater incidence of infectious complications and increased mortality (6), enteral nutrition (EN) is the preferred route of feeding for critically ill patients who require nutritional support therapy (5). Moreover, EN preserves the intestinal integrity, prevents mucosal atrophy and bacterial translocation (7, 8). There are several methods of administering EN, of which gastric tube feeding is the most commonly applied route of access. However, administering EN via the gastric route in patients suffering from bowel motility disorders may lead to high gastric residual volumes and consequently to pulmonary aspiration (9). This problem can be overcome by inserting a jejunal feeding tube. Metheny *et al*. observed an 18 % lower aspiration percentage when the feeding tube is placed in the fourth portion of the duodenum and beyond (10).

Prior research in patients following gastrectomy or Roux-en-Y gastric bypass (RYGB) indicate that bypassing/eliminating the stomach does not per se lead to malabsorption (11, 12). After operation-associated metabolic sequelae have been resolved, there is no evidence that exclusive jejunal feeding results in protein malnutrition (13). However, bypassing the stomach and the duodenum obviates important endocrine and exocrine functions of these organs. Knowledge on the effect of the site of EN delivery on gastrointestinal hormones is crucial, especially for critically ill, because of their altered hormone response and delayed gastric emptying. This study will give us insight on whether during continuous administration of EN, the site of nutrient delivery affects the magnitude of gastrointestinal hormone secretion in response to nutrients.

Thus, the aim of this study was to compare the impact of gastric versus jejunal administration of EN on circulating plasma glucose and amino acids concentrations and the associated endocrine response ***in vivo*** in humans. By administering a polymeric EN and by frequent blood sampling we compared the impact of gastric versus jejunal feeding on gastrointestinal hormone responses and nutrient digestion and absorption in twelve healthy young men.

SUBJECTS AND METHODS

Subjects

Twelve healthy men (mean ± SD, 21 ± 2 y) participated in the present study. Subjects were randomly assigned to either gastric-jejunal or jejunal-gastric treatment sequence in a cross-over design. Inclusion criteria were: aged between 18 and 45 y, a body mass index (BMI) between 18 and 27, 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.

The 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. All authors had access to the study data and reviewed and approved the final manuscript.

TABLE 1. *Subjects' characteristics.*

Baseline characteristics	***n* = 12**
Age (y)	21 ± 2
Body height (m)	1.9 ± 0.1
Body weight (kg)	77.3 ± 10.8
Body mass index (kg/m^2)	22.3 ± 2.4
Basal plasma glucose (mmol/l)	5.4 ± 0.4
Basal plasma insulin (pmol/L)	52.8 ± 19.2
HOMA-IR	1.8 ± 0.7
Basal energy expenditure (kcal/24h)	2499 ± 223
Enteral nutrition (kcal)	1251 ± 104
Enteral nutrition per kg weight (kcal/kg)	16.3 ± 1.0

All values are means ± SDs.

Diet and physical activity prior to testing

All volunteers were instructed to refrain from alcohol consumption, exhaustive physical activity and to keep a constant diet 3 days before the trial. All subjects consumed a standardized meal the day before the experiment.

Experiment

According to a cross-over design, each subject received EN through a nasogastric tube (NGT) and a nasojejunal tube (NJT), separated by at least a 4-week wash-out period. In healthy subjects with normal small intestinal motility, nasojejunal tubes, once they are correctly positioned in the stomach, will migrate in a caudad direction during the phase III migrating motor complex. Abdominal X-rays were performed to confirm that the NGT's were correctly positioned in the stomach and the NJT approximately 30-40 cm distal to the ligament of Treitz**.**

Protocol

Following an overnight fast, a polyurethane catheter was placed in a dorsal hand vein for frequent blood sampling. Administration of the EN through a NGT or NJT was started directly after basal blood sampling. Venous blood samples were collected frequently during a 12 h postprandial period at 15, 30, 45, 60, 90, 120, 150, 180, 210, 240, 480, and 720 min. Venous blood glucose analyzes were performed immediately. Blood samples were collected into EDTA-containing tubes, serum tubes, heparin tubes, and P800 tubes (K_2EDTA tube with a proprietary cocktail of protease, esterase and DPP-IV inhibitors, BD Diagnostics, USA), and centrifuged within 10 min after sampling at 1770g for 12 min at 4°C. Aliquots of plasma were frozen and stored at –80°C.

Enteral nutrition

The amount of EN was determined using the Harris Benedict equation, with stress factor 'none' and activity factor 'bed rest' (14). For healthy male subjects with minimal activity the Basal Energy Expenditure (BEE) can be estimated with the Harris Benedict equation multiplied by 1.3 (BEE (kcal/24h) = (66.473 + 13.7516 · Weight (kg) + 5.0033 · Height (cm) - 6.755 · Age (y)) · 1.3) (15). The mean amount administered per subject over a period of 12 hours was 1251 ± 104 kcal (mean ± SD). Per kg body weight each subject received 16.3 ± 1.02 kcal (mean ± SD). Subjects received continuous feeding with fiber enriched EN containing four protein sources (25% casein, 35% whey, 20% pea protein and 20% soy protein; Nutrison Multi Fibre, Nutricia N.V., Zoetermeer, The Netherlands) (see Table 2 for the detailed composition).

TABLE 2. *Composition of a standard enteral nutrition with multi fibre*[A].

Ingredients	per 100ml
Energy kcal (kJ)	**103 (430)**
Protein equivalent (16 %E) (g)	**4.0**
Nitrogen (g)	0.6
Carbohydrate (47%E) (g)	**12.3**
Polysaccharides (g)	11.3
Sugars (g)	0.8
- Lactose (g)	<0.025
Fat (34%E) (g)	**3.9**
Saturates (g)	1.0
- Of which MCT (g)	0.6
EPA (mg)	19.5
DHA (mg)	14.0
Dietary fibre (3%E) (g)	**1.5**
Soluble (g)	0.7
Insoluble (g)	0.8
Minerals	
Sodium (mg)	100
Potassium (mg)	150

Ingredients	per 100ml
Chloride (mg)	125
Calcium (mg)	80
Phosphorus (mg)	72
Magnesium (mg)	23
Iron (mg)	1.6
Zinc (mg)	1.2
Copper (µg)	180
Manganese (mg)	0.33
Fluoride (mg)	0.10
Molybdenum (µg)	10
Selenium (µg)	5.7
Chromium (µg)	6.7
Iodine (µg)	13
Vitamins	
Vitamin A (µg RE)	82
Vitamin D (µg)	1.0
Vitamin E (mg α-TE)	1.3
Vitamin K (µg)	5.3
Thiamin (mg)	0.15
Riboflavin (mg)	0.16
Niacin (mg NE)	1.8
Pantothenic acid (mg)	0.53
Vitamin B6 (mg)	0.17
Folic acid (µg)	27
Vitamin B12 (µg)	0.21
Biotin (µg)	4.0
Vitamin C (mg)	10
Others	
Carotenoids (mg)	0.20
Choline (mg)	37
Water (g)	**83**
Osmolarity (mOsmol/l)	250
Osmolality (mOsmol/kg H_2O)	300
Potential renal solute load (mOsmol/l)	369

[A]Composition of Nutrison Multi Fibre® (Nutricia N.V., Zoetermeer, the Netherlands).

Ingredients: Water, maltodextrin, vegetable oils, dietary fibres (soy polysaccharides, resistant starch, inulin, arabic gum, cellulose, oligofructose), whey protein concentrate (from milk), sodium caseinate (from milk), pea protein isolate, soy protein isolate, emulsifier (soy lecithin), acidity regulator (citric acid), sodium chloride, fish oil, tri calcium phosphate, tri potassium citrate, di potassium hydrogen phosphate, potassium hydroxide, potassium chloride, carotenoids ((contains soy) b-carotene, lutein, lycopene), choline chloride, calcium hydroxide,

magnesium hydroxide, magnesium hydrogen phosphate, sodium L-ascorbate, ferrous lactate, zinc sulphate, nicotinamide, retinyl acetate, DL-a tocopheryl acetate, copper gluconate, sodium selenite, manganese sulphate, calcium D-pantothenate, chromium chloride, D-biotin, cholecalciferol, pteroylmonoglutamic acid, thiamin hydrochloride, pyridoxine hydrochloride, sodium molybdate, riboflavin, sodium fluoride, potassium iodide, phytomenadione, cyanocobalamin. Contains omega 3 fatty acids from fish oils (EPA and DHA).

Plasma analysis

Plasma glucose concentrations were analyzed with the HemoCue® Glucose 201 DM Analyser (HemoCue Diagnostics BV, Waalre Netherlands). 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-phtaldialdehyde and fluorimetry (Nutricia Research, Utrecht, The Netherlands). Plasma insulin, C-peptide, cholecystokinin (CCK), plasma peptide YY (PYY3-36), and ghrelin were determined by the Department of Clinical Chemistry, VU University Medical Center, Amsterdam, The Netherlands. Plasma insulin and C-peptide concentrations were analyzed by luminescence immunometric assay (Advia Centaur, Siemens Medical Solutions Diagnostics, USA). CCK concentrations were analyzed by radioimmunoassay (Euro-Diagnostica, Sweden). PYY3-36 concentrations were analyzed by radioimmunoassay (Millipore, USA). Plasma ghrelin concentrations were analyzed by radioimmunoassay (Linco Research Inc., St.Charles Missouri, USA). GLP-1 and GLP-2 concentrations in plasma were measured by radioimmunoassays after extraction of plasma with 70 % ethanol (vol/vol, final concentration). Carboxy-terminal GLP-1 immunoreactivity was determined using antiserum 89390 which has an absolute requirement for the intact amidated carboxy-terminus of GLP-1 7-36amide and cross reacts less than 0.01% with carboxy-terminally truncated fragments and 89% with GLP-1 9-36amide (16). GLP-2 concentrations were measured using a radioimmunoassay employing antiserum code no. 92160 and standards of human GLP-2 (proglucagon 126-158, a gift from Novo Nordisk A/S) and monoiodinated Tyr-12 GLP-1, specific activity > 70 MBq/nmol (17). The antiserum is directed aganist the N-terminus of GLP-2 and therefore measures only fully processed GLP-2 of intestinal origin. Sensitivity for both assays was below 5 pmol/l, and intra-assay coefficient of variation below 10 %.

Statistics

This is an exploratory study; the primary parameters have not been reported in healthy subjects or patients before. Therefore the expected difference between the study groups and its variance is an estimate, based on the results published by Ledeboer *et al* (18, 19). They studied a similar group of healthy subjects; however, they compared gastric feeding with duodenal feeding where in this study gastric feeding is compared to jejunal feeding. Differences in hormone response of CCK on 2 time points (20 min and 240 min) reported by these authors were used to calculate the expected difference in change between gastric and jejunal feeding. Based on these data, it was assumed that the expected mean difference in change of CCK after 20 minutes of administrating EN between gastric and jejunal feeding for CCK was approximately 3.3 pmol/L (6.6 v 3.3 pmol/L). The within-group standard deviation of

the change was expected to be between 0.7 and 2.4 pmol/L (based on a correlation of r=0.8 between gastric and jejunal feeding). Applying a significance level (alpha) of 0.050, a paired t-test, and a power of 80%, the proposed sample size of 5 was thought to be sufficient to detect a statistically significant result between the groups.

It was assumed that the expected mean difference in change of CCK after 240 minutes of administrating EN between gastric and jejunal feeding for CCK was approximately 0.8 pmol/L (3.7 v 2.9 pmol/L). The within-group standard deviation of the change was expected to be between 1.0 and 1.2 (based on a correlation of r=0.8 between gastric and jejunal feeding). Applying a significance level (alpha) of 0.050, a paired t-test, and a power of 80%, the proposed sample size of 9 was thought to be sufficient to detect a statistically significant result between the groups.

Using the above-mentioned estimates, a significance level (α) of 0.05, and a power of 80%, a sample size of n=9 was assumed to detect a statistically significant difference applying Statpower (20, 21). Assuming 20% drop-out, in total 12 subjects were needed.

Baseline characteristics are expressed as means ± SD, P-values are based on Student's t-test for independent samples. All efficacy data are expressed as means ± SEMs. The mean time to peak is calculated from every subject's specific time to peak. 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-values 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, insulin and C-peptide (Table 3 and Figure 1)

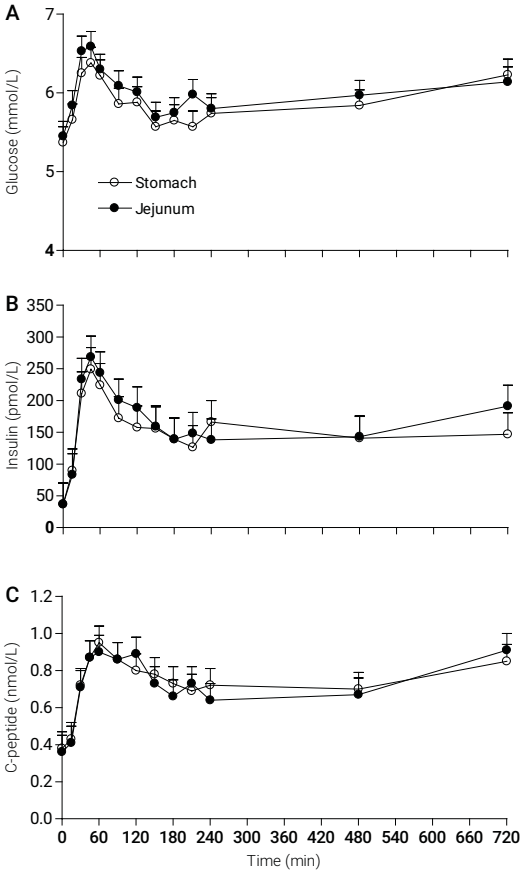
Plasma glucose concentrations increased immediately following the onset of EN administration in both groups. Time to peak, peak value and iAUC of plasma glucose concentrations did not differ significantly between regimens. Plasma insulin concentrations increased rapidly in both groups reaching peak levels of respectively 174 ± 68 and 162 ± 63 min. Peak plasma insulin concentrations were significantly higher following jejunal when compared with gastric feeding. The insulinogenic index (ratio of insulin concentration at 30 min minus fasting insulin to the difference of glucose at same time) showed no significant difference between regimens. C-peptide concentrations were not different between groups. Significant correlations were observed between peak insulin and peak C-peptide concentrations following gastric ($r=0.84$, $P < 0.05$) and jejunal ($r=0.85$, $P < 0.05$) feeding.

TABLE 3. *Plasma glucose, insulin and C-peptide concentrations. Baseline, peak value, time to peak, iAUC, and insulinogenic index following either gastric (n = 11) or jejunal feeding (n = 12).*

	Glucose (µmol/L)		Insulin (pmol/L)		C-peptide (µmol/L)	
	NGT	NJT	NGT	NJT	NGT	NJT
Baseline (µ or pmol/L)	5.4 ± 0.2	5.5 ± 0.2	37 ± 34	36 ± 33	0.38 ± 0.1	0.36 ± 0.1
Peak value (µ or pmol/L)	7.0 ± 0.2	7.2 ± 0.2	326 ± 54	392 ± 53 *	1.2 ± 0.1	1.2 ± 0.1
Time to peak (min)	194 ± 85	297 ± 80	174 ± 68	162 ± 63	185 ± 85	311 ± 80
iAUC (µ or pmol/L·720min)	402 ± 82	456 ± 78	89 ± 16	94 ± 15	295 ± 44	270 ± 43
Insulinogenic index (T30)			105 ± 21	102 ± 20		

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

FIGURE 1. *Mean (± SEM) glucose (A), insulin (B), and C-peptide (C) plasma concentrations following either gastric (n = 11) or jejunal feeding (n = 12). Data were analyzed with repeated-measures mixed model ANOVA. There was no significant difference between regimens.*



Plasma amino acids (Table 4 and Figure 2)

Time to peak, peak value and iAUC of the sum of all AA, sum of all EAA, phenylalanine, leucine, glutamine and citrulline did not differ between feeding regimens. Within time analysis showed significant higher concentrations for the sum of all AA and phenylalanine at 45 and 60 min following jejunal when compared with gastric feeding**.**

Within time analysis showed significant higher concentrations for glutamine at 45 min, and for citrulline at 210 min following gastric when compared with jejunal feeding.

Gastrointestinal hormones (Table 5 and Figure 3)

Peak plasma CCK concentrations were significantly higher following jejunal feeding (12 ± 2 pmol/L) when compared to gastric feeding (4 ± 2 pmol/L; $P < 0.05$). The iAUC was significantly higher following jejunal feeding (2551 ± 542 pmol/L) when compared to gastric feeding (907 ± 574 pmol/L; $P < 0.05$). The time to peak was not different between groups, however CCK concentrations were significantly higher at 30, 90, 120, 180 and 480 min following jejunal feeding.

Peak plasma PYY concentrations were higher following jejunal feeding (81 ± 7 pg/mL) when compared to gastric feeding (65 ± 7 pg/mL; $P < 0.05$). The time to peak and iAUC were not significantly different, however PYY concentrations were significantly higher at 30, 60, 120, 150, 180, 210, 240 and 480 min following jejunal feeding.

Plasma ghrelin concentrations declined similarly following EN administration in both feeding regimens. The times to peak, peak values and iAUCs were also similar.

Peak plasma GLP-1 concentrations were significantly higher following jejunal feeding (22 ± 1 pmol/L) when compared to gastric feeding (17 ± 1 pmol/L; $P < 0.05$). The iAUC was significantly higher following jejunal feeding (2212 ± 371 pmol/L) when compared to gastric feeding (1033 ± 574 pmol/L; $P < 0.05$). The time to peak was not different between groups, however GLP-1 concentrations were significantly higher at 60, 480 and 720 min following jejunal feeding.

Peak plasma GLP-2 concentrations were significantly higher following jejunal feeding (24 ± 2 pmol/L) when compared to gastric feeding (17 ± 2 pmol/L; $P < 0.05$). The iAUC and time to peak were not different between groups, however GLP-2 concentrations were significantly higher at 30, 90, 120, and 480 min following jejunal feeding.

Significant correlations were observed between peak values of plasma CCK and PYY concentrations following gastric feeding ($r=0.66$, $P < 0.05$). Peak values of plasma CCK and ghrelin concentrations correlated inversely with each other following jejunal feeding ($r=-0.75$, $P < 0.05$).

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. 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

TABLE 4. *Plasma amino acid concentrations. Baseline, peak value, time to peak, and iAUC following either gastric (n = 11) or jejunal feeding (n = 12).*

	Sum of all AA		Sum of all EAA		Phenylalanine		Leucine		Glutamine		Citrulline	
	NGT	NJT	NGT	NJT	NGT	NJT	NGT	NJT	NGT	NJT	NGT	NJT
Baseline (µmol/L)	2467 ± 54	2430 ± 52	976 ± 26	972 ± 25	550 ± 16	537 ± 15	34 ± 2	34 ± 2	57 ± 2	55 ± 2	139 ± 5	138 ± 5
Peak value (µmol/L)	2744 ± 50	2741 ± 47	1131 ± 26	1153 ± 25	592 ± 14	587 ± 14	42 ± 3	43 ± 3	66 ± 1	66 ± 1	173 ± 6	174 ± 6
Time to peak (min)	276 ± 93	373 ± 87	415 ± 97	359 ± 91	353 ± 90	255 ± 85	579 ± 60	552 ± 57	366 ± 101	361 ± 98	329 ± 94	289 ± 88
iAUC (mol/L·720min)	108 ± 30	100 ± 29	50 ± 14	52 ± 13	11 ± 4	14 ± 4	2 ± 0.5	3 ± 0.5	3 ± 0.7	3 ± 0.6	8 ± 2	9 ± 2

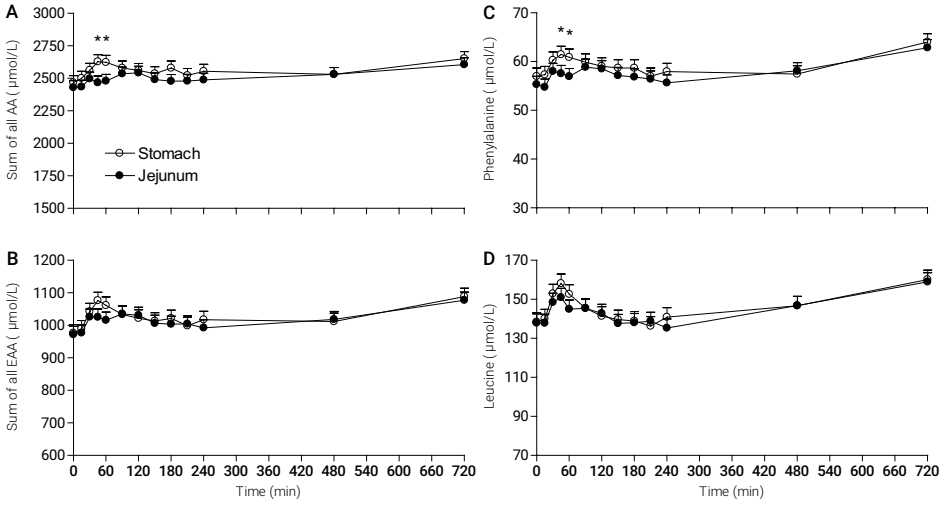
All values are means ± SEMs. There was no significant difference between regimens. AA, amino acids; EAA, essential amino acids; iAUC, incremental area under the curve; NGT, nasogastric tube; NJT, nasojejunal tube.

TABLE 5. *Gastrointestinal hormones. Baseline, peak value, time to peak, and iAUC following either gastric (n = 11) or jejunal feeding (n = 12).*

	Cholecystokinine		Peptide YY		Ghrelin		GLP-1		GLP-2	
	NGT	NJT	NGT	NJT	NGT	NJT	NGT	NJT	NGT	NJT
Baseline (µmol/L)	0.8 ± 0.8	0.8 ± 0.8	54 ± 7	61 ± 7	797 ± 59	763 ± 57	13 ± 1	13 ± 1	9 ± 2	10 ± 2
Peak value (µmol/L)	3.9 ± 1.6	12 ± 1.5 *	65 ± 7	81 ± 7 *	817 ± 64	835 ± 61	17 ± 1	22 ± 1 *	17 ± 2	24 ± 2 *
Time to peak (min)	204 ± 71	177 ± 67	226 ± 68	164 ± 63	54 ± 64	155 ± 59	163 ± 65	266 ± 61	273 ± 68	243 ± 66
iAUC (mol/L·720min)	1 ± 0.6	3 ± 0.5 *	4 ± 2	6 ± 1	1 ± 5	11 ± 5	1 ± 0.4	2 ± 0.4 *	3 ± 0.9	4 ± 0.8

All values are means ± SEMs. *Significantly different ($P < 0.05$) compared with gastric feeding. GI, gastrointestinal; GLP-1, glucagon-like polypeptide 1; GLP-2, glucagon-like polypeptide 2; iAUC, incremental area under the curve; NGT, nasogastric tube; NJT, nasojejunal tube.

FIGURE 2. *Mean (± SEM) sum of all AA (A), sum of all EAA (B), phenylanaline (C), and leucine (D) plasma concentrations following either gastric (n = 11) or jejunal feeding (n = 12). Data were analyzed with repeated-measures mixed model ANOVA. Interaction of time and treatment: P < 0.01. *Significantly different from jejunal feeding, P < 0.05.*



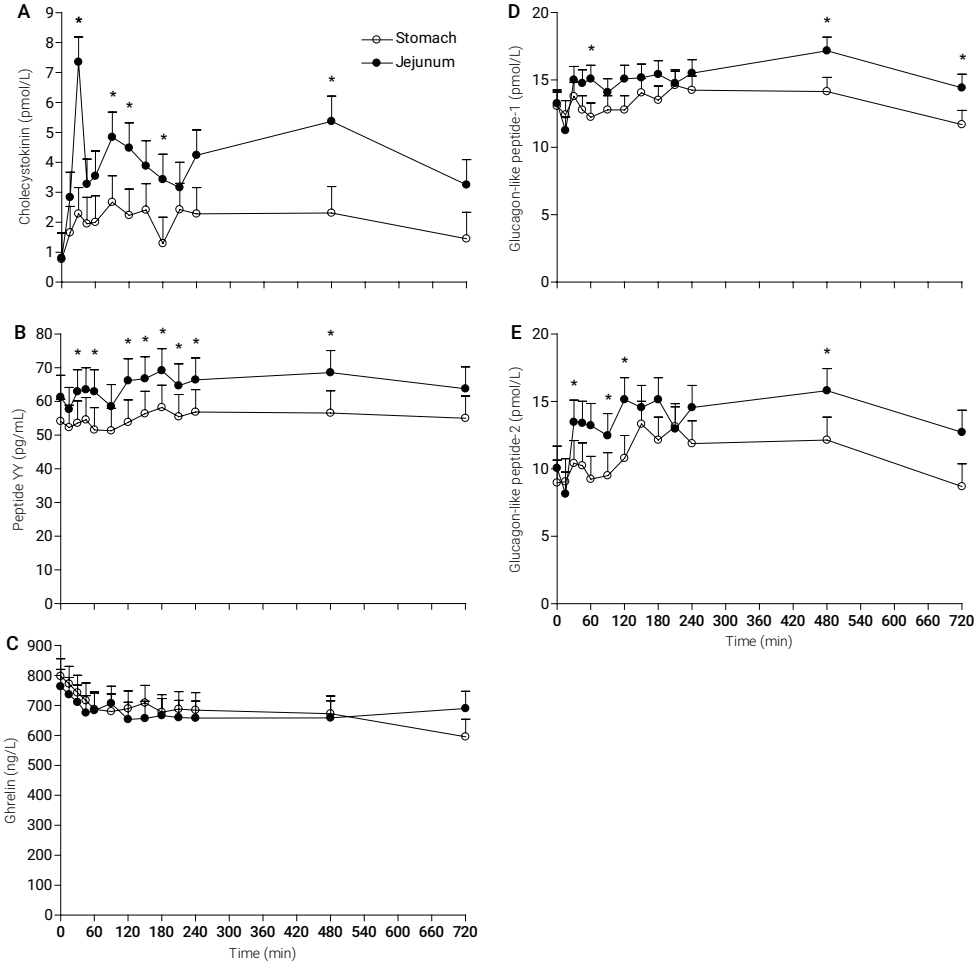
1 subject: 1 nausea) and 2 with jejunal feeding (occurring in 2 subjects: 1 nausea, 1 diarrhoea). The number of AEs was not significantly different between groups. Blood safety parameters all remained within the respective reference ranges and no clinically relevant changes in liver and kidney function were observed.

DISCUSSION

In the present study, we compared the impact of gastric versus jejunal administration of EN on circulating plasma glucose and amino acids concentrations and the associated endocrine response ***in vivo*** in humans. A non-coagulating polymeric EN did not result in different post-prandial plasma amino acid and glucose concentrations between regimens. However, the post-prandial endocrine response after administering EN differed substantially between jejunal and gastric feeding in healthy males, with higher peak plasma CCK, PYY, GLP-1, and GLP-2 concentrations following jejunal feeding.

In patients with gastric retention, nasogastric feeding is contraindicated and introduces the risk of regurgitation and pulmonary aspiration; jejunal feeding is an alternative route. Studies suggest that jejunal feeding requires a predigested rather than a polymeric diet (22-24). This is in contrast to our study, in which we administer a polymeric EN, and show similar post-prandial rise in circulating plasma amino acid concentrations between regimens. These results imply that adequate nutrition support can be obtained with jejunal feeding. This is also in contrast to the phenomenon of the 'ileal brake', which is thought to be activated by infusion of a polymeric EN distal to the ligament of Treitz, causing release of peptide YY and GLP-1. PYY inhibits exocrine pancreatic secretion leading to reduced absorption. In our study we also observed higher peak plasma PYY and GLP-1 concentrations following jejunal feeding distal

FIGURE 3. *Mean (± SEM) plasma cholecystokinine (A), peptide YY (B), ghrelin (C), GLP-1 (D), and GLP-2 (E) concentrations following either gastric (n = 11) or jejunal feeding (n = 12). Data were analyzed with repeated-measures mixed model ANOVA. Interaction of time and treatment: P < 0.01. *Significantly different from jejunal feeding, P < 0.05.*



to the ligament of Treitz, nevertheless it did not lead to a reduced digestion and absorption of nutrients as suggested by other authors (22). In our opinion the composition of EN determines nutrient digestion and absorption to a greater extent than the GI endocrine response; in which the proportion of casein is crucial for the EN not to coagulate in the acidic environment of the stomach (25). This is in line with our previous study in which we show that jejunal feeding with labeled casein is followed by more rapid protein digestion and amino acid absorption when compared with gastric feeding (26).

Thus, pre-digestion by gastric acid does not seem to be required for pancreatic proteases to effectively degrade the proteins and therefore to result in a similar rise in post-prandial amino

acid concentration with gastric and jejunal feeding. This is further supported by the glutamine and citrulline data. Continuous jejunal feeding does not lead to differences in the rise in circulating citrulline and glutamine when compared with gastric feeding; suggesting that in both feeding regimens there is an active intestinal enterocyte metabolic mass with access to post-prandial glutamine. Moreover, the higher levels of the intestinotropic hormone GLP-2 following jejunal feeding may even imply a protective effect on the intestine, when compared to gastric feeding. To substantiate that the increases in GLP-2 reflect an intestinotropic effect of jejunal feeding, a citrulline generation test with a dipeptide alanine-glutamine drink should be performed in humans (27).

Jejunal feeding also improves insulin secretion as indicated by the greater peak plasma insulin concentration, possibly due to higher peak plasma concentrations and iAUC of GLP-1 (28). It is known that higher levels of GLP-1 may reduce glucose levels in critically ill patients, leading to enhanced gastric emptying and a reduction in complications associated with insulin resistance (29). In our healthy volunteers, glucose levels were similar, possibly as a result of higher insulin responses. A similar effect is seen in bariatric surgery, in which dramatic improvements in glycemic control have been observed within one week, especially after RYGB surgery. Type 2 diabetes is improved or even reversed soon after these operations and well before significant weight loss occurs (30). This improvement is associated with a rise in GLP-1 levels (31). GLP-1 is known as an incretin hormone, responsible for part of the increase in insulin secreted after oral (opposed to intra venous) nutrient administration, and thereby reducing fasting and postprandial glycemia (32). With this study we were able to create conditions simulating nutritional administration after RYGB. Higher levels of GLP-1 following jejunal feeding may therefore improve glycemic control.

During postpyloric tube feeding, GI intolerance is observed more frequently than during prepyloric feeding, possibly by evoking a stronger GI response. This was observed by Ledeboer *et al*, showing that duodenal feeding elicited a stronger GI response than gastric feeding. They demonstrated an accelerated small-bowel transit time, more rapid and stronger gallbladder contractions, increased CCK, and pancreatic polypeptide release (18). These results are similar to our results of gastric and jejunal feeding, with higher peak plasma CCK concentrations being attained following jejunal feeding. CCK is involved in the regulation of gallbladder motility, exocrine pancreas excretion through relaxation of the sphincter of Oddi, gastric emptying, and intestinal motility (18). Intraluminal nutrients, especially fat and protein, stimulate CCK release, which signals the pylorus to reduce gastric emptying. The apparent differences in CCK following gastric and jejunal feeding may be attributed to various factors, ranging from gastric emptying to small intestine mucosa exposed to nutrients. In a previous study, we demonstrate by using labeled glucose ([6,6-$^{2}H_{2}$]glucose) that gastric emptying is less likely to attribute to the apparent differences (33). A more likely explanation for the increased CCK response after jejunal feeding is that nutrients were distributed over a larger area of CCK-releasing cells in the proximal small intestine.

Apart from CCK, the secretion of PYY was also significantly increased following jejunal feeding. Most effects of PYY are inhibitory, such as the inhibition of gastric, pancreatic and intestinal secretion or reduction in gastrointestinal motility, gall-bladder emptying and

gastric emptying. High concentrations of CCK and PYY are likely to contribute to delayed gastric emptying (34). The aim of this study was to compare ***in vivo*** endocrine and exocrine responses following jejunal versus gastric feeding with a polymeric EN in healthy young males. Gastric versus jejunal feeding with EN in healthy males does not result in different post-prandial plasma amino acid and glucose concentrations. The endocrine response to gastric versus jejunal feeding differs substantially, with higher peak plasma CCK, PYY GLP-1, and GLP-2 concentrations being achieved following direct jejunal feeding. This may result in an improved anabolic response, greater insulin sensitivity, and an improved intestinotropic effect. Nevertheless, it may also lead to delayed gastric emptying.

REFERENCES

1. Waitzberg DL, Caiaffa WT, Correia MI. Hospital malnutrition: the Brazilian national survey (IBRANUTRI): a study of 4000 patients. Nutrition 2001;17:573-80.
2. Correia MI, Waitzberg DL. The impact of malnutrition on morbidity, mortality, length of hospital stay and costs evaluated through a multivariate model analysis. Clin Nutr 2003;22:235-9.
3. Diks J, van Hoorn DE, Nijveldt RJ, Boelens PG, Hofman Z, Bouritius H, van Norren K, van Leeuwen PA. Preoperative fasting: an outdated concept? JPEN J Parenter Enteral Nutr 2005;29:298-304.
4. Schulman RC, JI. M. Can nutrition support interfere with recovery from acute critical illness? World Rev Nutr Diet 2013;105:69-81.
5. McClave SA, Martindale RG, Vanek VW, McCarthy M, Roberts P, Taylor B, Ochoa JB, Napolitano L, Cresci G. Guidelines for the Provision and Assessment of Nutrition Support Therapy in the Adult Critically Ill Patient: Society of Critical Care Medicine (SCCM) and American Society for Parenteral and Enteral Nutrition (A.S.P.E.N.). JPEN J Parenter Enteral Nutr 2009;33:277-316.
6. Heyland DK, MacDonald S, Keefe L, Drover JW. Total parenteral nutrition in the critically ill patient: a meta-analysis. JAMA 1998;280:2013-9.
7. Gianotti L, Nelson JL, Alexander JW, Chalk CL, Pyles T. Post injury hypermetabolic response and magnitude of translocation: prevention by early enteral nutrition. Nutrition 1994;10:225-31.
8. Luttikhold J, Oosting A, van den Braak CC, van Norren K, Rijna H, van Leeuwen PA, Bouritius H. Preservation of the gut by preoperative carbohydrate loading improves postoperative food intake. Clin Nutr 2013.
9. Heyland DK, Drover JW, MacDonald S, Novak F, Lam M. Effect of postpyloric feeding on gastroesophageal regurgitation and pulmonary microaspiration: results of a randomized controlled trial. Crit Care Med 2001;29:1495-501.
10. Metheny NA, Stewart BJ, McClave SA. Relationship between feeding tube site and respiratory outcomes. JPEN J Parenter Enteral Nutr 2011;35:346-55.
11. Sategna-Guidetti C, Bianco L. Malnutrition and malabsorption after total gastrectomy. A pathophysiologic approach. J Clin Gastroenterol 1989;11:518-24.
12. Brolin RE, LaMarca LB, Kenler HA, Cody RP. Malabsorptive gastric bypass in patients with superobesity. J Gastrointest Surg 2002;6:195-203; discussion 204-5.
13. Liedman B, Andersson H, Bosaeus I, Hugosson I, Lundell L. Changes in body composition after gastrectomy: results of a controlled, prospective clinical trial. World J Surg 1997;21:416-20; discussion 420-1.
14. MacDonald A, Hildebrandt L. Comparison of formulaic equations to determine energy expenditure in the critically ill patient. Nutrition 2003;19:233-9.
15. Weijs PJ, Kruizenga HM, van Dijk AE, van der Meij BS, Langius JA, Knol DL, Strack van Schijndel RJ, van Bokhorst-de van der Schueren MA. Validation of predictive equations for resting energy expenditure in adult outpatients and inpatients. Clin Nutr 2008;27:150-7.
16. Orskov C, Rabenhoj L, Wettergren A, Kofod H, Holst JJ. Tissue and plasma concentrations of amidated and glycine-extended glucagon-like peptide I in humans. Diabetes 1994;43:535-9.
17. Hartmann B, Johnsen AH, Orskov C, Adelhorst K, Thim L, Holst JJ. Structure, measurement, and secretion of human glucagon-like peptide-2. Peptides 2000;21:73-80.
18. Ledeboer M, Masclee AA, Biemond I, Lamers CB. Effect of intragastric or intraduodenal administration of a polymeric diet on gallbladder motility, small-bowel transit time, and hormone release. Am J Gastroenterol 1998;93:2089-96.
19. Ledeboer M, Masclee AA, Coenraad M, Vecht J, Biemond I, Lamers CB. Antroduodenal motility and small bowel transit during continuous intraduodenal or intragastric administration of enteral nutrition. Eur J Clin Invest 1999;29:615-23.
20. bavry JL. STATPOWER User's guide (2nd ed). Chicago: Scientific Software Inc. 1991.
21. Twisk JWR. Applied multilevel analysis. Cambridge University Press 2006.
22. Silk DB. Formulation of enteral diets for use in jejunal enteral feeding. Proc Nutr Soc 2008;67:270-2.
23. Silk DB. The evolving role of post-ligament of Trietz nasojejunal feeding in enteral nutrition and the need

for improved feeding tube design and placement methods. JPEN J Parenter Enteral Nutr 2011;35:303-7.
24. Luttikhold J, van Norren K, Buijs N, Rijna H, van Leeuwen PA. Gastrointestinal obstruction by solidification of enteral nutrition: a result of impaired digestion in critically ill patients. Nutritional Therapy Metabolism 2013;31:101-109.
25. van den Braak CC, Klebach M, Abrahamse E, Minor M, Hofman Z, Knol J, Ludwig T. A novel protein mixture containing vegetable proteins renders enteral nutrition products non-coagulating after in vitro gastric digestion. Clin Nutr 2013;32:765-71.
26. Luttikhold J, Van Norren K, Buijs N, Ankersmit M, Heijboer A, Gootjes J, Rijna H, Van Leeuwen PA, Van Loon LJ. Jejunal casein feeding is followed by more rapid protein digestion and amino acid absorption when compared with gastric feedin gin vivo in humans. Clinical Nutrition Abstracts book of the 36th ESPEN Congress, Geneva, Switzerland, 6-9 September 2014.
27. Peters JH, Wierdsma NJ, Teerlink T, van Leeuwen PA, Mulder CJ, van Bodegraven AA. The citrulline generation test: proposal for a new enterocyte function test. Aliment Pharmacol Ther 2008;27:1300-10.
28. Holst JJ. On the physiology of GIP and GLP-1. Horm Metab Res 2004;36:747-54.
29. Deane AM, Chapman MJ, Fraser RJ, Burgstad CM, Besanko LK, Horowitz M. The effect of exogenous glucagon-like peptide-1 on the glycaemic response to small intestinal nutrient in the critically ill: a randomised double-blind placebo-controlled cross over study. Crit Care 2009;13:R67.
30. Peterli R, Wolnerhanssen B, Peters T, Devaux N, Kern B, Christoffel-Courtin C, Drewe J, von Flue M, Beglinger C. Improvement in glucose metabolism after bariatric surgery: comparison of laparoscopic Roux-en-Y gastric bypass and laparoscopic sleeve gastrectomy: a prospective randomized trial. Ann Surg 2009;250:234-41.
31. de Carvalho CP, Marin DM, de Souza AL, Pareja JC, Chaim EA, de Barros Mazon S, da Silva CA, Geloneze B, Muscelli E, Alegre SM. GLP-1 and adiponectin: effect of weight loss after dietary restriction and gastric bypass in morbidly obese patients with normal and abnormal glucose metabolism. Obes Surg 2009;19:313-20.
32. Gautier JF, Fetita S, Sobngwi E, Salaun-Martin C. Biological actions of the incretins GIP and GLP-1 and therapeutic perspectives in patients with type 2 diabetes. Diabetes Metab 2005;31:233-42.
33. Luttikhold J, van Norren K, Buijs N, Ankersmit M, Heijboer AC, Gootjes J, Rijna H, van Leeuwen PA, van Loon LJ. Jejunal Casein Feeding Is Followed by More Rapid Protein Digestion and Amino Acid Absorption When Compared with Gastric Feeding in Healthy Young Men. J Nutr 2015;145:2033-8.
34. Nguyen NQ, Fraser RJ, Bryant LK, Chapman MJ, Wishart J, Holloway RH, Butler R, Horowitz M. The relationship between gastric emptying, plasma cholecystokinin, and peptide YY in critically ill patients. Crit Care 2007;11:R132.