Accelerated protein digestion and amino acid absorption after Roux-en-Y gastric bypass\textsuperscript{1,2}

Kirstine N Boisen-Møller\textsuperscript{3,5,9,*} Siv H Jacobsen\textsuperscript{3,5,9} Carsten Dirksen\textsuperscript{3,5} Nils B Jørgensen\textsuperscript{3,5} Søren Reitelseder\textsuperscript{7} Jens-Erik B Jensen\textsuperscript{3} Viggo B Kristiansen\textsuperscript{4} Jens J Holst\textsuperscript{5,6} Gerrit van Hall\textsuperscript{6,8} and Sten Madsbad\textsuperscript{3,5}

\textsuperscript{3}Department of Endocrinology and \textsuperscript{4}Department of Surgical Gastroenterology, Hvidovre Hospital, Copenhagen, Denmark; \textsuperscript{5}Novo Nordisk Foundation Centre for Basic Metabolic Research and \textsuperscript{6}Department of Biomedical Sciences, University of Copenhagen, Copenhagen, Denmark; \textsuperscript{7}Institute of Sports Medicine Copenhagen, Bispebjerg Hospital, Copenhagen, Denmark; \textsuperscript{8}Department of Clinical Metabolomics, Rigshospitalet, Copenhagen, Denmark

ABSTRACT

Background: Roux-en-Y gastric bypass (RYGB) involves exclusion of major parts of the stomach and changes in admixture of gastro-pancreatic enzymes, which could have a major impact on protein digestion and amino acid absorption.

Objective: We investigated the effect of RYGB on amino acid appearance in the systemic circulation from orally ingested protein and from endogenous release.

Design: Nine obese glucose-tolerant subjects, with a mean body mass index (in kg/m\textsuperscript{2}) of 39.2 (95\% CI: 35.2, 43.3) and mean glycated hemoglobin of 5.3\% (95\% CI: 4.9\%, 5.6\%), were studied before and 3 mo after RYGB. Leucine and phenylalanine kinetics were determined under basal conditions and during 4 postprandial hours by intravenous infusions of [3,3,3\textsuperscript{2}H\textsubscript{3}]leucine and [ring-\textsuperscript{2}H\textsubscript{3}]phenylalanine combined with ingestion of [1-\textsuperscript{13}C]-leucine intrinsically labeled caseinate as the sole protein source of the meal. Changes in body composition were assessed by dual-energy X-ray absorptiometry.

Results: After RYGB, basal plasma leucine concentration did not change, but marked changes were seen postprandially with 1.7-fold increased peak concentrations (before—mean: 217 \mu mol/L; 95\% CI: 191, 243 \mu mol/L; 3 mo—mean: 377 \mu mol/L; 95\% CI: 252, 502 \mu mol/L; P = 0.012) and 2-fold increased incremental AUC (before—mean: 4.1 mmol \cdot min/L; 95\% CI: 2.7, 5.5 mmol \cdot min/L; 3 mo—mean: 9.5 mmol \cdot min/L; 95\% CI: 4.9, 14.2 mmol \cdot min/L; P = 0.032). However, the postprandial hyperleucinemia was transient, and concentrations were below basal concentrations in the fourth postprandial hour. These concentration differences were mainly caused by changes in leucine appearance rate from orally ingested caseinate: peak rate increased nearly 3-fold (before—mean: 0.5 \mu mol/(kg fat-free mass \cdot min); 95\% CI: 0.4, 0.5 \mu mol/(kg fat-free mass \cdot min); 3 mo—mean: 1.4 \mu mol/(kg fat-free mass \cdot min); 95\% CI: 0.8, 1.9 \mu mol/(kg fat-free mass \cdot min); P = 0.002), and time to peak was much shorter (before—mean: 173 min; 95\% CI: 137, 209 min; 3 mo—mean: 65 min; 95\% CI: 46, 84 min; P < 0.001). Only minor changes were seen in endogenous leucine release after RYGB.

Conclusions: RYGB accelerates caseinate digestion and amino acid absorption, resulting in faster and higher but more transient postprandial elevation of plasma amino acids. Changes are likely mediated by accelerated intestinal nutrient entry and clearly demonstrate that protein digestion is not impaired after RYGB. This trial was registered at clinicaltrials.gov as NCT01559792. *Am J Clin Nutr* 2015;102:600–7.

Keywords: protein digestion, amino acid absorption, bariatric surgery, intrinsically labeled protein, caseinate, milk protein, gastric emptying, glucagon-like peptide-1, glucagon

INTRODUCTION

Roux-en-Y gastric bypass (RYGB)\textsuperscript{10} induces weight loss of 20–30\% that is maintained for at least 20 yr (1, 2). The surgery involves extensive rearrangements of the upper gastrointestinal tract, resulting in formation of a gastric pouch (25–50 mL) that drains directly into the lower part of the jejunum, thus excluding the remaining stomach, duodenum, and upper jejunum from food exposure. Secretions from the bypassed stomach, exocrine pancreas, and biliary system are mixed with food through an entero-entero anastomosis located approximately 100–150 cm distal to the pouch. RYGB was initially believed to inhibit food intake through restriction, but recent studies have demonstrated accelerated pouch emptying (3) and rapid absorption of the liquid phase marker, paracetamol (acetaminophen) (4), as well as glucose postoperatively (5–8). The absorption kinetics of lipids and proteins are largely unknown. Exclusion of major parts of the stomach and changed admixture of food with gastro-pancreatic enzymes have been suggested to impair or delay

\textsuperscript{1}Supported by the Danish Council for Independent Research for Medical Sciences, the Novo Nordisk Foundation, and the Strategic Counsel for the Capital Area of Copenhagen (Denmark). This work was carried out as part of the program of the UNIK: Food, Fitness & Pharma for Health and Disease (see www.foodfitnesspharma.ku.dk). The UNIK project is supported by the Danish Ministry of Science, Technology and Innovation. None of the funding sources had any influence on study design, implementation, analysis, or interpretation of the data.

\textsuperscript{2}Supplemental Material and Supplemental Figures 1–4 are available from the “Supplemental data” link in the online posting of the article and from the same link in the online table of contents at http://ajcn.nutrition.org.

\textsuperscript{9}These authors contributed equally to this work.

\textsuperscript{*To whom correspondence should be addressed. E-mail: kirstine.bojsen-moeller@regionh.dk.}

\textsuperscript{10}Abbreviations used: FFM, fat-free mass; GLP-1, glucagon-like peptide-1; Ra, rate of appearance; Rd, rate of disappearance; RYGB, Roux-en-Y gastric bypass.

Received February 13, 2015. Accepted for publication July 2, 2015.

First published online August 5, 2015; doi: 10.3945/ajcn.115.109298.
protein digestion (9). Therefore, we investigated the effect of RYGB on the appearance of amino acids in the systemic circulation from orally ingested protein as well as from endogenous sources during a mixed meal. Leucine and phenylalanine kinetics were determined under basal conditions and 4 h postprandially by intravenous infusions of [3,3,3-2H3]-leucine and [ring-2D5]-phenylalanine combined with ingestion of [1-13C]-leucine intrinsically labeled casein as the sole protein source of the meal. Importantly, leucine has been suggested as a regulator of anabolic and metabolic signaling in human skeletal muscles (10–12). Compared with leucine, phenylalanine has less special properties and is less abundant in casein, which potentially could influence systemic kinetics (13).

METHODS

Subjects

The study group included 10 obese subjects undergoing laparoscopic RYGB at Hvidovre Hospital (Copenhagen, Denmark). Before enrollment in the study, all participants fulfilled the inclusion criteria for bariatric surgery in Denmark and had completed a preoperative diet-induced total body weight loss of 8% required by health authorities. All subjects presented preoperative glycated hemoglobin (HbA1c) of 7.8 mmol/L after a 75-g oral glucose load, and fasting plasma glucose of 5.2 (4.9, 5.5) mmol/L, respectively with fasting plasma glucose of 6.0% (42 mmol/mol) (Table 1). One participant who was examined both before and after surgery experienced serious postoperative complications requiring prolonged hospitalization and was excluded from data analysis. Data on glucose absorption and metabolism in the same study population have been reported previously (5).

Ethics

Written, informed consent was obtained from all subjects, and the study was approved by the Municipal Ethical Committee of Copenhagen in accordance with the Helsinki declaration and by the study was approved by the Municipal Ethical Committee of Copenhagen in accordance with the Helsinki declaration and by the Danish Data Protection Agency and was registered at www.clinicaltrials.gov as NCT01559792.

Methods

Subjects were studied within 2 wk before RYGB and 3 mo after the operation. Each study session included a meal test and a dual-energy X-ray absorptiometry scan. On each study day, subjects arrived after an overnight fast (10–12 h) and were weighed and placed in a reclined position in a hospital bed allowing no physical activity. Cannulas were inserted in veins of each arm, one for tracer infusions and one for blood sampling. Primed continuous infusions of [3,3,3-2H3]-leucine [3 μmol/kg fat-free mass (FFM), 0.07 μmol/kg FFM per min] and [ring-2D3]-phenylalanine (1.5 μmol/kg FFM, 0.03 μmol/kg FFM - min)) were provided with use of a volumetric infusion pump (μVP5000; Arcomed ag). The exact, measured infusion concentration was used for later calculations. All stable isotopes (Cambridge Isotope Laboratories) were prepared under sterile conditions by the Capital Region Pharmacy (Herlev, Denmark). After 2 h of tracer infusions, the test meal was provided and infusions continued for 4 h postprandially.

Test meal was a semiliquid mixed meal (200 mL, 394 kcal, energy percentage: carbohydrate 50%, protein 15%, fat 35%) consisting of glucose (50 g), rapeseed oil (14.1 g), and [1-13C]-leucine intrinsically labeled calcium caseinate (15.2 g) with a [1-13C]-leucine to leucine ratio of 10.08%. Details on production of intrinsically labeled caseinate by the infusion of [1-13C]-leucine in cows have been described previously (14). Aroma was added to the mixed meal to disguise the distinct flavor of caseinate. The meal was consumed slowly over 30 min, and meal ingestion was supervised to secure that intake was evenly distributed over this period. Blood was sampled before initiation of tracer infusions to determine natural isotope abundance. Thereafter, blood samples were obtained at −30, −15, 0, 15, 30, 45, 60, 90, 120, 180, and 240 min relative to meal intake.

Dual-energy X-ray absorptiometry scans were performed by using a Hologic Discovery A (S/N 83487) dual-energy X-ray absorptiometry scanner (Hologic Inc.) with software package Apex 2.3 to determine fat percentage and FFM.

Surgical procedure

Operations were performed at the Department of Gastroenterology at Hvidovre Hospital with use of a standard laparoscopic RYGB technique. A gastric pouch was created (~25 mL) that drained directly into an alimentary jejunal limb of ~100 cm. The remaining stomach, duodenum, and first part of the jejunum...
Analytic procedures

Blood was collected into prechilled EDTA-coated tubes and was immediately centrifuged at 2000 × g for 10 min at 4°C. Plasma samples were frozen and stored at −80°C until analysis. A 200-μL aliquot of plasma was mixed with 100 μL internal standard (50 μmol/L L-[U-13C₆]-phenylalanine + 150 μmol/L [U-13C₆]-leucine) and 500 μL 50% acetic acid before being passed through a strong cation exchange column (Dowex AG 50W-X8; Bio-Rad). The purified amino acids were eluted with 3 mL 2 M NH₄OH, dried under a stream of N₂, and converted to their t-butyldimethylsilyl derivatives by adding 200 μL N-methyl-N-(tert-butyldimethylsilyl)-trifluoroacetamide and acetonitrile (1:3) and heating for 1 h at 70°C. The enrichment of the sample and the internal standards was determined with use of gas chromatography–tandem mass spectrometry (Tracer GC Ultra-TSQ Quantum; Thermo Scientific) with electron impact ionization and selective ion monitoring.

Calculations and statistical analysis

Data are expressed as means (95% CIs) of absolute values and means (95% CIs) of absolute postoperative change unless otherwise stated. Mean basal concentrations were calculated as a mean of the samples taken within the last 30 min before meal ingestion, and for the 4-h postprandial period, the total AUC was calculated by using the trapezoidal method with subtraction of basal concentrations to yield incremental AUC. Total rate of appearance (Ra) and disappearance (Rd) of leucine and phenylalanine were estimated with standard non–steady-state equations (15) by using enrichments of [3,3,3-²H₃]-leucine and [ring-²D₅]-phenylalanine, respectively. Oral leucine appearance rate was calculated from [1-¹³C]-leucine enrichments multiplied by total leucine appearance rate and corrected for time-dependent variations in plasma [1-¹³C]-leucine enrichment, and endogenous leucine appearance rate was calculated by subtracting the oral leucine Ra from the total leucine Ra. Total systemic appearance of leucine in the 4 postprandial hours (total AUC of oral leucine Ra) was expressed as a percentage of the total leucine content in the caseinate. Equations and enrichments are provided in the Supplemental Material and Supplemental Figure 1, respectively.

Single parameters (basal/peak concentrations and AUCs) were compared with paired t tests. Postprandial time courses of leucine and phenylalanine kinetics were analyzed by ANOVA in a linear mixed-effects model by using postprandial time (time) and study session (session) and the time × session interaction as fixed categorical effects and individual subjects as a random effect. Logarithmic transformation of data was used if required for optimal model fit. Time × session interaction was reported as the primary readout, and P < 0.05 was interpreted as a change in the postprandial time course after RYGB. Statistical analyses were performed in R version 3.0.2 (www.R-project.org).

RESULTS

Weight loss and changes in body composition after RYGB have been reported previously along with changes in oral and endogenous glucose kinetics and secretion of insulin, glucagon, glucagon-like peptide-1 (GLP-1), and glucose-dependent insulinotropic polypeptide (5). Three months after RYGB, patients had a mean reduced body weight of 18.5 kg, which included a mean of 6.6 kg of FFM (Table 1). Furthermore, patients experienced reductions in hepatic insulin resistance (mean relative change from before: −39%; 95% CI: −62%, −17%; P = 0.005) and fasting insulin concentration (mean relative change: −41%; 95% CI: −61%, −21%; P = 0.003), as well as accelerated postprandial glucose absorption (mean relative change in peak Ra of oral glucose: +165%; 95% CI: +96%, +236%; P < 0.001) and increased postprandial secretion (incremental AUC) of glucoregulatory hormones: insulin (mean relative change: +83%; 95% CI: +50%, +116%; P < 0.001), GLP-1 (mean relative change: +485%; 95% CI: +150%, +819%; P < 0.001), and glucagon (incremental AUC reversed from negative to positive, P = 0.026) (5).

Leucine concentration and kinetics

Basal plasma leucine concentration was unchanged 3 mo after RYGB (before—mean: 173 μmol/L; 95% CI: 148, 197 μmol/L; 3 mo—mean: 161 μmol/L; 95% CI: 132, 191 μmol/L; change—mean: −11 μmol/L; 95% CI: −38, 15 μmol/L; P = 0.345). Marked changes were seen in the postprandial leucine concentrations (Figure 1A and Supplemental Figure 2 for individual concentration curves) with a 1.7-fold increased peak concentration (before—mean: 217 μmol/L; 95% CI: 191, 243 μmol/L; change—mean: +160 μmol/L; 95% CI: +46, +274 μmol/L; P = 0.012) and 2-fold increased incremental AUC (before—mean: 4.1 mmol · min⁻¹ · L⁻¹; 95% CI: 2.7, 5.5 mmol · min⁻¹ · L⁻¹; 3 mo—mean: 9.5 mmol · min⁻¹ · L⁻¹; 95% CI: 4.9, 14.2 mmol · min⁻¹ · L⁻¹; change—mean: +5.4 mmol · min⁻¹ · L⁻¹; 95% CI: +0.6, +10.2 mmol · min⁻¹ · L⁻¹; P = 0.032). However, the postprandial hyperleucinemia was transient after RYGB, and in the fourth postprandial hour (at 180 and 240 min), leucine concentrations were statistically significantly below basal concentrations only after RYGB, resulting in unchanged total AUC after surgery (P = 0.531).

Basal leucine Ra and Rd did not change post-RYGB when corrected for changes in FFM [μmol/(kg FFM · min); Figure 1B, C] but was lower after surgery when expressed in absolute rates [μmol/min; Figure 1D, E]. The postprandial changes were largely independent of the corrections for FFM (Figure 1B–E). Postprandial total Ra and Rd of leucine changed after RYGB (Figure 1B, C) with 1.5-fold increased peak rates and ~20% lower nadir rates after surgery (P = 0.004). The mean (95% CI) total Ra peak before and 3 mo after surgery was 2.0 (1.8, 2.1) μmol/(kg FFM · min) and 3.0 (2.3, 3.8) μmol/(kg FFM · min), respectively, representing a change of +1.1 (+0.4, +1.8) μmol/(kg FFM · min) (P = 0.006). The mean (95% CI) total Rd peak before and 3 mo after surgery was 1.9 (1.8, 2.1) μmol/(kg FFM · min) and 2.9 (2.3, 3.5) μmol/(kg FFM · min), respectively, representing a change of +1.0 (+0.4, +1.5) μmol/(kg FFM · min) (P = 0.003). Total Ra and Rd were below basal at 180 min and 240 min both before and after surgery but were significantly lower after than before.
Endogenous Ra of leucine was unchanged in the basal state, but the postprandial time course changed after RYGB (Figure 1F) with ∼20% lower nadir values: the mean (95% CI) values before and 3 mo after surgery were 1.1 (1.0, 1.2) μmol/(kg FFM · min) and 0.9 (0.8, 1.0) μmol/(kg FFM · min), respectively, representing a change of −0.2 (−0.3, −0.1) μmol/(kg FFM · min) (P = 0.009). Maximal postprandial suppression from basal to nadir tended to increase (P = 0.079), but changes in the duration of postprandial suppression could not be determined from the present study because endogenous Ra of leucine at the end of the experiment (t = 240 min) remained suppressed at a similar rate (P = 0.910) before and after surgery.

Systemic appearance of leucine from orally ingested caseinate was dramatically changed after RYGB (Figure 1G and Supplemental Figure 3 for individual curves). Peak Ra of oral leucine increased nearly 3-fold, with mean (95% CI) values before and 3 mo after surgery of 0.5 (0.4, 0.5) μmol/(kg FFM · min) and 1.4 (0.8, 1.9) μmol/(kg FFM · min), respectively, representing a change of +0.9 (+0.4, +1.4) μmol/(kg FFM · min) (P = 0.002). The time to peak also was much shorter, with mean (95% CI) values before and 3 mo after surgery of 173 (137, 209) min and 65 (46, 84) min, respectively, representing a change of −108 (−158, −59) min (P < 0.001) and reflecting accelerated systemic uptake of leucine after RYGB. Expressing rate per kilogram of FFM or as whole-body Ra did not influence conclusions (data not shown). Total systemic appearance of leucine in the 4 postprandial hours was expressed as a percentage of the total leucine content in caseinate and increased after RYGB from a mean (95% CI) of 49% (40%, 58%) to 70% (61%, 79%), representing an absolute change of +21% (+10%, +31%) (P = 0.002), equivalent to the systemic appearance of leucine from 7.4 g and 10.6 g of the ingested protein, respectively. However, protein uptake was not completed within the 4 h of postprandial blood sampling neither before nor after surgery (Figure 1G).
Phenylalanine concentration and kinetics

Basal phenylalanine concentration was significantly lower 3 mo after RYGB (before—mean: 61 μmol/L; 95% CI: 56, 67 μmol/L; 3 mo—mean: 52 μmol/L; 95% CI: 46, 57 μmol/L; change—mean: −9 μmol/L; 95% CI: −15, −4 μmol/L; \( P = 0.003 \)), and changes in the entire postprandial period were prominent (Figure 2A). Preoperatively, phenylalanine concentrations increased slowly after meal intake, reaching a mean (95% CI) maximal increment (\( \Delta \text{peak-basal} \)) of 12 (10, 13) μmol/L without returning to basal values at 240 min (\( \Delta \text{basal-240} \) significantly below zero, \( P = 0.032 \)). After RYGB, the mean (95% CI) maximal increment was 37 (22, 51) μmol/L (\( P = 0.004 \)), and in the third and fourth postprandial hours, phenylalanine concentrations had reached basal values (\( \Delta \text{basal-180} \) and \( \Delta \text{basal-240} \) were not statistically significantly different from zero).

Systemic total Ra and Rd of phenylalanine declined in the basal state after RYGB, whether expressed as μmol/(kg FFM \( \cdot \) min) or μmol/min (Figure 2B–E). Postprandial kinetics of phenylalanine changed dramatically after RYGB (Figure 2B,C). Before surgery, the meal induced a minor increase in total Ra and Rd of phenylalanine in the first 4 postprandial hours, but after surgery peaks were clearly increased. For Ra, mean (95% CI) values before and 3 mo after surgery were 0.71 (0.64, 0.78) μmol/(kg FFM \( \cdot \) min) and 0.99 (0.79, 1.20) μmol/(kg FFM \( \cdot \) min), respectively, representing a change of +0.29 (+0.09, +0.48) μmol/(kg FFM \( \cdot \) min) (\( P = 0.010 \)). For Rd, mean (95% CI) values before and 3 mo after surgery were 0.70 (0.63, 0.77) μmol/(kg FFM \( \cdot \) min) and 0.99 (0.76, 1.22) μmol/(kg FFM \( \cdot \) min), respectively, representing a change of +0.29 (+0.08, +0.50) μmol/(kg FFM \( \cdot \) min) (\( P = 0.012 \)). Endogenous Ra and oral Ra of phenylalanine were not calculated because caseinate was only intrinsically labeled with leucine and not phenylalanine.

DISCUSSION

In this study, we demonstrate that RYGB results in marked changes of the systemic leucine and phenylalanine kinetics after ingestion of a mixed meal containing caseinate as the sole protein source. A faster and higher but more transient postprandial elevation of the amino acids was observed in the systemic circulation. The accelerated appearance of the meal protein–derived amino acids in plasma is most likely caused by accelerated entry of nutrients into the small intestine combined with accelerated protein digestion and amino acid absorption. A lower splanchnic extraction of the amino acids may contribute but is likely secondary...
to the accelerated amino acid absorption after RYGB (13, 16–18). Furthermore, our results confirm that the postprandial increases in plasma amino acids observed in previous RYGB studies (19, 20) result from accelerated appearance of amino acids from orally ingested protein and not from increased endogenous release.

Accelerated glucose absorption has previously been demonstrated after RYGB (5–8) and is a consequence of the surgical changes in gastrointestinal anatomy, resulting in faster passage of food through the pouch and the upper anastomosis and not weight loss in general (8). Accelerated amino acid absorption after protein intake is, however, somewhat surprising because protein in contrast to glucose needs to be digested. A major novel finding in this study is that protein digestion is not impaired after RYGB despite exclusion of major parts of the stomach and distal admixture of gastro-pancreatic secretions. This is likely a reflection of an almost instantaneous delivery of nutrients not only through the upper anastomosis but also all the way to the common limb. Scintigraphic studies after RYGB have shown detectable amounts of solid food in the small intestine 15 min after meal start but could not distinguish between the alimentary and common limb (3). Transit of liquids may be even faster as suggested by reports of cecal arriving time of 26 min after ingestion of a glucose drink (21) and maximal concentrations of paracetamol almost immediately after intake (4). Consistent findings of exaggerated secretion of L-cell products after RYGB (22) also support a rapid passage of nutrients to distal segments of the small intestine where L cells are located with the highest density (23). Studies in rodents have demonstrated significant hypertrophy of gut mucosa of the alimentary limb, which contributes to enhanced postprandial glucose uptake (24), but it has not been investigated whether these gut mucosal adaptations affect protein uptake. Intriguingly, the rates of appearances of oral leucine and oral glucose seem to be superimposable only after RYGB [Figure 1G and (5), Supplemental Figure 4 for superimposed curves]. However, protein uptake in the alimentary limb may be questionable because it would require brush-border enzymes to digest protein in the absence of gastro-pancreatic secretions. Gastric secretions of acid and pepsin may not be essential for protein digestion, because complete gastrectomy does not cause severe protein malabsorption (25). Exclusion of major parts of the stomach post-RYGB may in fact limit the acid-mediated clotting of casein, which is believed to delay gastric emptying (26, 27). In contrast, absence of secretions from the exocrine pancreas will cause severe protein malabsorption (28), although protein digestion can occur even with small outputs (28). Therefore, secretions from the exocrine pancreas must still be delivered in adequate amounts to the common limb for protein digestion not to be impaired after RYGB.

Accelerated protein digestion and amino acid absorption may have metabolic as well as nutritional consequences. Exaggerated postprandial releases of GLP-1 and insulin have been reported consistently after RYGB (22), including in the present study (5), and are important for improved glucose tolerance in patients with type 2 diabetes after surgery (29). Amino acids, especially leucine and phenylalanine, are potent stimulators of insulin secretion (15, 16, 30, 31) and are able to stimulate GLP-1 secretion as well (32). Accelerated digestion of protein may act in concert with glucose to enhance postprandial insulin and GLP-1 release in response to mixed meals after RYGB, although glucose seems to mediate substantial effects by itself (33). Moreover, high concentrations of amino acids could contribute importantly to the paradoxical increase in postprandial glucagon release (30, 34, 35), which is a consistent finding after RYGB (22), also in the present population (5). Indeed, the postprandial glucagon release is especially pronounced in response to mixed meals (33), although also observed after glucose alone (36). Recently, it has been demonstrated that post-RYGB hyperglycagonesia represents fully processed (and therefore biologically active) glucagon, but the source (L cells or α cells) and metabolic importance are still unknown (37).

The nutritional anabolic effect of ingested protein depends on the rate of digestion and the postprandial profile of plasma amino acids, as clearly demonstrated when assessing protein turnover after ingestion of slowly digested micellar casein compared with fast whey (14, 16, 17, 26, 27, 38) or casein hydrolysate (15, 16). Slow or fast digestion rates are, however, reflections of the gastric emptying rate and not the protein itself, as clearly demonstrated in this study, in which RYGB changed the digestion rate of caseinate. Protein turnover may be altered post-RYGB as a consequence of accelerated digestion but is also influenced by weight loss itself. Marked weight loss will inevitably decrease FFM along with fat mass, and in the present study, patients lost ~7 kg of FFM, equivalent to ~10% of initial FFM or ~36% of the total weight lost (5). Similar decreases in FFM have been found in other studies 3–6 mo after RYGB (36, 39), as well as after equivalent weight loss induced by gastric banding (8) or gastric sleeve (40). The degree of caloric restriction determines the proportion of FFM lost during major weight loss (40, 41) as well as the initial FFM (42). Severe protein malnutrition (hypoaalbuminemia) has been observed after RYGB but is rare (43, 44) and occurs primarily in patients with low protein intake (45), after severe postoperative complications, or after long limb procedures (43). Based on our results, decreased uptake of protein is not a likely contributor to protein loss after RYGB; however, the transient postprandial hyperaminoacidemia may potentially limit the anabolic phases over 24 h. Therefore, it seems reasonable to recommend small but frequent meals post-RYGB. As for the source of protein, this study demonstrates that the concept of slow and fast digested proteins may not apply to RYGB patients.

In the present study, protein uptake was not completed within the 4 h of postprandial blood sampling, which limits the evaluation of total protein uptake and splanchnic extraction, especially presurgery. To determine the nutritional consequences of RYGB, we need further studies investigating 24-h systemic protein turnover and in particular postprandial turnover rates of individual protein stores, particularly skeletal muscle. For the evaluation of protein turnover, patients should be studied more than 1 y post-RYGB to avoid the influence of calorie restriction. The strength of the study is the use of intrinsic labeled protein, which allows the investigation of the combined effect of digestion, absorption, and metabolism of protein. However, the use of intrinsic labeled protein excluded the use of a more everyday protein source such as meat, which may be digested more slowly due to the solid texture.

In conclusion, RYGB accelerates caseinate digestion and amino acid absorption, resulting in a faster and higher but also more transient postprandial elevation of plasma amino acids.
These findings demonstrate that protein digestion is not impaired after RYGB but in contrast accelerated as a result of the extensive changes in upper gastrointestinal anatomy. Furthermore, our results are consistent with gastric emptying rate as a rate-limiting step in protein digestion and supports previous findings of accelerated nutrient passage to distal intestinal segments after RYGB, potentially stimulating the secretion of L-cell products.

This work would not have been possible without the professional help of Dorthe Baunbjerg Nielsen, Susi Polmann, and Alis Sloth Andersen (Hvidovre Hospital, Denmark) and Nina Pluszak and Lene Foged (Clinical Metabolomics Core Facility, Rigshospitalet, Copenhagen, Denmark). We thank Steen Ladelund (chief statistician at Hvidovre Hospital, Denmark) for statistical support.

The authors’ responsibilities were as follows—KNB-M and SHJ: conducted the research; KNB-M: performed the data analysis, wrote the manuscript, and has primary responsibility for the final content of the manuscript; CD, NBJ, SR, J-EBJ, and VBK: contributed to the data analysis and discussion and reviewed/edited the manuscript; JHH, GvH, and SM: contributed to the design of the study, data generation and analysis, and discussion and reviewed/edited the manuscript; and all authors: approved the final version of the manuscript. KNB-M, SHJ, CD, NBJ, JHH, and SM are affiliated with the Novo Nordisk Foundation Centre for Basic Metabolic Research, which is funded by the Novo Nordisk Foundation. SHJ and NBJ have one-third of their PhD scholarship funded by Novo Nordisk. SR has received funding from Arla Foods Ingredients Group P/S and the Danish Dairy Research Foundation. J-EBJ, VBK, and GvH declared no conflicts of interest related to this study.

REFERENCES


